Parasitology

cambridge.org/par

Research Article

Cite this article: Davis NE, Blair D, Brant SV (2022). Diversity of *Trichobilharzia* in New Zealand with a new species and a redescription, and their likely contribution to cercarial dermatitis. *Parasitology* **149**, 380–395. https://doi.org/10.1017/S0031182021001943

Received: 13 August 2021
Revised: 10 October 2021
Accepted: 25 October 2021
First published online: 10 November 2021

Keywords:
Austropeplea; cercarial dermatitis; Lake Wanaka; museum vouchers; New Zealand; schistosomes; swimmer's itch; *Trichobilharzia*

Author for correspondence:
Sara V. Brant, E-mail: sbrant@unm.edu

Diversity of *Trichobilharzia* in New Zealand with a new species and a redescription, and their likely contribution to cercarial dermatitis

Norman E. Davis[1], David Blair[2] and Sara V. Brant[3]

[1]University of Otago, Dunedin, New Zealand; [2]James Cook University, College of Science and Engineering, Townsville, Australia and [3]Department of Biology, University of New Mexico Museum of Southwestern Biology Division of Parasites, Albuquerque, New Mexico 87111, USA

Abstract

In response to annual outbreaks of human cercarial dermatitis (HCD) in Lake Wanaka, New Zealand, ducks and snails were collected and screened for avian schistosomes. During the survey from 2009 to 2017, four species of *Trichobilharzia* were recovered. Specimens were examined both morphologically and genetically. *Trichobilharzia querquedulae*, a species known from four continents, was found in the visceral veins of the duck *Spatula rhynchotis* but the snail host remains unknown. *Cercaria longicauda* [i.e. *Trichobilharzia longicauda* (Macfarlane, 1944) Davis, 2006], considered the major aetiological agent of HCD in Lake Wanaka, was discovered, and redescribed from adults in the visceral veins of the duck *Aythya novaeseelandiae* and cercariae from the snail *Austropeplea tomentosa.* Recovered from the nasal mucosa of *Ay. novaeseelandiae* is a new species of *Trichobilharzia* that was also found to cycle naturally through *Au. tomentosa*. Cercariae of a fourth species of *Trichobilharzia* were found in *Au. tomentosa* but the species remains unidentified.

Introduction

Trichobilharzia Skrjabin and Zakharov, 1920 is a speciose genus within a unique family of digenetic trematodes, Schistosomatidae. This family consists of dioecious worms that infect the circulatory system and tissues of their bird or mammal host and use marine and freshwater snails as intermediate hosts. There are about 40 species of *Trichobilharzia* described mostly from waterfowl from all continents, except Antarctica (Horák *et al.*, 2002; Brant and Loker, 2009). Species confirmed as belonging to *Trichobilharzia* based on molecular data, use freshwater pulmonate snails in the families Physidae and Lymnaeidae as intermediate hosts (Brant and Loker, 2009; Horák *et al.*, 2012).

Species of *Trichobilharzia* have achieved notoriety as the leading aetiological agent of human cercarial dermatitis (HCD), or 'swimmer's itch' (Kolárová *et al.*, 2013; Soldanova *et al.*, 2013; Horák *et al.*, 2015). HCD is an allergic reaction (rash) in human skin to the penetration of schistosome cercariae that have emerged from the aquatic snail host. Most cases are contracted in freshwater environments and are due to avian schistosomes, but marine environments, particularly where gulls reside, are also suitable habitats (Horák *et al.*, 2012, 2015). Schistosome and host species identification is critical for outbreak management and targeted control. For example, *Trichobilharzia querquedulae* McLeod, 1937 in North America is found in physid snails and ducks in the genus *Spatula*. Thus, rather than targeting all species of snails and ducks in an environment, only the known host species need to be managed.

Just in the last few years, several papers have defined and redefined the genetic diversity within *Trichobilharzia* relative to the reported morphological diversity. As a result, several new species or new lineages (probably new species) have been recognized (Jouet *et al.*, 2010*a*, 2010*b*, 2015; Brant *et al.*, 2013, 2017; Kolárová *et al.*, 2013; Devkota *et al.*, 2014; Pinto *et al.*, 2014, 2017; Fakhar *et al.*, 2016; Ashrafi *et al.*, 2018, 2021).

The above works are important because morphological species identification of cercariae is difficult, and the identification of adults is problematic (Blair and Islam, 1983). Added to that, the avian hosts are often migratory, and the snail hosts can be common and widespread (e.g. Ebbs *et al.*, 2016; Ashrafi *et al.*, 2018, 2021). Morphological features of the cercariae are not sufficient for species discrimination. Cercarial behaviour, as well as minute structures only visible on live specimens, host use and interactions can be helpful in distinguishing species but are not always definitive (e.g. Rudolfová *et al.*, 2005; Podhorský *et al.*, 2009). Morphological features used for the differentiation of adult worms are subtle and few; often it is difficult to obtain whole worms or even worms of both sexes (Blair and Islam, 1983; Brant and Loker, 2009; Horák *et al.*, 2012). Matching of larval stages (in snails) with adults/eggs (from birds) can be done only experimentally, which is difficult and extremely time consuming (Blair and Islam, 1983; Brant *et al.*, 2006; Brant and Loker, 2009). Comparative DNA sequence analysis offers a sound method for organizing and quantifying genetic diversity as a proxy for species diversity (e.g. Vilas *et al.*, 2005; Brant *et al.*, 2006; Jouet *et al.*, 2010*a*, 2010*b*; Brant *et al.*, 2011; Aldhoun *et al.*, 2012; Brant and Loker, 2013; Kolárová *et al.*, 2013; Ebbs *et al.*, 2016; Fakhar *et al.*, 2016; Ashrafi *et al.*, 2018, 2021).

Table 1. Localities and hosts examined on South Island, New Zealand

	Snail	Prevalence	Duck	Prevalence	Latitude and longitude
New Zealand					
Lake Wanaka, Bremner Bay	*Austropeplea tomentosa*	30/1000	–	–	−44.6786, 169.1246
	Physa acuta	0/750	–	–	−44.6786, 169.1246
	Gyraulus corinna	0/600	–	–	−44.6786, 169.1246
	Galba truncata	0/71	–	–	−44.6786, 169.1246
	Lymnaea stagnalis	0/30	–	–	−44.6786, 169.1246
	Potamopyrgus antipodarum	0/50	–	–	−44.6786, 169.1246
	Glyptophysa variabilis	0/20	–	–	−44.6786, 169.1246
Lake Wanaka, Glendhu Bay	–	–	*Aythya novaeseelandiae*	32/34	−44.67158, 169.0189
Lake Wanaka, Roy's Bay	–	–	*Spatula rhynchotis*	2/2	−44.568863, 170.187
Lake Aviemore	*Austropeplea tomentosa*	0/4	–	–	−44.600, 170.200
	Gyraulus corinna	0/3	–	–	−44.600, 170.200
	Physa acuta	0/35	–	–	−44.600, 170.200
	Glpytophysa variabilis	0/5	–	–	−44.600, 170.200
	Potamopyrgus antipodarum	0/50	–	–	−44.600, 170.200
Lake Benmore	–	–	*Spatula rhynchotis*	5/5	−44.351241, 170.2091
Australia					
Mary River, Opium Creek Station, NT	*Austropeplea lessoni*	0/30	–	–	−12.577075, 131.7246
Townsville area, QLD	–	–	*Anas superciliosa*	1/1	–

The prevalence represents species of *Trichobilharzia* over a 10-year collecting range. *Spatula rhychotis* had only *Trichobilharzia querquedulae* and no other species of schistosome. Snails were screened by shedding only.

The avian schistosome diversity in New Zealand is little known despite annual HCD outbreaks, which have prompted much of the work done in the country (Macfarlane, 1944, 1949; Featherston and McDonald, 1988; Featherston *et al.*, 1988; Davis, 1998, 2000, 2006*a*, 2006*b*). *Cercaria longicauda* Macfarlane, 1944 was the suspected culprit in HCD outbreaks in the high-country lakes of the South Island (Macfarlane, 1944, 1949; Featherston and McDonald, 1988; Rind, 1991; Davis, 2000, 2006*a*, 2006*b*). First discovery of adults of the genus *Trichobilharzia* in New Zealand was by Featherston and McDonald (1988) from the ducks *Aythya novaeseelandiae* (Gmelin, 1789) and *Anas platyrhynchos* Linnaeus, 1758. The worms found by those authors were not identified to species (also there were no comments on morphology) or linked to a snail intermediate host. To resolve this, Davis (2006*a*) partially completed the life cycle by exposing the snail host of *C. longicauda*, *Austropeplea tomentosa* (L. Pfeiffer, 1855), formerly known as *Lymnaea tomentosa*, to miracidia from a visceral schistosome from *Ay. novaeseelandiae*. He subsequently described the adults and cercariae (Davis, 2006*a*). Davis (2006*a*) stated that the cercariae he recovered were morphologically closest to those of *C. longicauda* and the adult worms, while belonging to the genus *Trichobilharzia*, did not conform to any described species (Davis, 2006*a*). Since that time, efforts have been made to look for additional species of schistosomes such that the nasal tissues and feces of ducks were also examined, and a larger diversity of snails was examined for schistosome cercariae. As a result, herein, one host and range extension (of *Trichobilharzia querquedulae*), one new species, one redescription and one novel genetic lineage of *Trichobilharzia* are reported. The aetiology and epidemiology of HCD around the Lake Wanaka area is discussed considering these findings.

Materials and methods

Parasite and host collections

Schistosomes were collected as outlined in Davis (2006*a*, 2006*b*). From 2009 to 2017, the viscera or nasal mucosa of the following ducks were examined (Table 1) – *Aythya novaeseelandiae*, *Spatula rhynchotis* (Latham, 1802), *Anas platyrhynchos*, *Anas superciolios*a (Gmelin, 1789), as well as *An. platyrhynchos* x *An. superciliosa* hybrids and the goose *Branta canadensis* (Linnaeus, 1758). The following snails were examined for schistosomes – *Austropeplea tomentosa*, *Gyraulus corinna* (Gray, 1850), *Glyptophysa variabilis* (Gray, 1843), *Galba truncatula* (Müller, 1774), *Potamopyrgus antipodarum* (Gray, 1843), *Lymnaea stagnalis* (Linnaeus, 1758) and *Physa acuta* Draparnaud, 1805. Snails were identified by gross morphology (Boray, 1964; Pullan *et al.*, 1972; Featherston and McDonald, 1988; Featherston *et al.*, 1988), except for *P. acuta* for which genetic data were also considered (see Ebbs *et al.*, 2018). Snail specimens are available as museum vouchers for further examination both by morphology and genetic assays (Table 2). Specimens collected from 2009 to 2017 preserved in 95% ethanol were used in the genetic assay. Most of the specimens were collected from Lake Wanaka (Table 1).

Ducks examined for adult worms were donated by local hunters. The nasal mucosa, liver, hepatic portal vein and mesenteric veins were examined for schistosomes. In addition to the schistosomes, intestinal helminths were collected from two *Ay. novaeseelandiae* and deposited in the Museum of Southwestern Biology Division of Parasites. In 2002, there was an opportunity to sample black ducks, *Anas superciliosa*, from the Townsville region, Queensland, Australia. Fragments lacking important diagnosable

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

Table 2. Museum of Southwestern Biology (MSB) vouchers and GenBank accession numbers for samples recovered

Species of *Trichobilharzia*	Host	GenBank accession number 28S	ITS	CO1	Collector Number	MSB catalogue Number	MSB catalogue Number snail host
Trichobilharzia longicauda	*Aythya novaeseelandiae*		OK104154		W313	MSB: Para:32132	
	Aythya novaeseelandiae	OK104146	OK104155	OK357978	W314	MSB: Para:32133	
	Aythya novaeseelandiae	OK104147	OK104156	OK357979	W315	MSB: Para:32134	
	Aythya novaeseelandiae	OK104148	OK104157	OK357980	W316	MSB: Para:32135	
	Aythya novaeseelandiae	OK104149	OK104158	OK357976	W455	MSB: Para:31065	
	Aythya novaeseelandiae				W911	MSB: Para:24886	
	Austropeplea tomentosa	OK104150	OK104159	OK357977	W451	MSB: Para:29085	MSB:Host:23258
	Austropeplea tomentosa		OK104160	OK357981	T3-NZ	MSB: Para:24896	MSB:Host:21319
	Austropeplea tomentosa			OK357982	T7-N7	MSB: Para:24897	MSB:Host:21320
	Austropeplea tomentosa				TBB-NZ	MSB: Para:24894	
Trichobilharzia querquedulae	*Spatula rhynchotis*		KP788760	KU057183	Tshov	MSB: Para:20794	
	Spatula rhynchotis			KU057181	W703	MSB: Para:20792	
	Spatula rhynchotis			KU057182	W704	MSB: Para:20793	
	Spatula rhynchotis				W845	MSB: Para:24887	
	Spatula rhynchotis				W846	MSB: Para:24888	
	Spatula rhynchotis				W932	MSB: Para:29072	
	Spatula rhynchotis				W968	MSB: Para:31066	
Trichobilharzia novaeseelandiae n. sp.	*Aythya novaeseelandiae*			OK357971	W415	MSB: Para:31071	
	Aythya novaeseelandiae	OK104144	OK104161	OK357973	W440	MSB: Para:31069	
	Aythya novaeseelandiae				W441	MSB: Para:31072	
	Aythya novaeseelandiae	OK104143	OK104162	OK357972	W454	MSB: Para:31064	
	Aythya novaeseelandiae				W504	MSB: Para:25489	
	Austropeplea tomentosa	OK104142	OK104163	OK357974	W462	MSB: Para:31070	MSB:Host:21253
	Austropeplea tomentosa				W501	MSB: Para:25494	
	Austropeplea tomentosa	OK104145			W781	MSB: Para:24892	MSB:Host:21316
	Austropeplea tomentosa				W784	MSB: Para:24893	MSB:Host:21317

(*Continued*)

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

Table 2. (*Continued.*)

		GenBank accession number			Collector	MSB catalogue	MSB catalogue
Species of *Trichobilharzia*	Host	28S	ITS	CO1	Number	Number	Number snail host
Trichobilharzia sp. J	*Austropleplea tomentosa*			OK357985	W782	MSB: Para:24890	MSB:Host:21314
	Austropleplea tomentosa			OK357984	W783	MSB: Para:24889	MSB:Host:21313
	Austropleplea tomentosa	OK104140		OK357983	T2-NZ	MSB: Para:24895	MSB:Host:21318
	Austropleplea tomentosa				W780	MSB: Para:24891	MSB:Host:21315
Trichobilharzia australis	*Anas superciliosa*	OK104141		OK357975	W5009	MSB: Para:32136	
Snails vouchered with no schistosome infection		**16S**					
	Austropeplea tomentosa	OK104151			SW451	MSB: Para:29085	MSB:Host:23258
							MSB:Host:21875
							MSB:Host:21879
							MSB:Host:21975
	Austropeplea lessoni	OK104152			S606		MSB:Host:15620
	Physa acuta	see Ebbs *et al.* (2018)					MSB: Host:21750-21758
							MSB:Host:21874
							MSB:Host:21878
							MSB:Host:21900
							MSB:Host:22022
							MSB:Host:22024
	Gyraulus corinna						MSB:Host:21231
							MSB:Host:21246
							MSB: Host:21321-21322
							MSB: Host:21968-21969
	Lymnaea stagnalis						MSB:Host:21233
							MSB:Host:21877
	Potamopyrgus antipodum						MSB: Host:21263-21264
							MSB:Host:21876
							MSB:Host:22035
	Glyptophysa variabilis	OK104153					MSB:Host:21880
							MSB:Host:21970
	Planorbidae						MSB:Host:22023

The Arctos database has additional specimen information https://arctos.database.museum/SpecimenSearch.cfm.

features of schistosomes were obtained from the nasal mucosa. These were assumed to represent *T. australis*, since the specimens came from the type locality and host (Blair and Islam, 1983). Feces were collected opportunistically when birds were observed on the beach and were examined for miracidia following McMullen and Beaver (1945).

Snails were collected individually by hand or using a kitchen sieve from the shallow edges of the Lake Wanaka, mainly at Bremner Bay (Table 1). Snails were also collected by snorkelling since *Au. tomentosa* has been found on deeper water vegetation in the lake, at 3–4 m (Davis, 2000). All snails were brought back and immediately processed for cercarial shedding by placing

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

them in individual wells of Corning Costar flat-bottomed cell-culture plates with either lake water or spring water and exposed to natural light. Parasites and snails were vouchered in the Museum of Southwestern Biology Division of Parasites (Table 2).

Morphological characterization of the worms

Morphological characterizations of the adult worms were made from ethanol-preserved or formalin-fixed fragments, stained in aqueous alum carmine and mounted in Canada Balsam. Images of the cercariae were made from 80% ethanol-preserved specimens. Measurements and images of cercariae were made from ethanol-preserved specimens. Unfortunately, at the time of the collections, a microscope was not available to document features (e.g. flame cells) only seen in live specimens. Drawings were made with a camera lucida attached to Olympus BX53 then traced with Huion H1060P drawing tablet (Huion Science and Technology Park, Shenzhen City, China).

Sequencing data and phylogenetic analysis

Genetic data were obtained from both the snail hosts and worms. DNA was extracted from small adult worm fragments or 1–2 cercariae with the QIAamp DNA Micro Kit (Qiagen, Valencia, California, USA) according to the manufacturer's guidelines, except that samples were eluted with 30 μL of buffer. DNA was amplified by PCR (TaKara Ex Taq kit, Takara Biomedicals, Otsu, Japan) and sequenced using previously published primers [28S nDNA region (U178, L1642), ITS1-5.8S-ITS2 nDNA region (BDF1, BDR2, 3S and 4S), and mtDNA region *cox*1 (Cox1_Schisto_5, Cox1_Schisto_3); for primers see Bowles and McManus, 1993; Bowles *et al*., 1995; Lockyer *et al*., 2003; Brant *et al*., 2006; Brant and Loker, 2009]. For the snails, a small piece of tissue was taken from the head-foot of individual snails from a couple of different localities. DNA was extracted using the E.Z.N.A. Mollusc DNA kit (Omega Bio-Tek, Norcross, Georgia, USA) following the manufacturer's protocol. The *16S* mitochondrial DNA loci was amplified with the primers Brh: 5′-CCGGTCT GAACTCAGATCACGT-3′ and Arl: 5′-CGCCTGTTTAACAAA AACAT-3′ (Palumbi *et al*., 1991). An effort was made to obtain *cox1* sequences from the snails, but it was not successful. Thermocycling conditions were as follow for the schistosomes (a) *28S* conditions were 94°C for 6 min; 3 cycles for each annealing temperature 55–49°C then 20 cycles 50°C with denaturation 94°C for 30 s and extension 72°C for 2 min, and a final extension at 72°C for 5 min; (b) *cox1* conditions were 94°C for 6 min; 3 cycles for each annealing temperature 51–47°C then 20 cycles 46°C with denaturation 94°C for 30 s and extension 72°C for 2 min, and a final extension at 72°C for 5 min; and (c) *ITS* conditions were 94°C for 6 min; 3 cycles for each annealing temperature 65–61°C then 20 cycles 60°C with denaturation 94°C for 30 s and extension 72°C for 2 min, and a final extension at 72°C for 5 min. For snails *16S* thermocycling conditions were 94°C for 2 min; 35 cycles of 94°C for 15 s, 45°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 7 min.

PCR products were visualized on 1.0% TBE agarose gel stained with GelRed® (Biotium, Fremont, California, USA). PCR products were purified with E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek) and sequenced using the Applied Biosystems BigDye direct sequencing kit, version 3.1 (Applied Biosystems, Foster City, California, USA). Sanger DNA sequencing was completed at the University of New Mexico. Chromatograms were edited in Sequencher v 5.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and sequences were aligned by eye in Se-Al v 2.0a11 (http://tree.bio.ed.ac.uk).

Phylogenetic analyses of the parasite and snail nuclear *28S*, *ITS* and mitochondrial *cox1* and *16S* sequence datasets were performed using Bayesian inference in MrBayes (Huelsenbeck and Ronquist, 2001) with default priors for *16S*, *28S* and *ITS1-5.8S-ITS2* (Nst = 6, rates = gamma, ngammacat = 4) and *cox1* (parameters un-linked so each partition by codon has its own set of parameters; Nst = 6 rates = invgamma). Partitions by codon evolved under different rates (preset applyto = (all) ratepr = variable). Model selection was done using ModelTest (Posada and Crandall, 1998). Four chains were run simultaneously for 5×10^5 generations, the first 5000 trees with pre-asymptotic likelihood scores were discarded as burn-in, and the retained trees were used to generate 50% majority-rule consensus trees and posterior probabilities. Outgroups used have been defined in previous analyses (see Brant and Loker, 2013). The new sequences generated in this study have been deposited in GenBank (see Table 2). Parasites and their snail host vouchers (see Thompson *et al*., 2021) were deposited in the Museum of Southwestern Biology Division of Parasites (MSB). Additionally, snail vouchers other than those positive for schistosomes also have been deposited in MSB with the catalogue numbers MSB Host:21230-21264, 21313-21322, 21654, 21750-21756, 21758, 21874-21880, 21900, 21968-21970, 21975, 22022-22024, 22035, 22247, 22263, 22264, 23246, 24232, 24235-24238.

Ethical statement

All ducks used in the present study were killed by licensed hunters in accordance with the game laws in New Zealand and with the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of New Mexico, USA (IACUC # 11-100553-MCC, Animal Welfare Assurance # A4023-01).

Results

Identification of specimens

During the survey period spanning 2009–2017 around the greater Lake Wanaka area, schistosomes were found only in the snail *Austropeplea tomentosa* and the ducks *Spatula rhynchotis* and *Aythya novaeseelandiae* ducks (Table 1). Feces from *Tadorna variegata* (Gmelin, 1789), *Anas platyrhynchos*, *S. rhynchotis*, *Ay. novaeseelandiae* were examined for miracidia and positive samples were found only for *Ay. novaeseelandiae.* Davis (2000) however did find schistosome eggs in the livers of ducks examined in his 1998 survey (*T. variegata*, *An. platyrhynchos*, *Ay. novaeseelandiae*, *S. rhynchotis*). The morphological and molecular analysis of the specimens resulted in the recognition of four distinct clades of *Trichobilharzia* (see Table 2). Adults of *T. longicauda* were recovered from the visceral veins and further characterized (Davis, 2006*a*) and a new species from the nasal mucosa, *T. novaeseelandiae* n. sp., is described and life cycle defined from *Ay. novaeseelandiae* and *Au. tomentosa.* The widespread *T. querquedulae* was recovered from *S. rhynchotis* (see Ebbs *et al*., 2016) and a lineage, likely a distinct species, known only from cercariae from *Au. tomentosa* was recovered but did not group with a previously defined genetic clade.

Description

Taxonomic summary

Phylum: Platyhelminthes Claus, 1887

Class: Trematoda Rudolphi, 1808

Subclass: Digenea Carus, 1863

Family: Schistosomatidae Stiles and Hassall, 1898

Genus: *Trichobilharzia* Skrjabin and Zakharow, 1920

Species: *Trichobilharzia longicauda* (Macfarlane, 1944) Davis, 2006

Macfarlane (1944) described *Cercaria longicauda* based on cercariae from lymnaeid snails in Lake Wanaka, New Zealand. No type specimens were designated in that paper, or in a later,

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

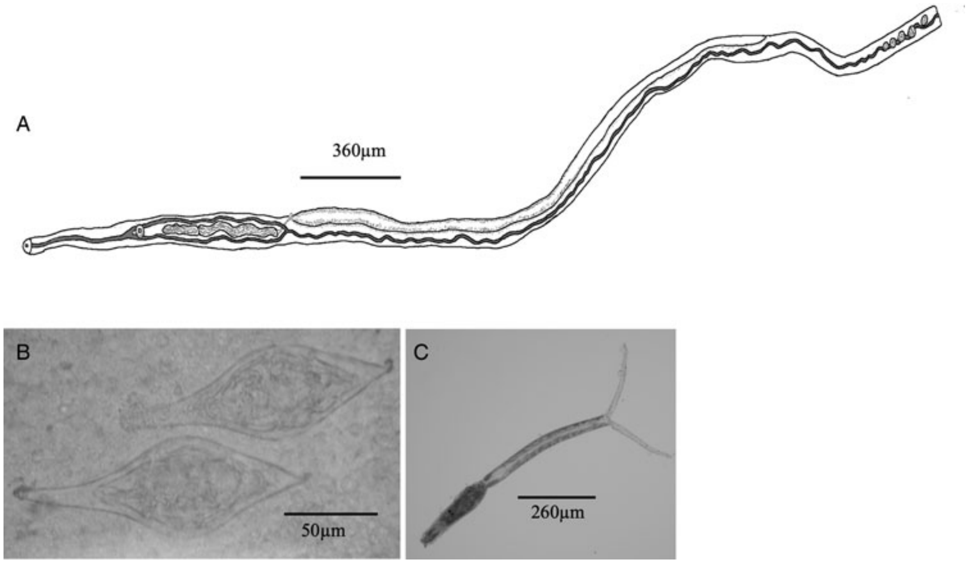

Fig. 1. Morphology of *Trichobilharzia longicauda* (A) anterior portion of adult male, (B) eggs from feces, (C) cercaria from a natural infection of *Austropeplea tomentosa*.

more detailed description by Macfarlane (1949). Davis (2006*a*) implied that Macfarlane's (1944) cercaria belonged to *Trichobilharzia* and we regard that as the first use of the combination *Trichobilharzia longicauda*. In our opinion, therefore, the correct name for the species should be *Trichobilharzia longicauda* (Macfarlane, 1944) Davis, 2006.

Diagnosis: *Adult male* (Fig. 1; measurements Table 3). Body uniform length except wider at gynecophoric canal and spatulate posterior. Tegument rugose except spinose ventral sucker and half inner surface of gynecophoric canal (Fig. 1A). Spines not observed on genital pore or oral sucker. Oral opening subterminal, ventral; intestinal bifurcation immediately anterior to ventral sucker; cecal reunion between the posterior external seminal vesicle and anterior to the gynecophoric canal (Fig. 1A), single caecum terminates close to the posterior end of the body. Testes spherical or slightly elliptical, beginning posterior to the gynecophoric canal arranged both in straight row or zig-zag between caecum, extending almost to end of reunited caecum. Seminal vesicle undulates, divided into external and internal portion occupying most space between ventral sucker and gynecophoric canal (Fig. 1A). Ejaculatory duct at posterior end of internal seminal vesicle, muscular until close to genital pore were thin-walled and terminates with muscular bulb. Also see Davis (2006a). *Adult female* ($n = 1$; measurements Table 3). Fragments of single female recovered, without distinguishable features. Eggs, like most visceral species in Clade Q (*sensu* Brant and Loker, 2009), spindle-shaped, with one pole longer than other (Fig. 1B). *Cercariae* (Fig. 1C; measurements Table 4). *Cercariae* ($n = 2$) body 290–310 μm in length, two eyespots and large muscular anterior organ; tail stem 460–470 μm in length, two furcae 240–250 μm in length but finfolds not observed. No live specimens observed for flame cell counts. Upon emergence from snail, cercariae swim towards the strongest light source and attach to sides or bottom of well with their ventral sucker.

Remarks: Trichobilharzia longicauda can be distinguished from all the other described species of *Trichobilharzia*, and from the new species described in the present work, most notably by the length of the gynecophoric canal (1390–1470 μm). The gynecophoric canal is long relative to other species except for *T. anatina* Fain, 1956 from Ruanda-Burundi (1300–1500 μm), which was found in the intestinal veins of *Anas undulata*. The cecal reunion, a relatively stable distinguishing feature (Fain, 1956; Blair and Islam, 1983; Horák *et al*., 2002; Rudolfová *et al*., 2005; Brant and Loker, 2009), is between the seminal vesicle and the gynecophoric canal in *T. longicauda*, whereas in *T. anatina*, it is located between the internal and external seminal vesicles. Unfortunately, there were no eggs recovered for *T. anatina* for comparison. The prevalence of *T. longicauda* in *Ay. novaeseelandiae* examined from Lake Wanaka was 91% (20/22) and 1.3% (13/1000) in *Au. tomentosa*. In 12 ducks both the nasal and the visceral species were present.

Type host (definitive): *Aythya novaeseelandiae* (Gmelin, 1789)

Site in definitive host: hepatic portal and mesenteric veins

Type host (intermediate): *Austropeplea tomentosa* (L. Pfeiffer, 1855)

Type locality: Bremner Bay, Lake Wanaka, New Zealand

Type specimen: Neotype an adult male MSB:Para:31803

Paratypes: fragments of males and females in ethanol and on slides MSB:Para:31802.

Vouchers: Adult worms-MSB:Para:24866, 31065; Cercariae-MSB: Para:29085, 24894, 24896, 24897; snail hosts-MSB:Host:23258, 21319, 21320.

All type and voucher specimens deposited in the Museum of Southwestern Biology Division of Parasites.

Etymology: The species is named after the original cercarial description, *Cercaria longicauda*, by Macfarlane (1944).

Species: *Trichobilharzia novaeseelandiae* n. sp. Davis and Brant

Diagnosis: *Adult male* (Fig. 2; measurements Table 3). Body uniform length except at gynecophoric canal and spatulate posterior extremity. Tegument rugose except spinose oral and ventral sucker and inner surface of gynecophoric canal (Fig. 2A). Spines not observed on genital pore; oral opening subterminal, ventral; intestinal bifurcation immediately anterior to ventral sucker; cecal reunion between posterior external seminal vesicle and anterior to gynecophoric canal, single caecum terminates close to posterior end of body (Fig. 2A). Testes spherical or slightly elliptical beginning posterior to gynecophoric canal arranged in straight row extending almost to end of caecum. Seminal vesicle undulates, divided into external and internal portion occupying most of the area between ventral sucker and gynecophoric canal. Ejaculatory duct at posterior end of internal seminal vesicle, muscular until close to genital pore where thin-walled. *Adult female* (measurements Table 3). Fragments of females recovered, without distinguishable features. Eggs, as for

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

Table 3. Morphological comparisons of the adult worms and cercariae

	Snail host	Bird host	Male	Male Length	Male OS	Male VS	Male OS to VS	Male CR	Male OS to GC	Male GC L X W	Male #Testes	Male Testes	Male ESV	Male ISV	Eggs *in utero*	Eggs in feces/nasal mucus	
Nasal species																	
***Trichobilharzia novaeseelandiae* n. sp.**	***Austropeplea tomentosa***	***Aythya novaesealandiae***	**New Zealand**	**3.5–5.8 mm**	**35–40 × 30–35**	**30–42.5 × 35–40**	**420–470 (445)**	**Possible SV and GC**	**1050–1100 (1075)**	**200–210 (205)**	**180 +**	**21–27.5 (25)**	**185–190**	**235–275**		**210–235 × 45–47 (*n* = 5)**	**This study**
Trichobilharzia australis	*Austropeplea lessoni*	*Anas superciliosa*	Australia	11.1 mm	40 × 30	30	380	SV and GC, 380	–	200	161–243	20 × 30	15 × 20		198 × 30	230 × 48	Blair and Islam (1983)
Trichobilharzia arcuata	*Austropeplea lessoni*	*Dendrocygna arcuata*	Australia	12.6 mm	40 × 30	40 × 30	400	ESV and ISV, 100	–	170	88–127	20 × 20	90 × 20	190 × 20	153 × 24	260 × 57, 125 × 55	Islam (1986)
Trichobilharzia regenti	*Radix balthica*	Anatidae	Germany	5.22 mm	39 × 32	34 × 29	333	SV and GC	–	280 × 79	120 +	39 × 28	140 × 34	104 × 23		199–274	Horák *et al.* (1998)
Trichobilharzia aureliani		Podicipediformes	Rwanda	16–17 mm	40 × 36	39 × 41	366–500	not observed	750–1000	230–275	125	20 × 30	130–150 × 20–25	250–300	150–180 × 30–35	175–220 × 32–40	Fain (1956)
Trichobilharzia rodhaini		*Bostrychia hagedash*	Rwanda	+ 6 mm frag	46 × 33	40	285	ESV and ISV	700	219	137 +	30 × 35	130	230		280–325 × 55–70	Fain (1956)
Trichobilharzia nasicola		*Anas undulata*	Rwanda	12–19 mm	38–46 × 30–38	40–47 × 35–41	380–475	ESV and ISV	1000–1300	300–350	170–200	30	200–250	250–340	200–230	280–330 × 50–70	Fain (1956)
Trichobilharzia spinulata		Egyptian and Spurwinged goose	Rwanda	15–21 mm	38–34	45 × 38	350–450	ESV and ISV	700–875	250–325	233	35–45	120–220	146–219		250–300 × 50–70	Fain (1956)
Trichobilharzia duboisi		*Nettapus auritus*	Rwanda	+ 1.4–2.2 mm	38 × 33	46 × 34	350	ESV and ISV	-	410–420 × 70–85	42 +	22–35	155 × 21–28	210 × 25–33		225–400 × 40–70	Fain (1959)
Visceral species																	
Trichobilharzia longicauda	***Austropeplea tomentosa***	***Aythya novaeseelandia***	**New Zealand**	**5.7 mm**	**–**	**–**	**–**	**SV and GC**	**–**	**1450 × 100**	**109**	**20 × 20**	**–**	**–**	**–**	**167 ± 10 × 44 ± 6 (*n* = 10)**	**This study; Davis, (2006*a*, 2006*b*)**
Trichobilharzia physellae	*Physa* sp.	Anatidae	North America	1.3–7.5 mm	28–40 × 24–28	16–32	160–340	VS and SV	–	100–190 × 56–80	96–160	4 × 4–28 × 32	–	–	–	–	Mcmullen and Beaver (1945)
Trichobilharzia querquedulae	*Physa* sp.	*Spatula* spp.	Worldwide	3.7- mm	64 × 56	73	274–375	SV and GC	678–880	375	210–240	-	–	–	–	–	McLeod (1937)
Trichobilharzia franki	*Radix auricularia*	Anatidae	Eurasia	3.2–4.0 mm	51–77 × 46–65	46–51 × 56–69	485–530	SV and GC	-	212–291 × 130–195	-	95–106 L	–	–	–	206 ± 25 × 69 ± 9	Muller and Kimmig (1994)
Trichobilharzia parocellata	*Austropeplea lessoni*	*Anas superciliosa*	Australia	4.4	40 × 30	40 × 30	300	ESV and ISV	580	240	41–64	30 × 40	130 × 20	120 × 20	–	170 ± 20 × 50 ± 6	Islam and Copeman (1986)
Jiilinobilharzia crecci		*Anas crecca*	China	3.6–4.5	44–59 × 36–50	40–59 × 59–67	325–450	SV and mid GC	–	735–1035	83–132	12–16 × 44	–	–	–	–	Lui and Bai (1976)
Trichobilharzia brevis	*Radix rubiginosa*	exp duck	Malaysia	2.1 and 4.3	35 × 50	35 × 50	260–350	SV and GC	–	not reported	45 and 51	-	–	–	–	225 ± 14 × 51 ± 5	Basch (1966)
Trichobilharzia brevis	*Austropeplea ollula*	exp domestic ducklings	Japan	3.6–3.9	21–40 × 26–37	12–25	262–447	SV and GC	–	72–113	66–95	-	–	–	–	–	Suzuki and Kawanaka (1980)
Trichobilharzia anatina		*Anas undulata*	Rwanda	7–8 mm	35–42 × 35	43–52 × 36–43	310–390	ESV and ISV	750–900	1300–1500	110–149	30	90–110	185–250	–	–	Fain (1956)
Trichobilharzia berghei		*Anas undulata*	Rwanda	4.4–5.8 mm	50 × 40	40 × 48	350–450	VS and SV	750–900	280–375	40–65	35–40	120–180	115–200	–	–	Fain (1956)
Trichobilharzia schoutedeni		*Thalassornis leuconotus*	Rwanda	5.1–6.8 mm	60 × 51	75	475	SV and GC	1200–1300	500–620	95–125	35 × 45	260–310	225–300	–	–	Fain (1956)

OS, oral sucker; VS, ventral sucker; CR, cecal reunion; GC, gynaecophoric canal; ESV/ISV, external/internal seminal vesicle; exp, experimental.
Specimens from this study in bold. Measurements in micrometers unless otherwise designated.

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

Table 4. Comparative measurements of cercariae

	N	Fixative	Snail host	Body length	Tail stem length	Furcae length	Ratio body:tail	Ratio tail: furcae	Country	Reference
***Trichobilharzia novaeseelandiae* n. sp.**	**3**	**80% ethanol**	***Austropeplea tomentosa***	**275–350 (316.6)**	**375–470 (418)**	**187–220 (200)**	**0.71**	**2.1**	**New Zealand**	**This study**
Trichobilharzia australis	10	Live	*Austropeplea lessoni*	331–371 (349)	379–433 (409)	225–241 (234)	0.85	1.75	Australia	Blair and Islam (1983)
Trichobilharzia arcuata	50	Live	*Austropeplea lessoni*	249–356 (297)	290–431 (361)	124–240 (205)	0.82	1.76	Australia	Islam (1986)
Trichobilharzia regenti			*Radix balthica*	225	331	206	0.68	1.61	Germany	Horak *et al*. (1998)
Trichobilharzia longicauda	**2**	**95% ethanol**	***Austropeplea tomentosa***	**290–310 (300)**	**460–470**	**240–250**	**0.65**	**1.91**	**New Zealand**	**This study**
Cercaria longicauda		95% ethanol	*Austropeplea tomentosa*	230–292 (262)	344–378 (361)	175–195 (185)	0.73	1.95	New Zealand	Davis (2000)
Cercariae longicauda		Boiling formalin	*Austropeplea tomentosa*	322 ± 22	484 ± 38	253 ± 9	0.58	1.64	New Zealand	Macfarlane (1944)
Cercariae longicauda	27	Boiling formalin	*Austropeplea tomentosa*	322 ± 21.6	484 ± 28.3	252 ± 95	0.67	1.9	New Zealand	Macfarlane (1949)
Cercariae longicauda	15	Hot formalin	*Austropeplea tomentosa*	252 ± 25.4	493 ± 24.4	254 ± 17.3			New Zealand	Macfarlane (1949)
Cercariae longicauda	10	Cold formalin	*Austropeplea tomentosa*	250 ± 26.4	593 ± 17.7	336 ± 13.6			New Zealand	Macfarlane (1949)
Cercariae longicauda			*Austropeplea tomentosa*	200–308 (255.2 ± 34)	490.43 ± 55.65	247.23 ± 25.94	0.52	1.98	New Zealand	Rind (1991)
Trichobilharzia brevis			*Radix rubiginosa*	237	304	218	0.78	1.39	Malaysia	Basch (1966)
***Trichobilharzia* sp. J**	**3**	**80% ethanol**	***Austropeplea tomentosa***	**230–260 (247)**	**310–330 (321.7)**	**150–170 (163.3)**	**0.77**	**1.98**	**New Zealand**	**This study**
Cercaria herini			*Radix natalensis*	360	450	250	0.8	1.8	Rwanda	Fain (1955)
Cercaria herini			*Radix natalensis*	398	598	294	0.66	2.03	South Africa	Appleton (2003)
Cercaria ocellata			*Radix natalensis*	420–470	370–430	230–310	1.1	1.5	South Africa	Porter (1938)
Trichobilharzia parocellata		Live	*Austropeplea lessoni*	232–348 (288)	282–406 (354)	174–273 (227)	0.81	1.56	Australia	Islam and Copeman (1986)

Specimens from this study in bold.

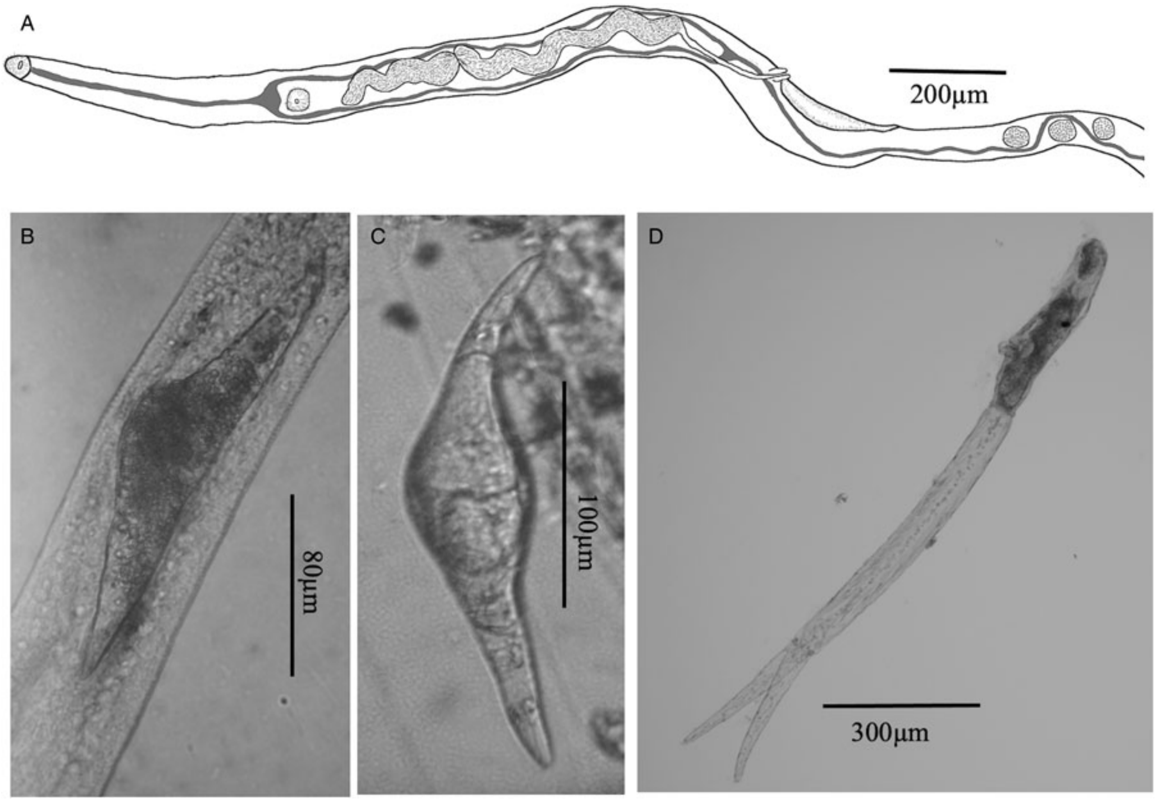

Fig. 2. Morphology of *Trichobilharzia novaeseelandiae* n. sp. (A) anterior portion of adult male, (B) eggs in utero, (C) eggs from feces, (D) cercaria from a natural infection of *Austropeplea tomentosa.*

most nasal species, sigmoid or boomerang shaped, with long polar ends (Fig. 2B and C). *Cercariae* (Fig. 2D; measurements Table 4). *Cercaria* ($n = 3$) body length 275–350 μm, two eyespots, large muscular anterior organ; tail stem length 375–470 μm, two furcae length 187–220 μm; finfolds not observed. No live specimens observed for flame cell counts. Upon emergence from snail, cercariae swim towards the strongest light source and attach to sides or bottom of well with their ventral sucker, and body flexed dorsally.

Remarks: Trichobilharzia novaeseelandiae n. sp. can be distinguished from six of the eight nasal species of *Trichobilharzia* described from Ruanda-Burundi and Australia by the position of the cecal reunion between the internal seminal vesicle and gynecophoric canal, *vs* between the internal and external seminal vesicles in four of the African species (*T. nasicola* Fain, 1956; *T. rodhaini* Fain, 1956; *T. spinulata* Fain, 1956, *T. duboisi* Fain, 1959; it was not observed in *T. aureliani* Fain, 1956) and one Australian species (*T. arcuata* Islam, 1986). The new species is closest to *T. regenti* Horák *et al.*, 1998 from Europe, *T. aureliani* Fain, 1956 from Rwanda, and *T. australis* Blair and Islam, 1983 from Australia. The new species is different from *T. regenti* by tail morphology, which is broadened, and coil shaped (see Fig. 1 in Horák *et al.*, 1998) and genetically (Fig. 3), otherwise they are morphologically very similar. The differences in adult male worms between this new species and *T. australis* and *T. aureliani* are not as distinguishable. The three species are quite similar in overall length/proportion and character of the gynecophoric canal, position of cecal reunion (except where observed), overall shape and size of the eggs; sigmoid/boomerang and location of spines. The overall proportional measurements for *T. australis* are different from the new species here in that the former are smaller and were measured from live specimens, which tend to be larger than fixed specimens. Measurements of the new species here were based on specimens preserved in both 80% ethanol and 10% formalin, and thus are proportionally smaller than live specimens. The prevalence of *T. novaeseelandiae* n. sp. in *Ay. novaseeladiae* examined from Lake Wanaka was 100% (12/12) and 1.3% (13/1000) in *Au. tomentosa*. In 12 ducks both the nasal and the visceral species were present.

Type host (definitive): *Aythya novaeseelandiae* (Gmelin, 1789)

Site in definitive host: nasal mucosa

Type locality: Glendhu Bay, Lake Wanaka, New Zealand

Type host (intermediate) *Austropeplea tomentosa* (L. Pfeiffer, 1855)

Type locality: Bremner Bay, Lake Wanaka, New Zealand

Type specimens: Holotype MSB:Para:31072 that includes anterior part of male worm that ends about 20 testes posterior to the gynecophoric canal.

Paratypes: adult worm fragments MSB:Para: 31071

Vouchers: Adult worms-MSB:Para:25489, 31069; Cercariae-MSB: Para:24892, 24893, 25494, 31070; snail hosts-MSB:Host:21253, 21316, 21317.

All type and voucher specimens deposited in the Museum of Southwestern Biology Division of Parasites.

Etymology: The species is named both for the Latin for New Zealand as well as the specific epithet of the type host, *Aythya novaeseelandiae*.

Species: *Trichobilharzia querquedulae* (McLeod, 1937)

Remarks: Trichobilharzia querquedulae was found in 7/7 *S. rhynchotis* examined, a prevalence similar to that in its North American hosts (see Ebbs *et al.*, 2016). Mostly fragments of adult worms were recovered and slides were not made. The snail host remains unknown.

Host (definitive): *Spatula rhynchotis* (Latham, 1801)

Site in definitive host: hepatic portal and mesenteric veins

Locality: Lake Wanaka, New Zealand

Vouchers: Adult worms-MSB:Para:20792, 20793, 20794, 24887, 24888, 29072, 31066.

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

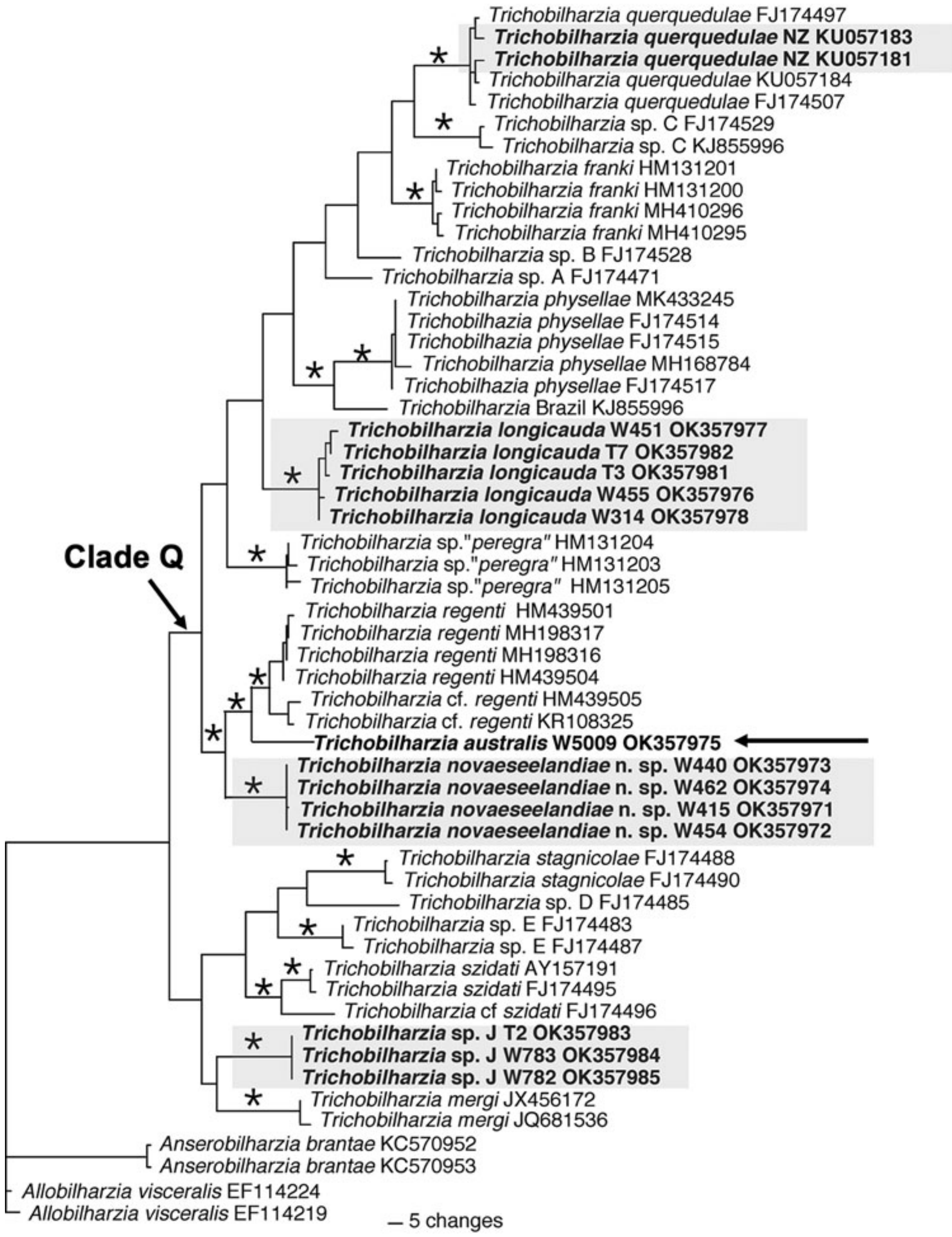

Fig. 3. Phylogenetic tree based on *cox*1 sequences placing the New Zealand samples among available sequences of *Trichobilharzia* species. Specimens from this study are in bold and those from New Zealand are in grey boxes. Clade Q *sensu* Brant and Loker (2009). Black arrow points to the position of the Australian nasal species, relative to the new species from this study. The '*' represents significant (values lower than 0.95 are not shown) posterior probability support for the Bayesian analysis. GenBank accession numbers follow the taxon names.

All type and voucher specimens deposited in the Museum of Southwestern Biology Division of Parasites

Taxon: *Trichobilharzia* sp. J

Diagnosis: (measurements Table 4). *Cercaria* ($n = 3$) body length 230–260 μm, two eyespots, large muscular anterior organ; tail stem length 310–330 μm, two furcae length 150–170 μm; finfolds not observed. No live specimens observed for flame cell counts and too few cercariae for reasonable images.

Remarks: This species of *Trichobilharzia* was found only as cercariae in the snail host. The behavior of these cercariae was not recorded. They were smaller than other cercariae of *Trichobilharzia* described from Australia and New Zealand, except for *Trichobilharzia parocellata* Islam and Copeman, 1986. Cercariae of *T. parocellata* were measured live by those authors, but if they had been measured in ethanol, the size might be more like that of the species found in New Zealand (Table 4). In the field, distinguishing this species from cercariae of *T. novaeseelandiae* n. sp. will be difficult as the overall size and proportions are similar. But the cercariae of *T. longicauda* overall is larger than this cercariae, most notably the length of the tail (Table 4). The prevalence of this species was 4/1000 (0.4%) *Austropeplea tomentosa* in Bremner Bay.

Host (intermediate): *Austropeplea tomentosa* (L. Pfeiffer, 1855)

Locality: Bremner Bay, Lake Wanaka, New Zealand

Vouchers: Cercariae-MSB:Para:24889, 24890, 24891, 24895. Snail hosts-MSB:Host:21315-21315, 21318.

All type and voucher specimens deposited in the Museum of Southwestern Biology Division of Parasites

Phylogenetic results

The phylogenetic results of both the mitochondrial *cox1* (Fig. 3) and the nuclear *28S* and *ITS* trees (nuclear DNA trees not shown) indicate that our samples from Australia and New

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

Table 5. The average uncorrected '*p*' distances within among species of *Trichobilharzia* based on partial *cox1* sequences

COX1	1	2	3	4	5	6	7	8	9	10	11
1. *Trichobilharzia novaeseelandiae* n. sp.	**0.1%**										
2. *Trichobilharzia regent*	7.7%	0.4%									
3. *Trichobilharzia australis*	**8.8%**	4.8%	–								
4. *Trichobilharzia longicauda*	**12.1%**	11.8%	**12.2%**	**0.9%**							
5. *Trichobilharzia franki*	12.6%	11.4.%	–	10.5%	0.6%						
6. *Trichobilharzia physellae*	11.8%	–	–	10.6%	10.2%	0.7%					
7. *Trichobilharzia querquedulae*	12.5%	–	–	10.6%	–	–	0.9%				
8. *T. querquedulae* New Zealand	–	12.8%	–	–	–	–	1.5%	0.7%			
9. *Trichobilharzia* sp. J	**13.6%**	11.6%	**12.5%**	**12.9%**	12.0%	12.6%	12.1%	12.1%	**0.0%**		
10. *Trichobilharzia mergi*	11.9%	–	–	–	–	–	–	–	11.5%	0.7%	
11. *Trichobilharzia szidati*	12.9%	–	–	–	–	–	–	–	11.1%	12.4%	0.5%
12. *Trichobilharzia stagnicolae*	–	–	–	–	–	–	–	–	13.0%	12.6%	11.4%

Samples from this study in bold. '–' comparison was not calculated.

Zealand came from four species of *Trichobilharzia*, three of which did not group with other species in the trees (Fig. 3). *Trichobilharzia querquedulae* from *Spatula rhynchotis* grouped with the other specimens of *T. querquedulae* from the Americas and South Africa as a monophyletic group (also see Ebbs *et al.*, 2016). *Trichobilharzia longicauda* and *T. novaeseelandiae* n. sp. formed unique monophyletic groups to the exclusion of any other genetic lineage available, thus supporting their status as distinct species, both falling within Clade Q (*sensu* Brant and Loker, 2009). The species that occur in the nasal mucosa of waterfowl, *T. regenti*, *T.* cf. *regenti*, and the new species described here, *T. novaeseelandiae* n. sp. (Fig. 3) also form a clade. This clade also includes a sequence putatively from *T. australis*, which is distinct from the new species. The nasal species *T. australis* was first cycled through lab-reared *Austropeplea lessoni* in northeastern Australia (Blair and Ottesen, 1979; Blair and Islam, 1983). This species was also found in wild snails and used successfully to infect lab-reared ducks (Blair and Ottesen, 1979). The fourth lineage from Lake Wanaka comprised only of cercariae, fell outside of Clade Q and clustered with *T. stagnicolae*, *T. szidati* and *T. mergi* but remains undescribed.

Pairwise comparisons of *cox1* uncorrected *p*-distances among the species of *Trichobilharzia* are given in Table 5. This analysis involved 55 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The final dataset included 552 positions. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018; Stecher *et al.*, 2020). These values are used as a proxy for species delineations: at least within avian schistosomes uncorrected *p*-distances seem consistent across studies for intra- and interspecific comparisons. Most species differ from others by at least 10–13% different, except for the nasal species; *T. novaeseelandiae* n. sp. and *T. regenti*, which are 8% different and *T. australis* and *T. regenti*, which are only 5.4% different. A provisional value of <5% for partial *cox1* sequences in schistosomes is often used where species designations might become questionable without further data (Vilas *et al.*, 2005; Brant and Loker, 2009; Ebbs *et al.*, 2016; Fakhar *et al.*, 2016). Unfortunately, there were no morphologically diagnosable fragments of *T. australis* available to us.

The *cox1* did not amplify for any of the snail samples, and only two snails worked for the *16S* (Fig. 4; also see Puslednik *et al.*, 2009 Fig. 4). There is still debate on the taxonomy of *Austropeplea* Cotton, 1942. However, *Au. tomentosa* is the type species and was originally described from New Zealand. Our New Zealand sequence (OK104151) grouped with sequences from specimens collected from the locality of *Au. tomentosa* (EU556236-37) in New Zealand (Puslednik *et al.*, 2009). Snails were not collected from the type locality of the putative *T. australis*, but specimens of *Au. lessoni* were available from Northern Territory, Australia (*cox1* sequence OK104152), which grouped within the other conspecifics. Puslednik *et al.* (2009) had a specimen of *Au. lessoni* from the general area of the type locality for *T. australis* (EU556259), suggesting that the snail host was likely *Au. lessoni*, as earlier defined by morphological examination (Blair and Ottesen, 1979; Blair and Islam, 1983).

Discussion

This is the first effort to characterize the life cycle, morphology and genetic diversity of avian schistosomes in New Zealand (Fig. 5). The diversity of *Trichobilharzia* in the snail host was notable since three of the four species found cycle through the same species of snail, *Austropeplea tomentosa*. It is not yet known what snail host *T. querquedulae* uses. In North America, this schistosome species cycles through *Physa gyrina* and *P. acuta* (Brant and Loker, 2009). Although *P. acuta*, a widespread invasive

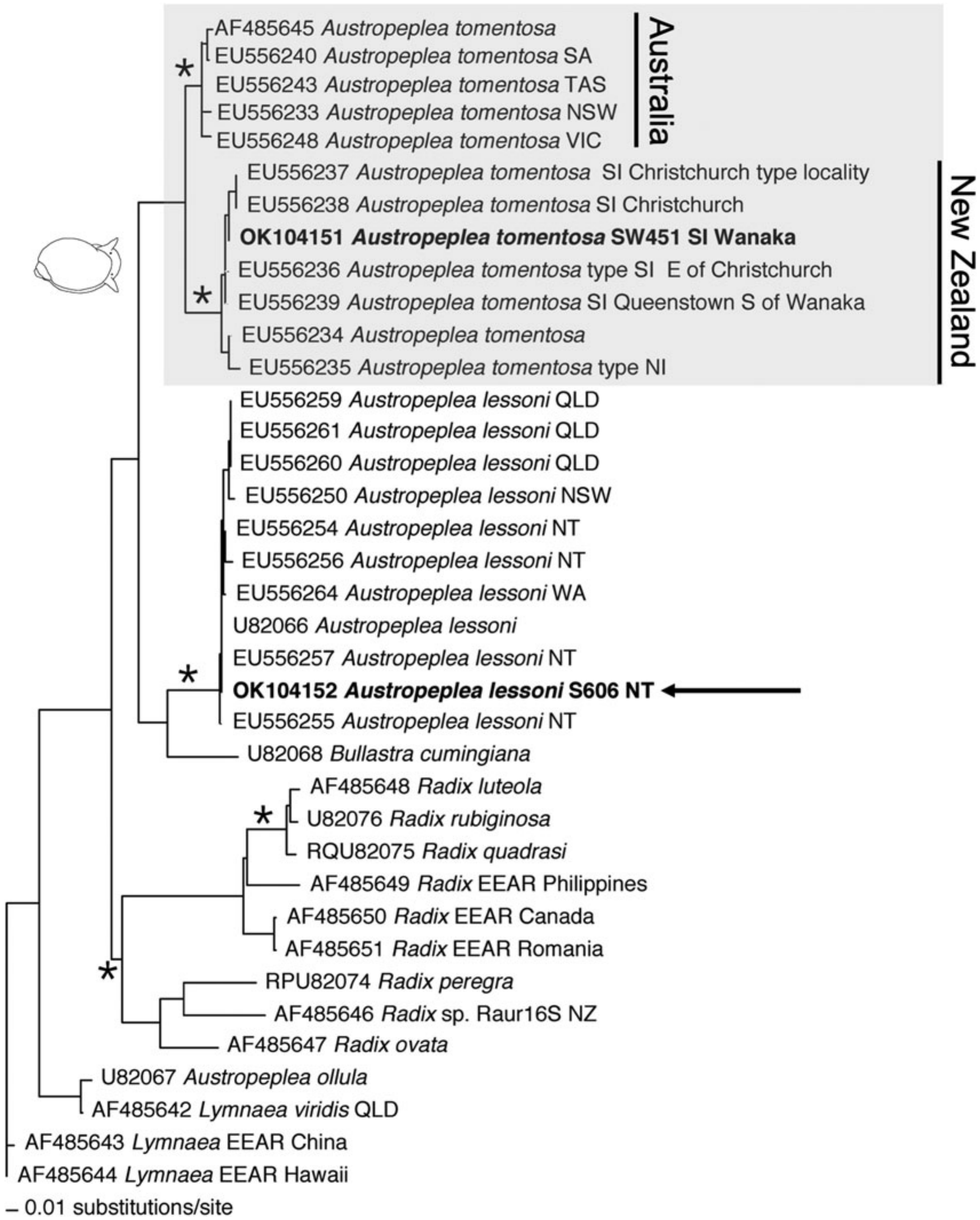

Fig. 4. Phylogenetic tree based on mitochondrial *16S* sequences placing the New Zealand snail sample within Lymnaeidae. Specimens from this study are in bold. *Austropeplea tomentosa* specimens are in the grey box that includes two clades, one with specimens from Australia (black arrow) and the other clade from New Zealand. The '*' represents significant posterior probability support (up to 0.95) for the Bayesian analysis. GenBank accession numbers precede the taxon names. SA, South Australia; TAS, Tasmania; NSW, New South Wales; VIC, Victoria; QLD, Queensland; NT, Northern Territory; WA, West Australia; NZ, New Zealand; SI, South Island; NI, North Island.

snail (Ebbs *et al.*, 2018), has been found in New Zealand since at least the 1940s (Macfarlane, 1944; Featherston and McDonald, 1988), thus far no infected snails have been found, in this or previous studies (Table 1). Based on the molecular phylogenetics, all four species of schistosomes were unequivocally placed within the genus *Trichobilharzia* (Fig. 3). It is also the case in Australia, that *Au. lessoni* is host to three described species of *Trichobilharzia*, two from the nasal tissues and one from the viscera (Macfarlane, 1952; Blair and Ottesen, 1979; Blair and Islam, 1983; Islam, 1986). Morphology alone did not provide sufficient diagnosable features to recognize the species diversity in Lake Wanaka (Figs 1 and 2). Combinations of both adult and larval features, intermediate host use, and more significantly, genetic data, contributed to understanding the species diversity in the snails and ducks.

The definitive hosts of the nasal species *T. australis* and *T. novaeseelandiae* n. sp. are Anseriformes (ducks, geese and swans) and for *T. aureliani* are Podicipediformes (grebes), but bird host-specificity is not known, except for *T. regenti*. Spanning Eurasia, several species of ducks, geese and swans are hosts for *T. regenti* or worms that are genetically very similar, but thus far other orders of birds examined did not have *T. regenti* (Rudolfová *et al.*, 2002, 2005; Jouet *et al.*, 2008, 2010*b*, Maleki *et al.*, 2012; Skírnisson *et al.*, 2012; Fakhar *et al.*, 2016; Ashrafi *et al.*, 2018). Possibly, specificity for the definitive

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

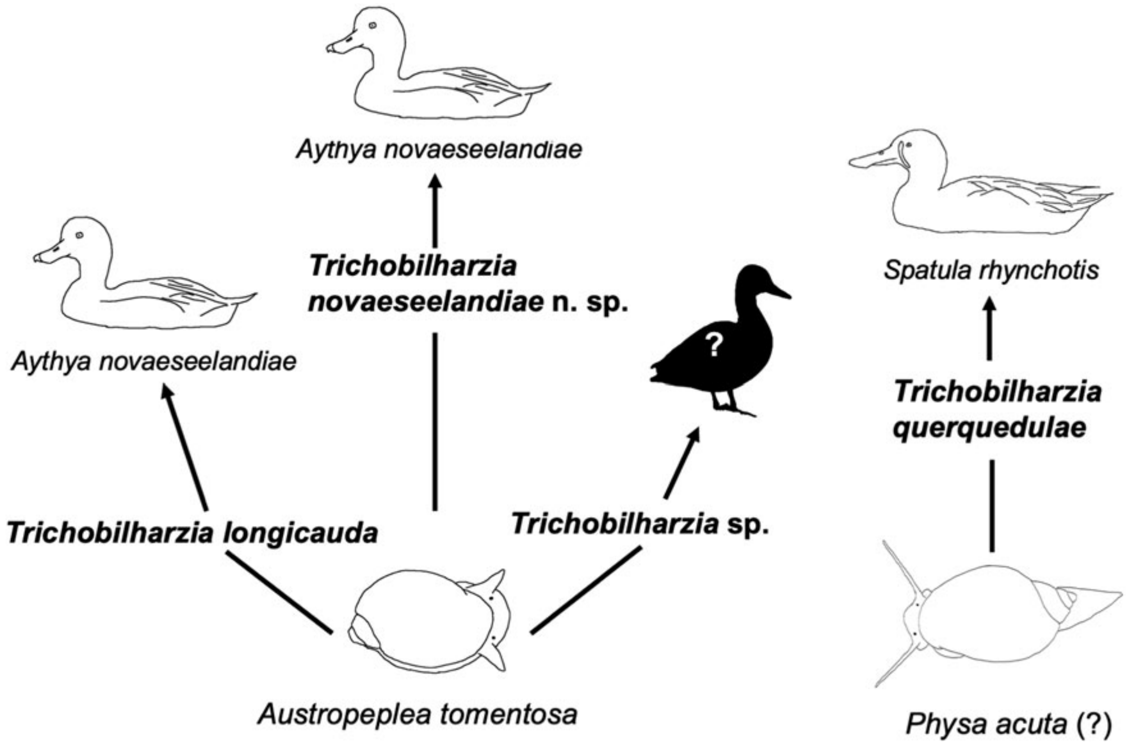

Fig. 5. Life cycle of species of *Trichobilharzia* in Lake Wanaka.

host might separate *T. novaeseelandiae* n. sp. from *T. aureliani* in the absence of other recorded features. To date, there are no named, but one genetically confirmed species of *Trichobilharzia* from snails from an African country (Moema *et al.*, 2019). There have been three reports of putative *Trichobilharzia* sp. from *Lymnaea natalensis* from South Africa (Appleton, 1984 – *Trichobilharzia* type 1; Moema *et al.*, 2008) that were also responsible for causing HCD. Moema *et al.* (2019) genetically confirmed a species of *Trichobilharzia* from *Lymnaea natalensis* but provided only *28S* sequence data and thus it is not possible to know to what species clade it might belong. However, in the *28S* gene tree, the specimen from *L. natalensis* did not group in Clade Q (data not shown). Avian schistosomes usually have a narrow range of intermediate host snails, even if several congeners can serve as hosts. It might be that, in the absence of the preferred snail species, these schistosomes can use related species (e.g. Manzoli *et al.*, 2021), which could be the case for *T. regenti* (in Europe uses *R. balthica*) and *T. franki* (in Europe uses *R. auricularia*) which are also found in domestic and wild ducks in Iran (Fakhar *et al.*, 2016; Ashrafi *et al.*, 2018, 2021). Also, for avian schistosome species, as far as is known, rarely do species in more than one genus or family of snail serve as natural hosts. Thus, likely the schistosome species from *Austropeplea* may be distinct from those using species of *Radix* or *Lymnaea*, snails that are phylogenetically distant (Vinarski *et al.*, 2020). The definitive host of *T. longicauda* is *Ay. novaeseelandiae*, but it is not known if other ducks can also host this species, or the un-named cercaria species in this study. Species of *Trichobilharzia* have previously been found in visceral veins/liver of *An. superciliosa*, *S. rhynchotis* (likely *T. querquedulae*) and *An. platyrhynchos* in New Zealand, but were not described (Featherstone and McDonald, 1988; Rind, 1991; Davis, 2006*b*). The duck species listed above can all be found feeding in the shallow waters where most of the snail hosts live.

The biogeography of species of *Trichobilharzia* is still a way from being understood until more specimens and their hosts, particularly from the African continent, are revealed. Certainly, one significant means of geographic movement for birds other than migration is introductions of ducks and geese for sport hunting. In New Zealand, *Branta canadensis* and *Anas platyrhynchos* were introduced as game birds starting in the early 1900s sourced from both the UK and the USA (Spurr *et al.*, 2005; Dyer and Williams, 2010; Guay *et al.*, 2015). These birds could have been sources of schistosomes at that time, though most of them were raised in captivity. In the UK, the prevalence of *Trichobilharzia* particularly in Anseriformes is not as well-known as in continental Europe, even though HCD in the UK has been reported (e.g. Fraser *et al.*, 2009; Morley, 2009; Lawton *et al.*, 2014). To date, only one species of avian schistosome (*T. franki*) has been confirmed in the UK from snails but there have been no reports from *An. platyrhynchos* or *B. canadensis* (Lawton *et al.*, 2014). In the USA, to date there have been no nasal species recovered from waterfowl, but a visceral species (*T. physellae*) has been found in *An. platyrhynchos* (Brant and Loker, 2009) and *Anserobilharzia brantae* has been recovered from *B. canadensis* (Brant *et al.*, 2013). What is notable is that most continents appear to have their own endemic species of *Trichobilharzia* but also at least one species that is more geographically widespread, most likely hosted by migratory birds.

Epidemiology of HCD on South Island of New Zealand

For this study, samples were collected in the same areas as previous workers (Macfarlane, 1944; Rind, 1991; Featherston *et al.*, 1988). Three species of *Trichobilharzia* utilizing *Au. tomentosa* were found. It is not possible to know if all three species were present when research was first conducted on Lake Wanaka. However, these three species each belongs to a separate clade, suggesting that they are endemic in *Au. tomentosa* in New Zealand. The single sample from Australia was most closely related to *T. regenti*, rather than to the nasal species from New Zealand. New samples that include a genetic characterization of species from Africa and more from Australia will weave a more comprehensive biogeographic history and confirm the presence of endemic species in Australia and New Zealand.

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

Macfarlane (1944) stated that prior to the 1920s there were few complaints of HCD, and by 1925 there were several cases a summer, but usually restricted to Roy's Bay on Lake Wanaka. At the time of his study, cases were from the south end of Lake Wanaka, and it was assumed the definitive host was a vertebrate, likely a water bird. By 1949, Macfarlane (1949) expanded his description of the aetiological agent, *Cercaria longicauda*, from *Au. tomentosa* and noted that the snails were associated with the aquatic plants, *Isoetes* sp. and *Juncus* sp., as were the ducks, *Ay. novaeseelandiae* and *An. superciliosa*, which spent most of the summer in those plant beds. About 40 years later, Featherston *et al.* (1988), after extensive surveys, listed the conditions they felt were the most important in the transmission dynamics of HCD on Lake Wanaka: (a) snails *Au. tomentosa* more than 3 mm long; (b) an aquatic plant often associated with the presence of *Au. tomentosa*, *Isoetes alpinus*; (c) sediment layer on the leaves of the *I. alpinus*; and (d) presence of the scaup, *Ay. novaeseelandiae*, which rest in great numbers in such a habitat. Bays of Lake Wanaka in previous years that were parasite-free still had snails present, but the scaup were absent; and (e) prevalence in snails was highest when temperatures were over 13°C (Featherston and McDonald, 1988; Featherston *et al.*, 1988). Over time it was found that more and more snails were infected, which correlated with the scaup moving into those areas, perhaps in response to needing a refuge from the increased recreational use of the lake. However, Davis (2000) did not find that *Au. tomentosa* had a predilection for any plant species and found snails grazing in the absence of macroscopic plants. Though other species of schistosomes (unknown spp. of *Dendritobilharzia*, *Ornithobilharzia*) have been found in snails (*Gyraulus* sp.) and ducks in New Zealand, thus far only cercariae from *Au. tomentosa* have been implicated in outbreaks (Rind, 1974, 1991; Davis, 2006*b*).

Conclusion

Four species of *Trichobilharzia* occur in ducks on Lake Wanaka. Three of the schistosome species use the same snail host, *Austropeplea tomentosa*. The New Zealand scaup, *Aythya novaeseelandiae*, was host to *Trichobilharzia longicauda* that is redescribed here and to a new species of nasal schistosome, *T. novaeseelandiae* n. sp. The New Zealand shoveler (*Spatula rhynchotis*) was also examined for both nasal and visceral schistosomes, but had only *T. querquedulae*, a species once thought to only occur in North American species of *Spatula*, but has now been found in New Zealand, Argentina and South Africa (Ebbs *et al.*, 2016). Only cercariae were obtained of the fourth species, and thus the bird host is unknown. Future efforts should be made to characterize the transmission dynamics of each species and its relative contribution to outbreaks of HCD.

Acknowledgements. Thank you for the cooperation at the New Zealand Fish and Game and Hawkes Bay District Council who facilitated the collections of ducks and snails.

Financial support. Field collections for this research received no specific grant from any funding agency, commercial or not-for-profit sectors and were mostly conducted by NED in New Zealand and DB in Australia. The University of New Mexico supported the work done in New Mexico through a National Science Foundation grant to SVB (DEB 1021427) and funds provided by the College of Arts and Sciences at UNM. Technical assistance at UNM Molecular Biology Facility was supported by NIH grant 1P20RR18754 from the Institute Development Award program of the National Center for Research Resources.

Conflict of interest. None.

References

Aldhoun J, Podhorský M, Kolická M and Horák P (2012) Bird schistosomes in planorbid snails in the Czech Republic. *Parasitology International* **61**, 250–259.

Appleton CC (1984) Schistosome dermatitis-an unrecognized problem in South Africa. *South African Medical Journal* **65**, 467–469.

Appleton CC (2003) The avian Schistosomatidae of sub-Saharan Africa with particular reference to *Cercariae herini*, a cause of dermatitis in people. *Proceedings of Workshop on African Freshwater Malacology*, 9-12 September, Kampala, Uganda, pp. 213–233.

Ashrafi K, Nouroosta A, Sharifdini M, Mahmoudi MR, Rahmati B and Brant SV (2018) Genetic diversity of an avian nasal schistosome causing cercarial dermatitis in the Black Sea-Mediterranean migratory route. *Parasitology Research* **117**, 3821–3833.

Ashrafi K, Sharifdini M, Darjani A and Brant SV (2021) Migratory routes, domesticated birds and cercarial dermatitis: the distribution of *Trichobilharzia franki* in Northern Iran. *Parasite* **28**, 4.

Basch PF (1966) The life cycle of *Trichobilharzia brevis* n. sp., an avian schistosome from Malaya. *Zeitschrift für Parasitenkunde* **27**, 252–259.

Blair D and Islam KS (1983) The life cycle and morphology of *Trichobilharzia australis* n. sp. (Digenea: Schistosomatidae) from the nasal blood vessels of the black duck (*Anas superciliosa*) in Australia, with a review of the genus *Trichobilharzia*. *Systematic Parasitology* **5**, 89–117.

Blair D and Ottesen P (1979) Nasal schistosomiasis in Australian anatids. *Journal of Parasitology* **65**, 982–984.

Boray JC (1964) Studies on the ecology of *Lymnaea tomentosa*, the intermediate host of *Fasciola hepatica*. 1. History, geographical distribution, and environment. *Australian Journal of Zoology* **12**, 217–223.

Bowles J and McManus DP (1993) Rapid discrimination of *Echinococcus* species and strains using a PCR-based RFLP method. *Molecular and Biochemical Parasitology* **57**, 231–239.

Bowles J, Blair D and McManus DP (1995) A molecular phylogeny of the human schistosomes. *Molecular Phylogenetics and Evolution* **4**, 103–109.

Brant SV and Loker ES (2009) Molecular systematics of the avian schistosome genus *Trichobilharzia* (Trematoda: Schistosomatidae) in North America. *Journal of Parasitology* **95**, 941–963.

Brant SV and Loker ES (2013) Discovery-based studies of schistosome diversity stimulate new hypotheses about parasite biology. *Trends in Parasitology* **29**, 449–459.

Brant SV, Morgan JAT, Mkoji GM, Snyder SD, Rajapakse RPVJ and Loker ES (2006) An approach to revealing blood fluke life cycles, taxonomy, and diversity: provision of key reference data including DNA sequence from single life cycle stages. *Journal of Parasitology* **92**, 77–88.

Brant SV, Bochte CA and Loker ES (2011) New intermediate host records for the avian schistosomes *Dendritobilharzia pulverulenta*, *Gigantobilharzia huronensis*, and *Trichobilharzia querquedulae* from North America. *Journal of Parasitology* **97**, 946–949.

Brant SV, Jouet D, Ferté H and Loker ES (2013) *Anserobilharzia* gen. n. (Digenea, Schistosomatidae) and redescription of *A. brantae* (Farr & Blankemeyer, 1956) comb. n. (syn. *Trichobilharzia brantae*), a parasite of geese (Anseriformes). *Zootaxa* **3670**, 139–206.

Brant SV, Loker ES, Casalins L and Flores V (2017) Phylogenetic placement of a schistosome from an unusual marine snail host, the false limpet (*Siphonaria lessoni*) and gulls (*Larus dominicanus*) from Argentina with a brief review of marine schistosomes from snails. *Journal of Parasitology* **103**, 75–82.

Davis NE (1998) Population dynamics and larval trematode interactions with *Lymnaea tomentosa* and the potential for biological control fo schistosome dermatitis in Bremner Bay, Lake Wanaka, New Zealand. *Journal of Helminthology* **72**, 319–324.

Davis NE (2000) *Cercarial Dermatitis and the Possibility of Biological Control in Lake Wanaka, New Zealand* (A thesis submitted for the degree of Doctor of Philosophy at the University of Otago). Dunedin, New Zealand.

Davis NE (2006a) Identification of an avian schistosome recovered from *Aythya novaeseelandiae* and infectivity of its miracidia to *Lymnaea tomentosa* snails. *Journal of Helminthology* **80**, 225–253.

Davis NE (2006b) A survey of waterfowl for echinostomes and schistosomes from Lake Wanaka and the Waitaki River watershed, New Zealand. *Journal of Helminthology* **80**, 33–40.

Devkota R, Brant SV, Thapa S and Loker ES (2014) Two avian schistosome cercariae from Nepal, including a *Macrobilharzia*-like species from *Indoplanorbis exustus*. *Parasitology International* **63**, 374–380.

Dyer J and Williams M (2010) An introduction most determined: Mallard (*Anas platyrhynchos*) to New Zealand. *Notornis* **57**, 178–195.

Ebbs ET, Loker ES, Davis NE, Flores V, Veleizan A and Brant SV (2016) Schistosomes with wings: how host phylogeny and ecology shape the global distribution of *Trichobilharzia querquedulae* (Schistosomatidae). *International Journal for Parasitology* **46**, 669–677.

Ebbs ET, Loker ES and Brant SV (2018) Phylogeography and genetics of the globally invasive snail *Physa acuta* Draparnaud 1805, and its potential to serve as an intermediate host to larval digenetic trematodes. *BMC Evolutionary Biology* **18**, 103.

Fain A (1955) Recherches sur les schistosomes d'oiseaux au Ruanda-Urundi (Congo belge). *Revue de Zoologie et de Botanique Africaines* **51**, 373–387.

Fain A (1956) Les schistosomes d'oiseaux du genre *Trichobilharzia* Skrjabin et Zakharov, 1920 au Ruanda Urundi. *Revue de Zoologie et de Botanique Africaines* **54**, 147–178.

Fain A (1959) Un nouveau schistosome du genre *Trichobilharzia* dans les fosses nasales du canard nain. *Revue de Zoologie et de Botanique Africaines* **60**, 227–232.

Fakhar M, Ghobaditara M, Brant SV, Karamian M, Gohardehi S and Bastani R (2016) Phylogenetic analysis of nasal avian schistosomes (*Trichobilharzia*) from aquatic birds in Mazandaran Province, northern Iran. *Parasitology International* **65**, 151–158.

Featherston DW and McDonald TG (1988) Schistosome dermatitis in Lake Wanaka: survey of the snail population, 1976–77. *New Zealand Journal of Zoology* **15**, 439–442.

Featherston DW, Weeks PJ and Featherston N (1988) Schistosome dermatitis in Lake Wanaka: *Cercaria longicauda* prevalence in *Lymnaea tomentosa*, 1978–1983. *New Zealand Journal of Zoology* **15**, 381–386.

Fraser SJ, Allan SJR, Roworth M, Smith HV and Holme SA (2009) Cercarial dermatitis in the UK. *Clinical and Experimental Dermatology* **34**, 344–346.

Guay PJ, Williams M and Robinson RW (2015) Lingering genetic evidence of North American mallards (Anas platyrhynchos) introduced to New Zealand. *New Zealand Journal of Ecology* **39**, 103–109.

Horák P, Kolárová L and Dvořák J (1998) *Trichobilharzia regenti* n. sp. (Schistosomatidae, Bilharziellinae) a new nasal schistosome from Europe. *Parasite* **5**, 349–357.

Horák P, Kolárová L and Adema CM (2002) Biology of the schistosome genus *Trichobilharzia*. *Advances in Parasitology* **52**, 155–233.

Horák P, Schets FM, Kolárová L and Brant SV (2012) Chapter 42. Trichobilharzia. In Lui D (ed.), *Molecular Detection of Human Parasitic Pathogens*. New South Wales, Australia: RCPA Biosecurity QAP. CRC Press, pp. 455–465.

Horák P, Mikeš L, Lichtenbergová L, Skála V, Soldánová M and Brant SV (2015) Avian schistosomes and outbreaks of cercarial dermatitis. *Clinical Microbiology Reviews* **28**, 165–190.

Huelsenbeck JP and Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England)* **17**, 754–755.

Islam KS (1986) The morphology and life-cycle of *Trichobilharzia arcuata* n. sp. (Schistosomatidae: Bilharziellinae) a nasal schistosome of water whistle ducks (*Dendrocygna arcuata*) in Australia. *Systematic Parasitology* **8**, 117–128.

Islam KS and Copeman DB (1986) The morphology and life-cycle of *Trichobilharzia parocellata* (Johnston & Simpson, 1939) Islam & Copeman, 1980 from the visceral blood vessels of Australian anatids. *Systematic Parasitology* **8**, 39–49.

Jouet D, Ferté H, Depaquit J, Rudolfová J, Latour P, Zanella D, Kaltenback ML and Léger N (2008) *Trichobilharzia* spp. in natural conditions in Annecy Lake, France. *Parasitology Research* **103**, 51–58.

Jouet D, Skírnisson K, Kolárová L and Ferté H (2010a) Molecular diversity of *Trichobilharzia franki* in two intermediate hosts (*Radix auricularia* and *Radix peregra*): a complex of species. *Infection, Genetics and Evolution* **10**, 1218–1227.

Jouet D, Skírnisson K, Kolárová L and Ferté H (2010b) Final hosts and variability of *Trichobilharzia regenti* under natural conditions. *Parasitology Research* **107**, 923–930.

Jouet D, Kolárová L, Patrelle C, Ferté H and Skírnisson K (2015) *Trichobilharzia anseri* n. sp. (Schistosomatidae: Digenea), a new visceral species of avian schistosomes isolated from graylag good (*Anser anser* L.) in Iceland and France. *Infection Genetics and Evolution* **34**, 298–306.

Kolárová L, Skírnisson K, Ferté H and Jouet D (2013) *Trichobilharzia mergi* sp nov (Trematoda: Digenea: Schistosomatidae), a visceral schistosome of *Mergus serrator* (L.) (Aves: Anatidae). *Parasitology International* **62**, 300–308.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549.

Lawton SP, Lim RM, Dukes JP, Cook RT, Walker AJ and Kirk RS (2014) Identification of a major causative agent of human cercarial dermatitis, *Trichobilharzia franki* (Müller and Kimmig 1994), in southern England and its evolutionary relationships with other European populations. *Parasite Vectors* **7**, 277.

Lockyer AE, Olsen PD, Ostergaard P, Rollinson D, Johnston DA, Attwood S W, Southgate VR, Horák P, Snyder SD, Le TH, Agatsuma T, Mcmanus DP, Carmichael AC, Name S and Littlewood DTJ (2003) The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858. *Parasitology* **126**, 203–224.

Lui Z and Bai G (1976) On bird schistosomes from Jilin Province: *Jilinobilharzia crecci* gen. nov., sp. nov. (Schistosomatidae: Bilharziellinae) with a discussion on the taxonomy of the subfamily Bilharziellinae. *Acta Zoologica Sinica* **22**, 385–392.

Macfarlane WV (1944) Schistosome dermatitis in the southern lakes: an investigation of swimmer's itch. *New Zealand Medical Journal* **43**, 136–140.

Macfarlane WV (1949) Schistosome dermatitis in New Zealand: part 1. The parasite. *American Journal of Hygiene* **50**, 143–151.

Macfarlane WV (1952) Schistosome dermatitis in Australia. *The Medical Journal of Australia* **20**, 669–672.

Maleki SH, Athari A, Haghighi A, Taghipour N, Gohardehi SH and Tabaei SS (2012) Species identification of bird nasal *Trichobilharzia* in Sari, north of Iran. *Iran Journal of Parasitology* **7**, 82–85.

Manzoli DE, Saravia-Pietropaolo MJ, Arce SI, Percara A and Beldomenico PM (2021) Specialist by preference, generalist by need: availability of quality hosts drives parasite choice in a natural multihost–parasite system. *International Journal of Parasitology* **51**, 527–534.

Mcleod JA (1937) Two new schistosomid trematodes from water birds. *Journal of Parasitology* **23**, 456–466.

Mcmullen DB and Beaver PC (1945) Studies of schistosome dermatitis. IX. The life cycles of three dermatitis-producing schistosomes from birds and a discussion of the subfamily Bilharziellinae (Trematoda: Schistosomatidae). *American Journal of Hygiene* **42**, 128–154.

Moema EB, King PH and Baker C (2008) Cercariae developing in *Lymnaea natalensis* Krauss, 1848 collected in the vicinity of Pretoria, Gauteng Province, South Africa. *Onderstepoort Journal of Veterinary Research* **75**, 215–233.

Moema EB, King PH and Rakgole JN (2019) Phylogenetic studies of larval digenean trematodes from freshwater snails and fish species in the proximity of Tshwane metropolitan, South Africa. *Onderstepoort Journal of Veterinary Research* **86**, a1726.

Morley N (2009) Cercarial dermatitis in the UK: a long established history. *Clinical and Experimental Dermatology* **34**, E443.

Müller V and Kimmig P (1994) *Trichobilharzia franki* n. sp.-the cause of swimmer's dermatitis in southwest German dredged lakes. *Applied Parasitology* **35**, 12–31.

Palumbi S, Martin A, Romano S, Wo MM, Stice L and Grabowski G (1991) *The Simple Fool's Guide to PCR*. Honolulu, Hawaii. https://searchworks.stanford.edu/view/9267895.

Pinto HA, Brant SV and de Melo AL (2014) *Physa marmorata* (Mollusca: Physidae) as a natural intermediate host of *Trichobilharzia* (Trematoda: Schistosomatidae), a potential causative agent of avian cercarial dermatitis in Brazil. *Acta Tropica* **138**, 38–43.

Pinto HA, Pulido-Murillo EA, de Melo AL and Brant SV (2017) Putative new genera and species of avian schistosomes potentially involved in human cercarial dermatitis in the Americas, Europe and Africa. *Acta Tropica* **176**, 415–420.

Podhorský M, Hůzová Z, Mikeš L and Horák P (2009) Cercarial dimensions and surface structures as a tool for species determination of *Trichobilharzia* spp. *Acta Parasitologica* **54**, 28–36.

Porter A (1938) The larval Trematoda found in certain South African Mollusca with special reference to schistosomiasis (bilharziasis). *Publications of the South African Institute for Medical Research* **62**, 1–492.

Posada D and Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Pullan NB, Climo FM and Mansfield CB (1972) Studies on the distribution and ecology of the family Lymnaeidae (Mollusca: Gastropoda) in New Zealand. *Journal of the Royal Society of New Zealand* **2**, 393–405.

Puslednik L, Ponder WF, Dowton M and Davis AR (2009) Examining the phylogeny of the Australasian Lymnaeidae (Heterobranchia: Pulmonata: Gastropoda) using mitochondrial, nuclear and morphological markers. *Molecular Phylogenetics and Evolution* **52**, 643–659.

https://doi.org/10.1017/S0031182021001943 Published online by Cambridge University Press

Rind S (1974) Some helminth parasites of freshwater birds from the South Island, New Zealand, with particular reference to trematodes of ducks. *Mauri ora* **2**, 139–146.

Rind S (1991) Three ocellate schistosome cercariae (Trematoda: Schistosomatidae) in *Gyraulus corinna*, with reference to *Cercaria longicauda* Macfarlane, 1944 in *Lymnaea tomentosa*. *New Zealand Journal of Zoology* **18**, 53–62.

Rudolfová J, Sitko J and Horák P (2002) Nasal schistosomes of wildfowl in the Czech Republic. *Parasitology Research* **88**, 1093–1095.

Rudolfová J, Hampl V, Bayssade-Dufour C, Lockyer AE, Littlewood DTJ and Horák P (2005) Validity reassessment of *Trichobilharzia* species using Lymnaea stagnalis as the intermediate host. *Parasitology Research* **95**, 79–89.

Skírnisson K, Kolárová L, Horák P, Ferté H and Jouet D (2012) Morphological features of the nasal blood fluke *Trichobilharzia regenti* (Schistosomatidae, Digenea) from naturally infected hosts. *Parasitology Research* **110**, 1881–1892.

Soldanová M, Selbach C, Kalbe M, Kostadinova A and Sures B (2013) Swimmer's itch: etiology, impact, and risk factors in Europe. *Trends in Parasitology* **29**, 65–74.

Spurr EB, Coleman JD and Whenua M (2005) *Review of Canada Goose Population Trends, Damage and Control in New Zealand*. Lincoln, NZ: Manaaki Whenua Press, Landcare Research.

Stecher G, Tamura K and Kumar S (2020) Molecular evolutionary genetics analysis (MEGA) for macOS. *Molecular Biology and Evolution*. https://doi.org/10.1093/molbev/msz312.

Suzuki N and Kawanaka M (1980) *Trichobilharzia brevis* Basch, 1966, as a cause of an outbreak of cercarial dermatitis in Japan. *Japanese Journal of Parasitology* **29**, 1–11.

Thompson CW, Phelps KL, Allard MW, Cook JA, Dunnum JL, Ferguson AW, Gelang M, Khan FAA, Paul DL, Reeder DM, Simmons NB, Vanhove MPM, Webala PW, Weksler M and Kilpatrick CW (2021) Preserve a voucher specimen! The critical need for integrating natural history collections in infectious disease studies. *mBio* **12**, e02698–20.

Vilas R, Criscione CD and Blouin MS (2005) A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Parasitology* **131**, 839–846.

Vinarski MV, Aksenova OV and Bolotov IN (2020) Taxonomic assessment of genetically-delineated species of radicine snails (Mollusca, Gastropoda, Lymnaeidae). *Zoosystematics and Evolution* **96**, 577–608.