Journal of Helminthology (2006) **80,** 225–233 DOI: 10.1079/JOH2005327

Identification of an avian schistosome recovered from *Aythya novaeseelandia* and infectivity of its miracidia to *Lymnaea tomentosa* snails

N.E. Davis*

Department of Zoology, University of Otago, PO Box 56, Dunedin, New Zealand

Abstract

A partial life cycle involving miracidia hatched from the eggs of *Trichobilharzia* sp. recovered from New Zealand scaup (*Aythya novaeseelandia*) to the release of furcocercariae by laboratory snails (*Lymnaea tomentosa*) was accomplished. Challenges with five and ten miracidia per snail were lethal. Challenge with three miracidia resulted in development to the daughter sporocyst stage and death in five, development to furcocercarial stage and death in one, and shedding of furcocercariae in one of seven snails. Observed lethality of schistosome miracidia to *L. tomentosa* may explain the low infection prevalence observed in the wild. Future work should plan challenge exposures of three or fewer miracidia to ensure snail survival and successful recovery of furcocercariae. The *Trichobilharzia* sp. found in the New Zealand scaup does not key morphologically to the literature. It may be a new species and further work is needed.

Introduction

Avian schistosome life cycle

The life cycle of avian schistosomes is similar to that of human schistosomes. Definitive hosts are usually Anseriformes. Dioecious adult *Trichobilharzia ocellata* reside in hepatic veins. Females migrate to the vessels of the intestinal wall where eggs are deposited. Eggs penetrate the gut wall and are voided, fully embryonated, in the faeces (Bourns *et al.*, 1973). In Lake Wanaka, each egg hatches to release a fully developed miracidium within 15 min of immersion in freshwater (MacFarlane, 1949). The miracidium must find a suitable snail host within 12 h of hatching to achieve infection (Prah & James, 1977; Anderson *et al.*, 1982; Waadu, 1991). Upon entering a snail, the parasite migrates to the heart and digestive gland where it transforms into successive stages of sporocysts. Several weeks later, daughter sporocysts produce thousands of furcocercariae which escape from the snail and seek the definitive host to complete the life cycle. The cercariae swim freely in a zig-zag pattern, are negatively geotropic, positively phototropic and rest occasionally by grasping the water surface or debris with the ventral acetabulum (Rind, 1991). Feiler & Haas (1988) reported a complex swimming behaviour influenced directionally by light and shadow with attachment to the host influenced by temperature gradient and chemical exudates of the host skin. Water temperature and exposure to sunlight are principal determinants of cercarial life span. Cercarial die-off increases during hot, sunny days (Mulvihill & Burnett, 1990). If the definitive host is not penetrated within 24 h, cercariae will die since they depend upon endogenous energy stores. Once within the definitive host they use exogenous metabolites to survive (Bayer, 1954). Upon encountering a prospective host, cercariae attach themselves to the skin with the ventral acetabulum, lose their tails and penetrate the skin to become schistosomules. In the definitive host, schistosomules migrate through the blood vessels to the lungs and then to the hepatic veins where sexual maturation occurs (Bourns *et al.*, 1973).

*Fax: +64 3 689 7867
E-mail: davisn@xtra.co.nz

Basch (1966) exposed ducklings and white mice to cercariae of *Trichobilharzia brevis* shed by wild *Lymnaea rubiginosa* snails. The ducklings shed miracidia and schistosomules were found in the lungs of a mouse. Bourns *et al.* (1973) studied the migration and development of *T. ocellata* in the the duck and reported that female worms appeared to reside in the hepatic veins and migrate to and from the villi, where they deposited their eggs. Blair & Islam (1983) described the life cycle of *Trichobilharzia australis* and proposed a revision of the genus *Trichobilharzia.* Maintenance of the life cycle of *T. ocellata* was achieved in the laboratory by Meulemann *et al.* (1984). Life cycles of other non-human Schistosomatidae are described by Cort (1928) and McMullen & Beaver (1945). Among the *Trichobilharzia* sp. there is definitive host specificity (Blair & Islam, 1983). The cercariae can penetrate the skin of other animals but they may not mature. Horak & Kolarova (2000) reported that *Trichobilharzia szidati* and *Bilharziella polonica* may fully transform to schistosomulae and migrate to the lungs in murine hosts, where they persist for up to 10 days post-exposure.

Most of the known species of *Trichobilharzia* infect lymnaeid or physid snail intermediate hosts. Some species can have multiple snail intermediate hosts (Vicente, 1979). Jarcho & Van Burkalow (1952) suggested that summer schistosome infections of snails continue development through the winter and birds may be exposed during the following summer to cercariae from snails infected the previous summer. In Lake Wanaka, however, avian schistosome infection of *Lymnaea tomentosa* snails was evident only between spring and autumn (Davis, 1998). Birds excrete viable *Trichobilharzia* sp. eggs as early as 2 weeks after infection. The seasonal rates of snail infection may vary widely and infection may be absent in some years (Brackett, 1941).

Identification of schistosomes of the genus *Trichobilharzia* to species level has not been accomplished in New Zealand. This genus is widely represented in Europe by the species *Trichobilharzia ocellata,* where the definitive host is the duck *(Anas platyrhynchos)* and lymnaeids are intermediate hosts (de Gentile *et al.*, 1996). MacFarlane (1944) suggested that *Cercaria longicauda* was a close relation to *Cercaria elvae* and *Cercaria ocellata.* Miracidia were recovered from one of two waterfowl, but no adult worms were found upon necropsy 3 months after exposing them to *C. longicauda* cercariae from three Lake Wanaka molluscs – *Myxas ampulla, M. arguta* and *Lymnaea alfredi* (MacFarlane, 1949). He identified these waterfowl as black teal, '*Fuligula novae zealandii*', now correctly identified as New Zealand scaup, *Aythya novaeseelandia*e (A. Scaife, personal communication). The three snail species have been reclassified together by Dell (1956) and finally by Climo & Pullen (1972) as *Lymnaea tomentosa.* MacFarlane (1949) did not recover adult schistosomes or miracidia from the domestic duck, pigeon, canary or domestic fowl following experimental infection with *C. longicauda*, and suggested that the New Zealand scaup is the main definitive host in Lake Wanaka. Featherston & McDonald (1988) recovered a male and a female *Trichobilharzia* from the liver and a male *Trichobilharzia* from the blood of a New Zealand scaup, while one *Trichobilharzia* (sex undetermined) was recovered from a mallard. Rind (1980) found the adults of two schistosome species (*Dendritobilharzia pulverulenta* and *Trichobilharzia* sp.) in teal, grey duck and mallard from the Wanaka/Queenstown area and stated that there was no substantiated link between adult schistosomes and *C. longicauda*.

The common merganser *Mergus merganser* and the snail *Stagnicola elrodi* are the natural hosts of *T. ocellata* in Flathead Lake, Montana, with prevalence of 84% and 2.0%, respectively (Loken *et al.*, 1995). The prevalence of adult schistosomes in New Zealand scaup and of larval schistosomes in *L. tomentosa* snails in Lake Wanaka (Davis, 1998, 2000) are comparable to Loken's findings. The New Zealand scaup is similar in behaviour to the common merganser and may be the best candidate for providing live schistosome eggs.

Adult schistosomes recovered from naturally infected definitive hosts may be identified morphologically with reference to a key. Farley (1971) developed a key to aid in identification of schistosomes excluding those found in mammals. He stated that the term *Trichobilharzia ocellata* should be used only in reference to the general complex of cercariae and adults from Europe, while suggesting that a similar complex may exist in North America. McDonald (1981) keyed the males only of the genus *Trichobilharzia* while reporting that taxonomy of these worms 'is difficult and in dispute'. Blair & Islam (1983) described the life cycle of *Trichobilharzia australis*, whose cercariae develop in lymnaeid snails, and provided a key for identification of adults of the genus *Trichobilharzia.*

Schistosome eggs recovered from the New Zealand scaup may be compared morphologically with those described in the literature. Basch (1966) described the eggs of *Trichobilharzia brevis*, stating that their characteristic shapes were similar to those described by McMullen & Beaver (1945). Appleton (1986) described the eggs of eight different avian schistosomes from birds in South Africa, and stated that the gross morphological features of these eggs may be used to place the parasites in one of four possible genera: *Austrobilharzia, Gigantobilharzia, Trichobilharzia* or *Ornithobilharzia.*

Successful infection by avian schistosome miracidia of a particular species of snail, in this case *L. tomentosa,* may provide a clue to the schistosome genus under investigation. Kalbe *et al.* (1997) found that miracidia of *Fasciola hepatica* and *Trichobilharzia ocellata* are highly selective in their choice of host snails, preferring water conditioned by their specific host snails, *Lymnaea truncatula* and *L. stagnalis* respectively, to water conditioned by other sympatric snail species. Guth *et al.* (1979) identified *Trichobilharzia* infections of Canada geese to the generic level based on egg shape, epidermal plate arrangement, host specificity, and behaviour of miracidia.

Cercariae from *L. tomentosa* experimentally infected by miracidia from schistosome eggs recovered from New Zealand scaup may be compared with *Cercaria longicauda*. Blair & Ottesen (1979) infected *Lymnaea lessoni* with miracidia from *Trichobilharzia* sp. Islam & Copeman (1986) successfully cycled *Trichobilharzia parocellata* through *Lymnaea lessoni* snails, pigeons and domestic Muscovy ducks. Islam (1986) described the development of *Trichobilharzia australis* in the snail *Lymnaea lessoni* and the Muscovy duck. Meuleman *et al.* (1984)

maintained *T. ocellata* using Peking ducklings (*Anas platyrhynchos*) and *Lymnaea stagnalis* snails as hosts.

The aims of the present study were to recover and identify adult avian schistosomes from the New Zealand scaup, to establish an experimental infection in *L. tomentosa* snails with miracidia hatching from eggs of these schistosomes and to identify and compare cercariae released by these snails with *C. longicauda* from Lake Wanaka, New Zealand.

Materials and methods

Schistosome / egg recovery

Schistosomes and eggs were found by dissection of hearts, lungs, livers, intestines and associated blood vessels of New Zealand scaup. Livers were crushed in 1% NaCl solution (saline) in 2-litre plastic containers using a thick based drinking glass as a pestle. Pieces of liver, hepatic blood vessels and blood vessels associated with the heart, lungs and intestines were teased apart in saline in a Petri dish under a dissecting microscope. Schistosomes were transferred into Petri dishes of cold (6°C) saline. Accumulated material (liver scraps and torn blood vessels) was homogenized in saline in a kitchen blender for three consecutive 15 s periods, pausing 15 s each time to allow settling. Intestines, stripped of contents, were cut into 25 mm strips and homogenized. Homogenized material was sedimented for 1 min per each 5 cm depth. Supernatant was resedimented twice and then discarded. Sediment was mixed in saline in a 250 ml beaker. Samples were then investigated for schistosome fragments and eggs, which were recovered into cold saline.

Treatment and identification of adult avian schistosomes

Specimens were either fixed in hot (80°C) buffered formalin for 24 h and stored in 4% formalin or recovered directly into 35% ethanol, then 30 min each through 50%, 70%, 85%, 90%, and stored in 95% ethanol. For staining, ethanol fixed material was brought through 90% and 85% ethanol (30 min each) to 70% ethanol. Formalin fixed material was brought through distilled water to 70% ethanol in 30 min each concentrations of 35% and 50%. Specimens in 70% ethanol were placed in dilute Semichon's acetic carmine overnight at room temperature. They were then differentiated in acid alcohol (1% HCl in 70% ethanol), and dehydrated in 85% ethanol for 30 min followed by three changes of 30 min in 100% ethanol. They were checked in cedar oil, rinsed in xylene and mounted in D.P.X. (Gurr - BDH). Specimens stored in 70% ethanol were also taken through decreasing concentrations of ethanol to distilled water prior to staining with Lillie-Mayer acid haemalum, diluted with 20 parts potassium alum for progressive staining of whole worms. They were stained overnight at room temperature. Excess stain was rinsed off in several changes of 35% ethanol, and the specimens were placed for at least an hour each in concentrations of 50% and then 70% ethanol. They were then differentiated in acid alcohol (1% HCl in 70% ethanol). The differentiation process was stopped by transfer briefly to alkaline alcohol (0.25 ml ammonium hydroxide in 250 ml 70% ethanol). Dehydration and mounting was accomplished as noted above for carmine staining.

Specimens were compared using keys (Farley, 1971; McDonald, 1981; Blair & Islam, 1983). Morphological features of these worms (body proportions, caecal bifurcation position, seminal vesicle features and length, caecal union position, gynecophoric canal length, size, shape and number of testes and shape of tail) were compared with similar features of candidates found in the keys and in the literature (Basch, 1966; Islam & Copeman, 1986; Müller & Kimmig, 1994).

Egg identification

Eggs were examined for shape and viability under the dissecting microscope (40 ×). They were identified to genus level (Appleton, 1986) and compared with descriptions of similar eggs by Basch (1966), Islam & Copeman (1986) and Müller & Kimmig (1994).

Experimental infection of snails with miracidia

Live embryonated eggs were placed in Petri dishes of stream water under incandescent light at room temperature (17°–22°C). Miracidia were captured by pipette as they hatched and placed with laboratory raised *L. tomentosa* snails isolated singly in individual flat-bottomed tubes (SAMCO 25 mm) one-third full of stream water, capped with polythene covers to prevent escape and pierced to facilitate gas exchange. Twelve snails were initially exposed to ten miracidia each for 24 h. The challenge level was then reduced to five and then to three miracidia per snail (four snails and seven snails, respectively) in an attempt to ensure snail survivability. Water was changed twice daily and the snails were fed a flake of oven-dried lettuce each once a day. Parasite development was observed twice daily through translucent snail shells under the dissecting microscope. Snails were maintained in their individual tubes until they died or released cercariae. Dead snails were dissected to determine their state of infection. Forty unchallenged snails were similarly maintained in individual tubes as controls.

Recovery and identification of schistosome furcocercariae

Cercariae were recovered as they escaped from an experimentally infected snail and placed into 35% ethanol, then progressively (30 min each) through 50%, 70%, 85%, 90% and 95% ethanol. They were photographed unstained at 100 × for comparison with measurements of *C. longicauda* (MacFarlane, 1944) and with the published measurements of similar cercariae.

Results

Identification of adult avian schistosomes

Adult male schistosomes from the liver and mesentery of the New Zealand scaup keyed to the genus *Trichobilharzia*. Both adult and juvenile worms, which were about one third the length of the adults, were found in liver squashes. Most worms were in fragments or

https://doi.org/10.1079/JOH2005327 Published online by Cambridge University Press

knotted fragments. One adult male worm was recovered whole (fig. 1). The main feature which sets this schistosome apart from all other avian schistosomes is the length of the gynecophoric canal (table 1). Other features differentiating this worm from other candidates are: seminal vesicle shape (single or dual organs) and length; caecal union location; size, positioning (staggered or in line) and number of testes, and shape of the tail. The only male *Trichobilharzia* with a similarly long gynecophoric canal is *T. anatina*, reported by Fain (1955). Other features of this parasite such as caecal union location and description of its external seminal vesicle eliminate *T. anatina* as a candidate.

Egg harvest and identification

Eggs were recovered from the liver and gut mucosa immediately above, immediately below, and including Meckle's diverticulum of the New Zealand scaup. Eggs in liver tissue were often encapsulated and deteriorating.

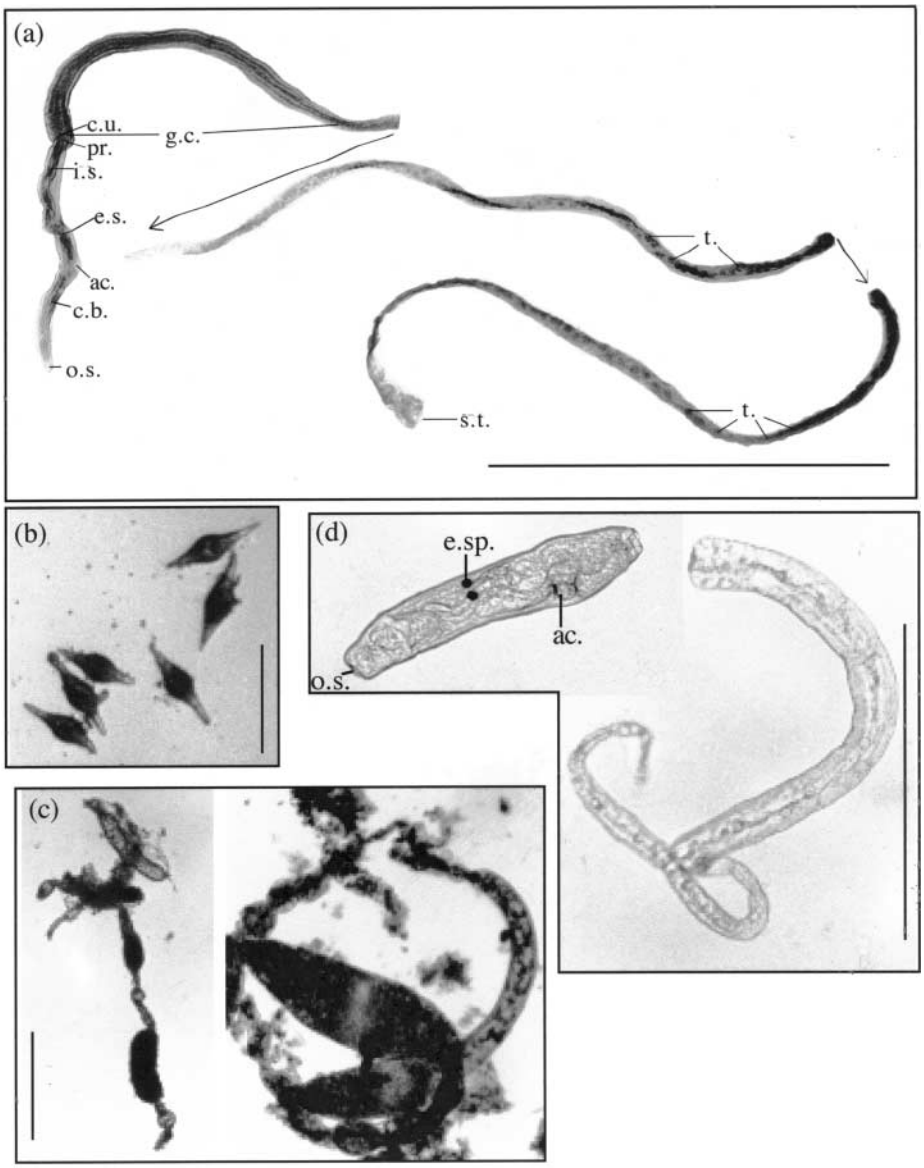

Fig. 1. Life cycle study of *Trichobilharzia sp.* recovered from *Aythya novaeseelandiae* and established in the snail intermediate host, *Lymnaea tomentosa.* (a) Adult male worm (photos of a whole specimen), bar = 1 mm; (b) eggs from liver and gut mucosa, bar = 150 μm; (c) left, mother sporocysts and, right, daughter sporocysts, note peristaltic constrictions giving sporocysts 'string of natural pearls' appearance, bar = 150 μm; (d) live furcocercaria, divested of its tail, bar = 250 μm. ac, acetabulum; c.b, caecal bifurcation; c.u, caecal union; e.s, external seminal vesicle; e.sp, eye spot; g.c, gynecophoric canal; i.s, internal seminal vesicle; o.s, oral sucker; pr, prostate; s.t, spatulate tail; t, testes.

https://doi.org/10.1079/JOH2005327 Published online by Cambridge University Press

Table 1. Comparison of adult male schistosomes from New Zealand scaup with keyed candidates. N.B. None of keyed species match in all features noted.

	New Zealand scaup schistosome	*Trichobilharzia parocellata*	*Trichobilharzia franki*	*Trichobilharzia brevis*
Reference	Davis, 2000	Islam & Copeman, 1994	Müller & Kimmig, 1994	Basch, 1966
Length (μm)	5700	2320–3480	3200–4000	2100–4000
Width (μm)	100	41–91	130	100
Caecal bifurcation	Prior to acetabulum	Prior to acetabulum	Prior to acetabulum	Prior to acetabulum
Seminal vesicle				
Form	Interior and exterior	Interior and exterior	Single organ	Single organ
Length	380–444	250 ± 60	265–315	220–260
Caecal union	Posterior to exterior seminal vesicle, immediately prior to gynecophoric canal	Mid length of interior seminal vesicle	Posterior to exterior seminal vesicle, immediately prior to gynecophoric canal	Prior to posterior of seminal vesicle, prior to gynecophoric canal
Gynecophoric canal				
Length (μm)	1450	240 ± 60	212–291	220–260
Width (μm)	100	90 ± 20	130–195	90–100
Testes				
Size (μm)	20 × 20	30 × 40	95 × 106	35 × 45
Arrangement	Staggered	Staggered	Single row	Single row
Position	Posterior to gynecophoric canal to tail end	Posterior to gynecophoric canal to tail end	Posterior to gynecophoric canal to tail end	Posterior to gynecophoric canal to tail end
Number	109	41–64	41–64	40–51
Tail shape	Club or spatulate with ear-like lateral lobes	Club or spatulate with ear-like lateral lobes	Club	Club or spatulate with ear-like lateral lobes

Table 2. Comparison of schistosome eggs from New Zealand scaup with those from literature. N.B. None of keyed species match in all features noted.

	New Zealand scaup schistosome	*Trichobilharzia parocellata*	*Trichobilharzia franki*	*Trichobilharzia. brevis*
Reference	Davis, 2000	Islam & Copeman, 1994	Müller & Kimmig, 1994	Basch, 1966
Length (μm)	167 ± 10	170 ± 20	206 ± 25	225 ± 14
Width (μm)	44 ± 6	50 ± 6	69 ± 9	51 ± 5
Number	10	40	18	6
Shape	Spindle	Spindle	Spindle	Spindle
Long axis	Straight to slightly curved	Straight	Straight	Straight to strongly curved
Tips	One sharper than other	One bluntly rounded other pointed	One blunt other pointed	One blunt other fine point
Terminal spine	No spine	Short spine	Short spine	No spine

Table 3. Observed life span in days (±standard error) of *Lymnaea tomentosa* snails exposed to three, five and ten miracidia hatched from schistosome eggs recovered from *Aythya novaeseelandia*.

Miracidial challenge	No. of snails exposed	Mean survival time (days p.e. ± SE)
3	7	33.57 ± 16.68
5	4	30.50 ± 19.82
10	12	9.58 ± 1.42

The majority of the eggs were either not embryonated (clear) or dead (opaque). They were similar in shape but smaller than eggs classed as 'type 3' by Appleton (1986), and similar in size, but not in shape to *Trichobilharzia parocellata* eggs as reported by Islam & Copeman (1986). Eggs of *T. franki* (Müller & Kimmig, 1994) and *T. brevis* (Basch, 1966) are longer than those recovered from the New Zealand scaup (table 2). Live fully embryonated eggs were easily recognized by observation of robust, rapidly rolling and tumbling miracidia within. Hatching occurred between 30 min and 6 h of egg placement in water at 17°C. The eggs were observed to swell to almost twice their original girth in the water. They would then split lengthwise, releasing miracidia which swam rapidly in straight lines punctuated by rapid random changes in direction.

Experimental infection of snails with miracidia

Attempts to infect snails with miracidia were successful (table 3). Twelve snails exposed to ten miracidia each died between 6 and 12 days (mean 9.58 days) post-exposure (p.e.). Four snails exposed to five miracidia each died between 3 and 45 days p.e. (mean 30.5 days). Of seven snails exposed to three miracidia each, five snails died between 12 and 32 days p.e. A sixth snail was sacrificed 56 days p.e. to investigate parasite development. The seventh snail started releasing furcocercariae 59 days p.e. and continued to do so until 70 days p.e. This snail succumbed 72 days p.e. to a massive proliferation of sporocysts which appeared to replace all snail digestive tissue and then degenerate as resources were depleted. All snails that died following exposure to miracidia showed evidence of infection with degenerated sporocysts and germ balls (embryos) both inside and outside sporocysts. Forty control snails that had been maintained and handled in a similar manner to snails exposed to miracidia survived indefinitely.

Development of parasites within snails

Robust sporocysts (fig. 1) were inserted in the snail digestive gland tissue at right angles to the gland's longitudinal axis. They were simple clear tubes containing germinal balls of dense granular material. Continuous peristaltic waves commenced sequentially at the distal end and moved down the body until they were lost from sight in the snail tissue. As a result of these waves, fixed sporocysts had the appearance of strings of misshapen natural pearls.

Furcocercariae recovered from the snail that had been sacrificed on day 56 p.e. were not fully developed. Sporocysts were numerous (>200) and appeared to be degenerating. Consequently, the diet of the one remaining infected snail was improved by adding ground wheat germ to the oven-dried ground lettuce (1:1 by volume). Developing sporocysts within this snail maintained their robust appearance until day 70 p.e. Release of furcocercariae (fig. 1) commenced on day 59 and was sporadic until day 70 p.e. Between day 70 and 72 p.e., the snail became sluggish and disinterested in food. No more furcocercariae were released and the snail died on day 72 p.e. by which time sporocysts had completely replaced the digestive gland tissue and were degenerating to collapsed thread-like strands as reported by Basch (1966). Furcocercariae (table 4) fixed in alcohol (Davis, 2000) were nearly similar in measurement to *C. longicauda* (Rind, 1991), *T. parocellata* (Islam & Copeman, 1986), and *C. stagnicolae,* (Talbot, 1936).

Discussion

Identification of adult avian schistosomes

The difficulty encountered in recovering whole adult worm specimens from the New Zealand scaup is supported by Basch (1966). The majority recovered were fragments and many worms were knotted together in uncountable masses. One whole male was recovered, and it was not possible to determine if there were any females included in the knotted worms. Fragments were teased apart, but no identifiable female fragments were found.

Table 4. Measurements (in microns) of avian schistosome cercariae from host lymnaeid snails.

Cercaria	Snail host	Body length	Tail length	Furca length	Reference
C. elvae	*Lymnaea stagnalis*	368	501	328	Miller, 1923*
C. ocellata		440	382	290	DuBois, 1929
C. stagnicolae	*L. emarginata*	256	374	196	Talbot, 1936
C. elvae	*L. stagnalis*	307 ± 97	400 ± 11	254 ± 11	Talbot, 1936
T. brevis	*L. rubiginosa*	237	304	218	Basch, 1966
T. ocellata	*L. peregra*	309 ± 4	527 ± 6	214 ± 5	Eklu-Natey *et al.*, 1985
T. parocellata	*L. stagnalis*	288 ± 40	354 ± 50	227 ± 34	Islam & Copeman, 1986
C. longicauda	*L. tomentosa*	255 ± 30	490 ± 56	247 ± 26	Rind, 1991
C. longicauda	*L. tomentosa*	260	510	300	Davis, 1998*
C. longicauda	*L. tomentosa*	262 ± 32	361 ± 17	185 ± 10	Davis, 2000

T., adult species; *C.* cercarial species; * Live measurement.

https://doi.org/10.1079/JOH2005327 Published online by Cambridge University Press

Adult avian schistosomes recovered from the liver and mesentery of the New Zealand scaup belong to the genus *Trichobilharzia*. Remarkably, the male specimens are unlike all but one of this genus found in the keys. The defining feature of this worm is the length of its gynecophoric canal (1450 μm), similar in that feature only to *Trichobilharzia anatina*, a rare species found in Africa (Fain, 1955; McDonald, 1981). Other features, such as seminal vesicle shape and length, rule out further consideration of *T. anatina*. The worm is longer than either *T. brevis* or *T. franki* by at least 1700 μm, and it is 500 μm longer than *T. parocellata*. Gynecophoric canals of the species mentioned above are shorter by at least 1150 μm than that of this specimen. Seminal vesicles are similar in construction (an internal (posterior) and external (anterior) seminal vesicle), however the combined length is greater than in *T. parocellata*. Seminal vesicles of both *T. franki* and *T. brevis* are single organs. In this specimen, the caecal union occurs posterior to the internal seminal vesicle and immediately prior to the gynecophoric canal. This does not match with the caecal union of *T. parocellata*, the only candidate with similar seminal vesicle construction. The club-shaped tail of some specimens equates to that of *T. franki*, while the spatulate shape matches that of *T. parocellata*. *Trichobilharzia brevis* can have either shape, as can this specimen.

Egg identification

The spindle shape of the eggs is identical to the classical shape attributable to the genus *Trichobilharzia* by Basch (1966) and Appleton (1986). The only eggs comparable in size to these eggs are those of *T. parocellata* (table 2). However, eggs of *T. parocellata* have one end bluntly rounded and a small terminal spine on the sharply pointed end, while these eggs have neither a bluntly rounded end nor a spine on the sharper point. Eggs recovered from the New Zealand scaup are attributable to the genus *Trichobilharzia*. They are different from others of this genus so far reported.

Experimental infection of snails with miracidia

All miracidia recovered were used in infection experiments. Snails exposed to five or more *Trichobilharzia* miracidia did not survive long enough to produce cercariae. High mortality of snails when challenged by these miracidia may explain the low prevalence (2.5%) of schistosome infection in wild snails reported by Davis (1998) and that (2.0%) reported by Loken *et al.* (1995). Further research using three and fewer challenge miracidia may result in a greater number of laboratory snails surviving to produce furcocercariae.

Development of parasites within snails

Peristaltic waves in developing sporocysts have not been reported elsewhere. Fortunately, the amber-coloured shell of *L. tomentosa* is nearly transparent. The snail tissue is also nearly transparent, except for the digestive gland and mantle tissue which is spotted with melanin. The aorta and heart are easily seen through the shell, and the heart is where mother sporocysts were first observed. As sporocysts develop they proliferate and, as they replace the tissue, more and more of the interior of the snail can be observed. Peristaltic waves may be useful in dispersing the absorbed nutrients among the developing daughter sporocysts or cercariae within. Peristalsis did not appear to be instrumental in moving the sporocysts within the snail, although they may also provide that function.

Release of cercariae

One snail (challenged with three miracidia) was sacrificed at 56 days p.e. because sporocyst activity was diminishing and the sporocysts were becoming thinner. This snail had many collapsed sporocysts and only a few undeveloped cercariae. The diminished size of the digestive gland and collapse of the sporocysts indicated that the sporocysts may have been exhausting their food resources. Improving the diet of the remaining snail appeared to enhance its survival. Release of cercariae from day 59 p.e. to day 70 p.e. (11 days) at a room temperature of 17–22°C can be compared to the 15-day period of release of cercariae by *T. brevis* at a temperature of 28°C (Basch, 1966). The higher ambient temperature in Basch's laboratory appears to have accelerated the development of the parasites within the snails which started releasing cercariae at 17 days p.e.

Cercarial measurements compare favourably with those of *C. longicauda* recovered from wild *L. tomentosa* snails. Measurements of cercariae by other workers may be influenced by fixing and slide-mounting methods, e.g. Basch (1966) fixed cercariae in hot formalin prior to measurement. For comparison, it is advisable to measure specimens that have been similarly fixed. Attempts have also been made to measure live cercariae. Results of these measurements can be highly variable, due to the extension and contraction of the body while the cercaria is moving. Differences in measurements reported in table 4 may be more attributable to fixing techniques than to the parasite species in question. Eklu-Natey *et al.* (1985) recorded minimum and maximum, as well as median plus or minus standard error, measurements of *Trichobilharzia* cf. *ocellata* cercariae released by *Lymnaea peregra* snails from Lac Leman, Switzerland. These measurements appear to be closest to those of *C. longicauda*. Caudal (481–567 μm) and furcal length (185–245 μm) correspond most closely with those of *C. elvae* (Miller, 1923), and of *C. longicauda* (Rind, 1991). The variability of measurements between *C. longicauda* and all other recorded cercariae makes any comparison and identification to species level based on cercarial morphology inconclusive.

Trichobilharzia *in* Aythya novaeseelandia – *a new species?*

The morphological differences noted between the adult male schistosome recovered from the New Zealand scaup and all others identified in the literature as well as the distinct size and shape of the eggs suggest that this parasite has not previously been formally identified. Further work is needed to identify this species.

https://doi.org/10.1079/JOH2005327 Published online by Cambridge University Press

Acknowledgements

I wish to thank Annette Davis, Mr Cliff Halford, Mr Graeme Hughes, Mr Herbie Illingworth, Mr Ken Miller, Mr Gerald Stokes, Dr David Wharton, Dr Stuart Mitchell, Dr Robert Poulin and Mr Arthur Scaife for their support and assistance. Correspondence was entered into with Professors Basch, Loker and Snyder. All were encouraging and helpful. A special tribute is here made to the late Dr Ken Mott, World Health Organization, who communicated his interest in and enthusiasm for this research in a manner that was inspirational and encouraging. He passed away while in the process of establishing a web site and compiling a comprehensive review of cercarial dermatitis.

References

Anderson, R.M., Mercer, J.G., Wilson, R.A. & Carter, N.P. (1982) Transmission of *Schistosoma mansoni* from man to snail: experimental studies of miracidial survival and infectivity in relation to larval age, water temperature, host size and host age. *Parasitology* **85**, 339–360.

Appleton, C.C. (1986) Occurrence of avian Schistosomatidae (Trematoda) in South African birds as determined by faecal survey. *South African Journal of Zoology* **21**, 60–67.

Basch, P.F. (1966) The life cycle of *Trichobilharzia brevis* n. sp., an avian schistosome from Malaya. *Zeitschrift für Parasitenkunde* **27**, 242–251.

Bayer, F.A.H. (1954) Larval trematodes in some freshwater snails: a suggested biological method of bilharzia control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **48**, 414–418.

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

Bourns, T.K.R., Ellis, J.C. & Rau, M.E. (1973) Migration and development of *Trichobilharzia ocellata* (Trematoda: Schistosomatidae) in its duck hosts. *Canadian Journal of Zoology* **51**, 1021–1030.

Brackett, S. (1941) Five new species of avian schistosomes from Wisconsin and Michigan with the life cycle of *Gigantobilharzia gyrauli* (Brackett, 1940). *Journal of Parasitology* **27**, 25–39.

Climo, F.M. & Pullen, N.B. (1972) A taxonomic review of the family Lymnaeidae (Mollusca: Gastropoda) in New Zealand. *Journal of the Royal Society of New Zealand* **2**, 5–13.

Cort, W.W. (1928) Schistosome dermatitis in the United States (Michigan). *Journal of the American Medical Association* **90**, 1027–1029.

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

Davis, N.E. (2000) Cercarial dermatitis and the possibility of biological control in Lake Wanaka, New Zealand. Thesis submitted for the degree of Doctor of Philosophy at the University of Otago Dunedin, New Zealand. Unpublished.

de Gentile, L., Picot, H., Bourdeau, P., Bardet, R., Kerjan, A., Piriou, M., Le Guennic, A., Bayssade-Dufour, C., Chabasse, D. & Mott, K.E. (1996) La dermatite cercarienne en Europe: un probleme de sante publique nouveau? *Bulletin de l'Organisation de la Sante* **74**, 159–163.

Dell, R.K. (1956) The fresh-water Mollusca of New Zealand. Parts II and III. Part II.- The species previously assigned to the genera *Lymnaea* and *Myxas*. *Transactions of the Royal Society of New Zealand* **84**, 71–90.

DuBois, G. (1929) Les cercaires de la région de Neuchatel. *Bulletin de la Societé Neuchateloise des Sciences Naturelles* **53**, 3–177.

Eklu-Natey, D.T., Al-Khudri, M., Gauthey, D., Dubois, J.P., Wuest, J., Vaucher, C. & Huggel, H. (1985) Epidemiology of swimmers' itch and morphology of *Trichobilharzia ocellata* in the lake of Geneva Switzerland. *Revue Suisse de Zoologie* **92**, 939–954.

Fain, A. (1955) Recherches Sur les Schistosomes d'oiseaux au Ruanda-Urundi (Congo belge). Découverte d'une nouvelle bilharziose aviaire: la trichobilharziose nasale, et description de schistosomes nouveaux. Note préliminaire. *Revue de Zoologie et Botanique Africaine* **51**, 373–387.

Farley, J. (1971) A review of the family Schistosomatidae: excluding the genus *Schistosoma* from mammals. *Journal of Helminthology* **4**, 289–320.

Featherston, D.W. & McDonald, T.G. (1988) Schistosome dermatitis in Lake Wanaka: survey of the snail population, 1976–77. *New Zealand Journal of Zoology* **15**, 439–442.

Feiler, W. & Haas, W. (1988) *Trichobilharzia ocellata*: chemical stimuli of duck skin for cercarial attachment. *Parasitology* **96**, 507–517.

Guth, B.D., Blankespoor, H.D., Reimink, R.L. & Johnson, W.C. (1979) Prevalence of dermatitis-producing schistosomes in natural bird populations of lower Michigan. *Proceedings of the Helminthological Society of Washington* **46**, 58–63.

Horak, P. & Kolarova, L. (2000) Survival of bird schistosomes in mammalian lungs. *International Journal for Parasitology* **30**, 65–68.

Islam, K.S. (1986) Development of *Trichobilharzia australis* Blair & Islam, 1983 in the snail, *Lymnaea lessoni* Deshayes and in an experimental definitive host, the Muscovy duck. *Journal of Helminthology* **60**, 301–306.

Islam, K.S. & Copeman, D.B. (1986) The morphology and life-cycle of *Trichobilharzia parocellata* (Johnston & Simpson, 1939) from the visceral blood vessels of Australian anatids. *Systematic Parasitology* **8**, 39–50.

Jarcho, S. & Van Burkalow, A. (1952) A geographical study of 'swimmers' itch in the United States and Canada. *Geographical Review* **42**, 212–226.

Kalbe, M., Haberl, B. & Haas, W. (1997) Miracidial host-finding in *Fasciola hepatica* and *Trichobilharzia ocellata* is stimulated by species-specific glycoconjugates released from the host snails. *Parasitological Research* **83**, 806–812.

Loken, B.R., Spencer, C.N. & Granath, W.O. Jr. (1995) Prevalence and transmission of cercariae causing schistosome dermatitis in Flathead Lake, Montana. *Journal of Parasitology* **81**, 646–649.

MacFarlane, W.V. (1944) Schistosome dermatitis in the Southern Lakes. An investigation of Swimmer's Itch. *New Zealand Medical Journal* **43**, 136.

MacFarlane, W.V. (1949) Schistosome dermatitis in New Zealand. Part 1. The parasite. *American Journal of Hygiene* **50**, 143–151.

McDonald, M.E. (1981) *Key to trematodes reported in waterfowl*. Resource Publication 142, Washington, DC, United States Department of the Interior, Fish and Wildlife Service.

McMullen, D.B. & Beaver, P.C. (1945) Studies of schistosome dermatitis. IX. The life cycles of three dermatitis-producing schistosomes from birds and a discussion of the sub-family Bilharziellinae (Trematoda: Schistosomatidae). *American Journal of Hygiene* **42**, 128–154.

Meuleman, E.A., Huyer, A.R. & Mooij, J.H. (1984) Maintenance of the life cycle of *Trichobilharzia ocellata* via the duck *Anas platyrhynchos* and the pond snail *Lymnaea stagnalis*. *Netherlands Journal of Zoology* **34**, 414–417.

Miller, Jr., H.M. (1923) Notes on some furcocercarious larval trematodes. *Journal of Parasitology* **10**, 35–46.

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

Mulvihill, C.A. & Burnett, J.W. (1990) Swimmer's Itch: a cercarial dermatitis. *Cutis* **46**, 211–213.

Prah, S.K. & James, C. (1977) The influence of physical factors on the survival and infectivity of miracidia of *Schistosoma mansoni* and *S. haematobium* I. Effect of temperature and ultra-violet light. *Journal of Helminthology* **51**, 73–85.

Rind, S. (1980) The biology of 'Wanaka Itch' – a report of work in progress. pp. 40–44 *in* Robertson, B.T. & Blair, I.D. (*Eds*) *The resources of Lake Wanaka*. New Zealand, Tussock Grasslands and Mountain Land Institute, Lincoln College.

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.

Talbot, S.B. (1936) Studies on schistosome dermatitis. II Morphological and life history studies on three dermatitis-producing schistosome cercariae, *C. elvae* Miller 1923, *C. stagnicolae*, n.sp., and *C. physelae*, n.sp. *American Journal of Hygiene* **23**, 372–384.

Vicente, S.F. (1979) Trematodos larvarios y sus moluscos hospedadores en Salamanca. *Revista Iberica de Parasitologia* **39**, 241–250.

Waadu, G.D.B. (1991) *Diplostomum spathaceum* (Rud. 1819): effect of miracidial age and lifespan on miracidial infectivity. *Journal of Helminthology* **65**, 28–30.

(Accepted 23 August 2005)