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A report of *Bilharziella polonica* cercariae in Knowsley Safari, Prescot, United Kingdom, with notes on other trematodes implicated in human cercarial dermatitis

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Abstract

As part of surveillance of snail-borne trematodiasis in Knowsley Safari (KS), Prescot, United Kingdom, a collection was made in July 2021 of various planorbid ($n = 173$) and lymnaeid ($n = 218$) snails. These were taken from 15 purposely selected freshwater habitats. In the laboratory emergent trematode cercariae, often from single snails, were identified by morphology with a sub-set, of those most accessible, later characterized by cytochrome oxidase subunit 1 (*cox*1) DNA barcoding. Two schistosomatid cercariae were of special note in the context of human cercarial dermatitis (HCD), *Bilharziella polonica* emergent from *Planorbarius corneus* and *Trichobilharzia* spp. emergent from *Ampullacaena balthica*. The former schistosomatid was last reported in the United Kingdom over 50 years ago. From *cox*1 analyses, the latter likely consisted of two taxa, *Trichobilharzia anseri*, a first report in the United Kingdom, and a hitherto unnamed genetic lineage having some affiliation with *Trichobilharzia longicauda*. The chronobiology of emergent cercariae from *P. corneus* was assessed, with the vertical swimming rate of *B. polonica* measured. We provide a brief risk appraisal of HCD for public activities typically undertaken within KS educational and recreational programmes.

Introduction

Human cercarial dermatitis (HCD), or swimmer's itch, is a nuisance disease in those who have contact with certain freshwater habitats. Within the British Isles, it remains unclear whether the avian schistosome *Bilharziella polonica* (Trematoda: Schistosomatidae) is a prominent cause (Fraser *et al.*, 2009; Morley, 2009). First detected in the United Kingdom as emergent cercariae from the great ram's horn snail, *Planorbarius corneus*, in Roath Park Lake, Cardiff by Iles (1959), its last formal report was by Khan (1961), again as emergent cercariae from *P. corneus* from Lake Meadows Park, Billericay. Other avian schistosomatids, particularly those within the genus *Trichobilharzia*, are assumed to be primarily responsible for HCD in the United Kingdom (Lawton *et al.*, 2014), across Europe (Soldánová *et al.*, 2013; Żbikowska & Marszewska, 2018; Juhász *et al.*, 2022) and elsewhere (Loker *et al.*, 2022), overshadowing any *B. polonica* incrimination(s). From a phylogenetic perspective, *B. polonica* is basal to a diverse clade of avian schistosomatids that includes the species-rich genus *Trichobilharzia*; the latter genus continues to expand upon increased populational sampling as novel genetic variants are encountered (Loker *et al.*, 2022). Of note, Prüter *et al.* (2017) found adult worms of *B. polonica* within the central nervous system of naturally infected mallards, raising a first concern of its underappreciated neurotropic behaviour.

Knowsley Safari (KS), Prescot is a 550-acre reserve with both drive-through and walk-through visitor areas, including a small lake occasionally used for recreational boating. Home to over 700 exotic animals, KS receives approximately 600,000 visitors a year (see fig. 1). KS visitors are also encouraged to engage with native species through activities such as mini-beast hunts and pond dipping activities as organized by KS' research and conservation and education teams, as well as enjoy rowing boat activities within their small recreational lake. In light of growing concerns about HCD in the United Kingdom, such water-contact activities might not be so benign (Fraser *et al.*, 2009; Lawton *et al.*, 2014). During July 2021, as part of ongoing surveillance for snail-borne diseases within KS, a collection was made of various freshwater snails at known water contact points, some of which were within public access areas. In the laboratory, emergent cercariae were studied from a selection of inspected snails. We draw attention upon our observations of *B. polonica* and *Trichobilharzia* spp. and its context of future risk management of HCD in KS.

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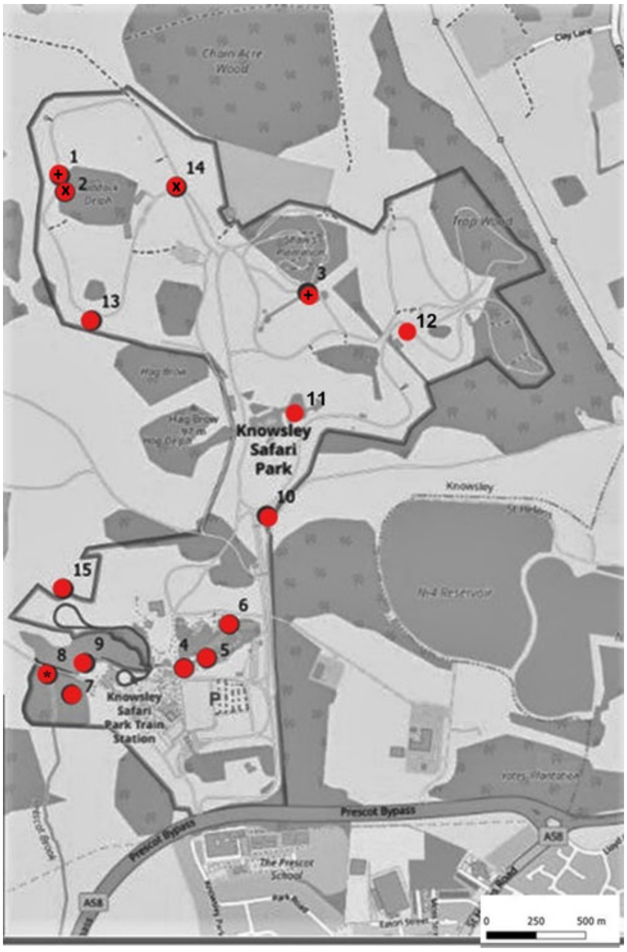

Fig. 1. The 15 sample sites, red circles, for the malacological survey performed at Knowsley Safari. The sites were selected upon discussions with the chief veterinarian and enclosure staff for water bodies that had known animal or human water contact. Cercariae of *Bilharziella polonica* were found at site 8 (denoted by *) within a public area. Cercariae of *Trichobilharzia* spp. at sites 1 and 3 (denoted by **+**) within a non-public enclosure. Sites 2 and 14 (denoted by X) contain *Galba truncatula*, the keystone intermediate snail host for *Fasciola hepatica*, within a non-public enclosure.

Material and methods

Collection and morphological description of snails and emergent trematode cercariae

Over three consecutive days in July 2021, a total 173 Planorbidae and 218 Lymnaeidae snails were collected from 15 sample sites at KS, north-west England (see fig. 1).

The sampling sites were purposefully chosen, upon discussions with KS veterinarians, to be representative of both public access areas and restricted staff access areas in specific animal enclosures. Snails were collected by hand and metal scoops, typically with 15–20 min of active searching by four people. Collected snails were identified through shell morphology according to the taxonomic keys of Rowson *et al.* (2021) which adopt the now favoured multi-generic and classification revision of United Kingdom lymnaeids, for example, *Ampullacaena balthica*.

Once in the laboratory, snails were grouped by species for an initial inspection for shedding cercariae in plastic beakers containing bottled drinking water with exposure to bright natural light. If cercariae were seen, snails were then separated individually into 6-well tissue culture plates and the next day were exposed to natural light between 10.00 and 16.00 to encourage the cercarial emergence. Cercariae were harvested by pipette, then observed under a light microscope, with either Lugol's iodine or Fuchsin staining. Emergent cercariae were identified, as far as possible to species-level, using keys from Combes *et al.* (1980), Frandsen & Christensen (1984) and Podhorský *et al.* (2009).

A selection of emergent cercariae were individually placed on Whatman FTA indicator cards for short-term storage (Whatman plc, Maidstone, UK) preceding total DNA extraction (see below). Collected snails were then crushed between two glass slides under a dissecting microscope to visualize any trematode sporocysts and rediae (parthenites) to estimate pre-patent prevalence of infection (s), although their species identity was presumed upon morphology of emergent cercariae.

Molecular identification of emergent cercariae

Genomic DNA was extracted from FTA punches using published protocols (Gower *et al.*, 2007), containing morphologically identified *B. polonica* cercariae from *P. corneus* and *Trichobilharzia* spp. cercariae from *Ampullacaena balthica*. The 340 base pair ASMIT region of the mitochondrial cytochrome oxidase 1 gene (*cox*1) was amplified with primers ASMIT 1 (5′ TTTTTTGGGC ATCCTGAGGTTTAT 3′) and ASMIT 2 (5′ TAATGCATMGGA AAAAAACA3′) (see Bowles *et al.*, 1992). Reactions were in 25 µL comprising 3 µl of extracted genomic DNA (∼100 ng), 1.5 µl of MyTaq red mix (Meridian Biosciences), 1 µl of each primer (5 pmols/µl) and 7.5 µl of double-distilled water. The thermal profile consisted of an initial denaturation step of 95°C for 1 min, followed by 40 cycles of 95°C for 15 s; 45°C for 30 s; and 72°C for 30 s. The polymerase chain reaction (PCR) was then finished with a terminal extension at 72°C for 2 min, then reactions stored at 4°C before electrophoresis. To check amplifications, products were separated in 2.0% agarose gels in Tris-Acetate-EDTA buffer gel, stained with SYBR™ safe. If suitable, reaction products were purified with Exo-SAP-IT (Applied Biosystems), and sent for Sanger sequencing at Source BioScience Sequencing, UK, using both forward and reverse ASMIT primers (Stothard *et al.*, 2009).

Analysis of cox1 sequences

Retrieved partial *cox*1 sequences were assembled using MEGA 11 (Kumar *et al.*, 2018), with base-calls corrected manually before submission to GenBank (Accession Numbers: ON987329-ON987334). A selection of comparable *cox*1 sequences were downloaded from GenBank. Nucleotide sequences were aligned with the software CLUSTAL W (Thompson *et al.* 1994). Maximum likelihood (ML) was performed – the analysis involved 36 nucleotide sequences. There was a total of 357 positions in the final dataset. The dataset was tested using MEGA 11 for the nucleotide substitution model of best fit – the Tamura–Nei substitution model was chosen by the Akaike information criterion. Bootstrap values based on 1000 re-sampled datasets were generated. The phylogenetic tree presented visualized using the tree explorer of MEGA 11 under the $TN93 + G + I$ model to give an outline appraisal of schistosomatid species identity and diversity. *Heterobilharzia americana* (MW425690) was chosen as an outgroup.

Chronobiology, swimming behaviour and vertical swimming rate (VSR) of cercariae

Planorbid snails found to be shedding cercariae were individually monitored further over a 24-h period on three separate days.

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Briefly, every two hours the infected snail was moved into a new well within the 6-well tissue culture tray. Water within each well was later harvested by syringe then filtered across a 10 µm nylon mesh filter and stained with Lugol's iodine to enumerate cercariae under a dissection microscope (×10).

The VSR for *B. polonica* cercariae was estimated following Morley (2020) whereby the phototaxis of swimming cercariae towards a light source is measured. A 30 ml clear glass specimen tube was surrounded by a dark card, apart from a small longitudinal viewing port slit. This slit enabled visualization of a white 4 mm card. Light was firstly shone from above or below the tube using a handheld torch for 30 s, thereby aggregating cercariae either at the top or bottom of the water column. After this the light was moved to the opposing side, causing positive phototaxis. Using a hand-lens, individual cercariae were measured for the time taken to cross the 4 mm white card, permitting a VSR in mms^{-1}. A box plot was presented of VSR in either descending or ascending swimming directions.

Results

Encountered snails with emergent trematode cercariae

Within KS, 15 aquatic locations were purposely selected and then examined in July 2021 (see fig. 1). Freshwater snails were found at nine locations (present at sites: 1, 2, 3, 4, 7, 8, 9, 13 and 14) with six locations devoid of snails (absent at sites: 5, 6, 10, 11, 12 and 15). Site 8 contained greatest snail species diversity. A total 391 freshwater snails were collected representative of planorbids ($n = 173$) and lymnaeids ($n = 218$) (see tables 1 and 2).

Four planorbid species, *Anisus vortex* ($n = 113$), *Planorbarius corneus* ($n = 35$), *Planorbis planorbis* ($n = 23$), *Gyraulus albus* ($n = 2$), and two lymnaeid species, *Ampullaceana balthica* ($n = 202$) and *Galba truncatula* ($n = 16$), were encountered. Across the 15 sites sampled, *P. corneus* and *A. balthica* were found at two and five sites, respectively, both in public access and in restricted access animal enclosure areas (tables 1 and 2).

Of the 173 planorbids inspected, 46 (26%) were infected with a larval trematode stage. Even though *P. corneus* was collected from two almost adjacent sites, infected snails were encountered in one location alone, site 8. This site presented with the widest diversity of snail and cercariae of at least three different species, noting echinostomes in both planorbids and lymnaeids (tables 1 and 2). Of the 218 lymnaeids inspected, 87 (40%) were infected with a larval trematode stage (table 2). Only one *P. corneus* was found to be actively shedding *B. polonica* (fig. 2.), which also enabled a comparative chronobiological investigation against *Cotylurus* sp., as being shed from another *P. corneus*. Several *A. balthica* were noted to shed *Trichobilharzia* spp. cercariae at sites 1 and 3.

Identification of emergent cercariae with cox1 sequences

A total of six partial *cox*1 were obtained for cercariae of *B. polonica* (BP1-3) and *Trichobilharzia* spp. (TB1-3), with a cursory ML analysis shown in fig. 3. While there was minimal sequence variation within *B. polonica* samples, the *Trichobilharzia* clearly represented two taxa upon their phylogenetic positions, TB1 viz. *Trichobilharzia anseri* and TB 2 and 3, an unnamed lineage with some affiliation with *Trichobilharzia longicauda* and *Tricholbilharzia physella* and *Allobilharzia visceralis*.

Chronobiology and VSR of trematode cercariae

The chronobiology of shedding cercariae from *P. corneus* was monitored over 24 h on three separate days taking advantage of two shedding snails with different emergent cercariae. The cercariae of *B. polonica* exhibited a rising trend from late afternoon through

Table 1. List of planorbid snails with emergent cercariae within Knowsley Safari.

Sample site	Planorbid species (number of snails)	Trematode species (number of infected snails)[a]
1. (N53.44911°, W2.81818°)	*Gyraulus albus* (2)	nil
2. (N53.44872°, W2.81798°)	nil	nil
3. (N53.44630°, W2.80820°)	nil	nil
4. (N53.43728°, W2.81318°)	nil	nil
5. (N53.43752°, W2.81226°)	nil	nil
6. (N53.43833°, W2.81135°)	nil	nil
7. (N53.43963°, W2.81723°)	*Planorbarius corneus* (8)	nil
8. (N53.43713°, W2.81867°)	*P. corneus* (29) *Planorbis planorbis* (23) *Anisus vortex* (110)	*Bilharziella polonica* (1) *Cotylurus* sp. (1) *Echinostoma* sp. (2) *Diplostomum* sp. (4) brevifurcate sp. (37)
9. (N53.43740°, W2.81716°)	*P. planorbis* (1)	brevifurcate sp. (1)
10. (N53.44094°, W2.80984°)	nil	nil
11. (N53.44328°. W2.80837°)	nil	nil
12. (N53.44530°, W2.80411°)	nil	nil
13. (N53.44563°, W2.81690°)	nil	nil
14. (N53.44884°, W2.81348°)	nil	nil
15. (N53.43918°, W2.81804°)	nil	nil

[a]estimation of infections takes into account data from snail crushing, though it is an imprecise identification method.

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Table 2. List of lymnaeid snails with emergent cercariae within Knowsley Safari.

Sample site	Lymnaeid species (number of snails)	Trematode species (number of infected snails) *
1. (N53.44911°, W2.81818°)	*Ampullaceana balthica* (90)	*Echinostoma* sp. (20) *Trichobilharzia* sp. (1)
2. (N53.44872°, W2.81798°)	*Galba truncatula* (3)	nil
3. (N53.44630°, W2.80820°)	*A. balthica* (80)	*Echinostoma* sp. (45) *Trichobilharzia* sp. (5)
4. (N53.43728°, W2.81318°)	*A. balthica* (18)	*Echinostoma* sp. (2)
5. (N53.43752°, W2.81226°)	nil	nil
6. (N53.43833°, W2.81135°)	nil	nil
7. (N53.43963°, W2.81723°)	nil	nil
8. (N53.43713°, W2.81867°)	*A. balthica* (1)	*Echinostoma* sp. (1)
9. (N53.43740°, W2.81716°)	nil	nil
10. (N53.44094°, W2.80984°)	nil	nil
11. (N53.44328°, W2.80837°)	nil	nil
12. (N53.44530°, W2.80411°)	nil	nil
13. (N53.44563°, W2.81690°)	*A. balthica* (13)	*Echinostoma* sp. (1)
14. (N53.44884°, W2.81348°)	*G. truncatula* (13)	*Echinostoma* sp. (12)
15. (N53.43918°, W2.81804°)	nil	nil

[a]estimation of infections takes into account data from snail crushing, though it is an imprecise identification method.

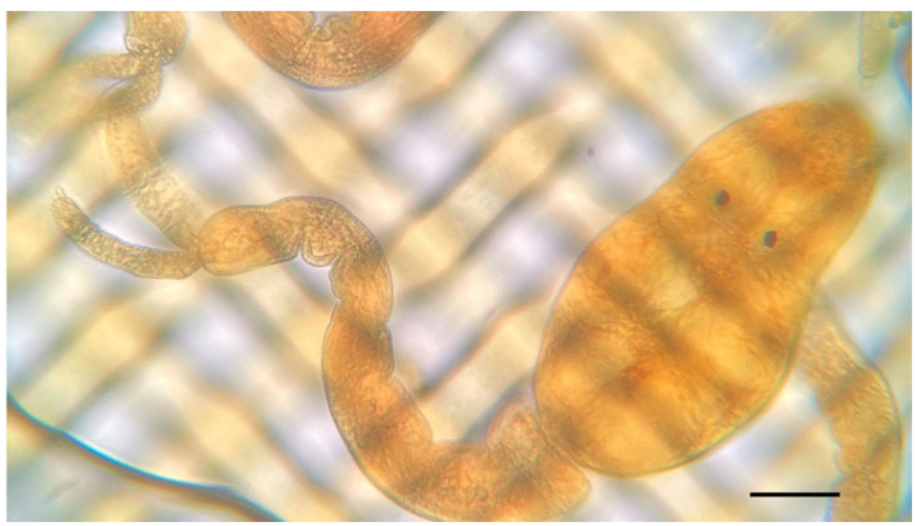

Fig. 2. Cercariae of *Bilharziella polonica*stained with Lugol's iodine. Scale bars = 50 µm.

to evening, peaking between 18:00 and 22:00. By contrast, *Cotylurus* sp. cercariae exhibited an earlier afternoon rise around 16:00, with numbers also consistently higher during afternoons. In all, the average number of *B. polonica* cercariae that were shed was 1142 cercariae per day as compared with an average of 872 cercariae for *Cotylurus* sp. The phototaxis response of *B. polonica* was manipulated to determine the VSR in both ascending or descending orientations, with a significantly faster downwards than upwards trending velocity (t-test, $t = -5.05$, df = 29.80, $P = 0.000023$) (see fig. 5).

Discussion

Collecting freshwater snails with inspection for emergent cercariae provides an effective first way to assess and alert for snail-borne trematodiases. For example, increased surveillance for HCD is sensible owing to its putative re-emergence due to ongoing climate change (Horák & Kolářová, 2011; Horák *et al.*, 2015; Lashaki *et al.*, 2020). While avian schistosomes have no doubt been present across the British Isles for decades (Morley, 2009), it is only recently that HCD has been flagged as of sporadic medical concern in the United Kingdom (Fraser *et al.*, 2009; Lawton *et al.*, 2014). In KS while there have been no formal reports of HCD, either in KS visitors or in staff, our snail–trematode surveys have revealed an underlying new risk to consider upon encounter of these three avian schistosome species.

Even though eDNA assays are having some application in monitoring HCD in environmental water bodies (Jothikumar *et al.*, 2015), such real-time PCR assays are currently only *Trichobilharzia*-specific. Less common avian-schistosomes, such as *B. polonica*, are overlooked as existing primers' combinations

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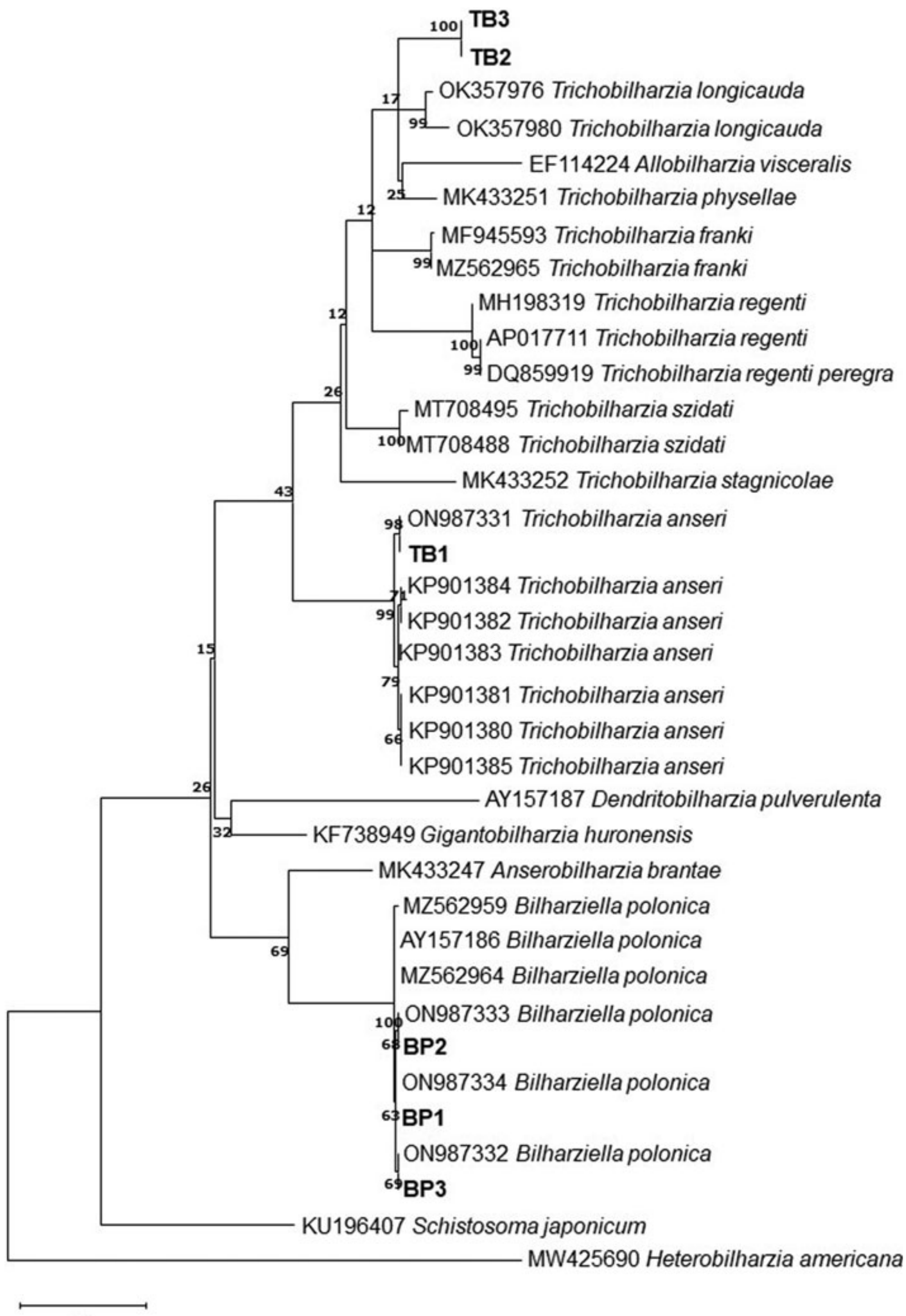

Fig. 3. Maximum likelihood tree based on partial cytochrome oxidase 1 gene sequences of *Bilharziella* (BP1–3) and *Trichobilharzia* (TB1–3) cercariae samples from the present study in relation to other schistostomatid sequences deposited in GenBank. Bootstrap values are given at the nodes. Samples from the present study are in boldface type. The scale bar indicates the expected number of substitutions per site.

do not target its eDNA. By contrast, our combination of more traditional snail and cercarial surveillance, as augmented by *cox*1 barcoding, has revealed the unexpected presence of this rather rare avian-schistosome. Its last reported presence in the United Kingdom was over 50 years ago, some 175 km further south, despite the more recent presence and study of avian schistosomatids elsewhere (Brant & Loker, 2009; Horák & Kolářová, 2011). Indeed, relatively little is known about this trematode and its natural history (Loker *et al.*, 2022). For example, a recent molecular epidemiological study from one of the major recreational lakes in Poland has suggested the presence of a cryptic *Bilharziella* sister species, which is not surprising given the general lack of targeted surveillance for this trematode (Stanicka *et al.,* 2021). Our report here hopes to stimulate some further interest and better awareness in the United Kingdom about this rather rare trematode.

In the context of KS, site 8, a small artificial plastic lined pond, is directly beneath the earthen dam of the recreational boating lake. It is sometimes used in pond dipping from the wooden walkway. Of note here, the recreational boating pond is home to several species of duck and goose. While *P. corneus* was not found in the recreational boating lake at the time of survey, this snail appears presently very restricted within KS to only two adjacent sites, each probably connected during flooding. As a likely consequence, *B. polonica* is also geographically very focalized, even down to a single snail, though such an infected snail can release copious numbers of highly motile cercariae within its microhabitat (see figs 4 and 5).

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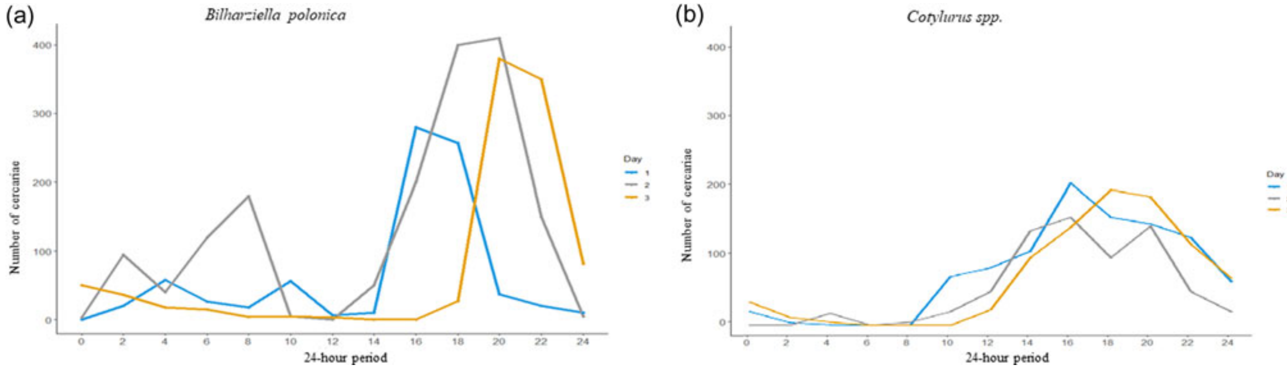

Fig. 4. Chronobiology of daily emergence of *Bilharziella polonica* (**A**) and *Cotylurus* sp. (**B**) cercariae during replicates on three consecutive days from two *Planorbarius corneus*carrying separate infections. A greater number of *B. polonica* were observed during the period of observation with greatest emergence toward early evening.

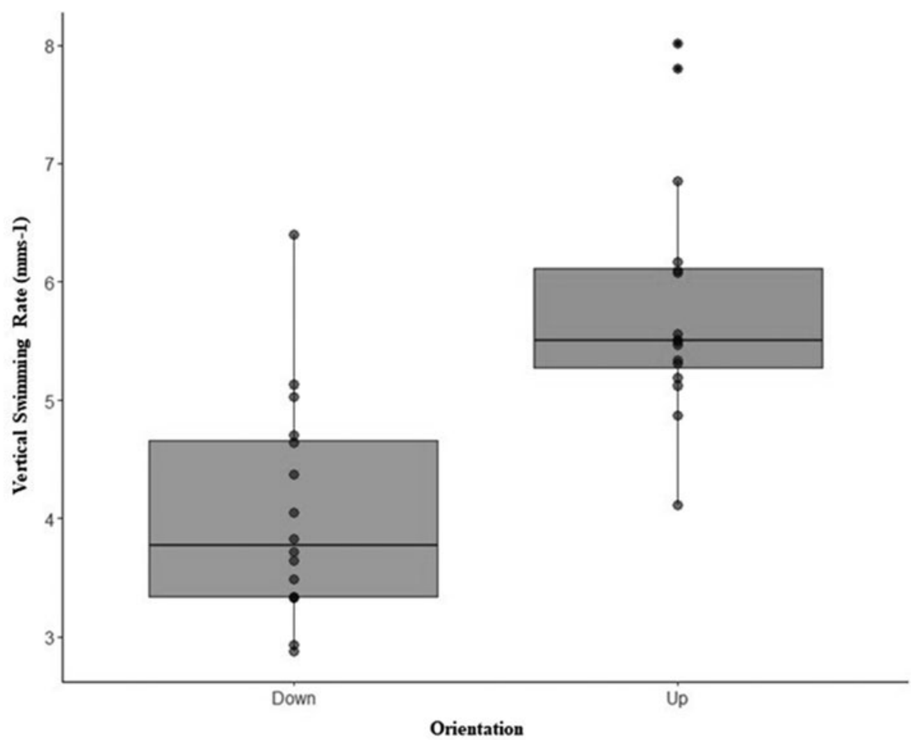

Fig. 5. Comparison of the vertical swimming rate of *Bilharziella polonica* cercariae when swimming either upwards or downwards orientations. There was a statistically significant effect between directions, likely representing a further impact of perhaps gravity or a positive geotaxis.

It is outside the scope of this paper to speculate upon the clinical risk that *B. polonica* might cause, particularly given its recently appreciated neurotropic behaviour (Prüter *et al.*, 2017). A sensible precautionary measure or an appropriate risk management strategy against HCD would be for adults and children to wear protective gloves when pond dipping. This should be alongside targeted introduction of basic educational information to raise awareness of HCD both in visitors and staff. Similarly, any immediate post-exposure application of ethanol-based disinfectant hand gels, now commonly available since the onset of the COVID-19 pandemic, to affected skin should be an efficacious knock-down of any avian schistosome cercariae adhered.

As mentioned, avian-schistosomes of the genus *Trichobilharzia* are more often implicated with HCD in freshwater (Loker *et al.*, 2022). Across the United Kingdom, precise incrimination of which species of *Trichobilharzia* is/are culpable is open to conjecture. For example, *Trichobilharzia franki* was implicated by Lawton *et al.* (2014) upon their inspection of Tundry Pond, Hampshire, but this species was not encountered here. Our *cox*1 analysis of emergent cercariae from *A. balthica* has now implicated two other taxa, *T. anseri*, a first report in the United Kingdom, and a novel genetic lineage with some affiliation with *T. longicauda*, alongside *T. physellae* and *Allobilharzia visceralis* (see fig. 3).

As proposed by Loker *et al.* (2022), with further genetic scrutiny of emergent cercariae additional novel *cox*1 lineages of *Trichobilharzia* will appear. This has also recently occurred with increased *B. polonica cox*1 lineage sampling (Stanicka *et al.*, 2021). Two decades ago, Kolárová *et al.* (1999) described *T. anseri* upon morphological features of cercariae which was then linked with an official description of *T. anseri* (Jouet *et al.*, 2015). Since then, this species has only been noted in Denmark in 2021 (Al-Jubury *et al.*, 2021) and our report here is a first report of *T. anseri* in the United Kingdom. Similarly, our TB2/3 lineage which has some affiliation with *T. longicauda*, is a tentative first United Kingdom report as we await later inspection of adult worms, or eggs thereof, obtained from local birds, for example, ducks and geese. These birds are sometimes available for dissection upon natural or accidental mortalities within KS.

Conclusion

Our snail survey of 15 freshwater habitats has set a contemporary biological baseline for future surveillance of HCD within KS alongside an outline risk appraisal. In total, three avian-schistosome species, as emergent cercariae from two freshwater snail species, were identified within KS park boundaries.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X22000694.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of invertebrates.

Author contributions. Inception and design of the study: BJ, NDW, EJLaC and JRS; snail collection and characterization: AJ, SEJB, HW, SJ, BJ, NDW and JRS; molecular *cox*1 barcoding and analyses: AJ, SEJB, HW, LCC, SJ and JRS; first draft of the paper: AJ, SEJB and HW; and all authors contributed writing, editorial revision and final approval of the manuscript.

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