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Expanding the swimmer's itch pool of the Benelux: a first record of the neurotropic *Trichobilharzia regenti* and potential link to human infection

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Abstract

Background Swimmer's itch, an allergic contact dermatitis caused by avian and mammalian blood flukes, is a parasitic infection affecting people worldwide. In particular, avian blood flukes of the genus *Trichobilharzia* are infamous for their role in swimmer's itch cases. These parasites infect waterfowl as a final host, but incidental infections by cercariae in humans are frequently reported. Upon accidental infections of humans, parasite larvae will be recognized by the immune system and destroyed, leading to painful itchy skin lesions. However, one species, *Trichobilharzia regenti*, can escape this response in experimental animals and reach the spinal cord, causing neuroinflammation. In the last few decades, there has been an increase in case reports across Europe, making it an emerging zoonosis.

Methods Following a reported case of swimmer's itch in Kampenhout in 2022 (Belgium), the transmission site consisting of a private pond and an adjacent creek was investigated through a malacological and parasitological survey.

Results Six snail species were collected, including the widespread *Ampullaceana balthica*, a well-known intermediate host for *Trichobilharzia* parasites. Shedding experiments followed by DNA barcoding revealed a single snail specimen to be infected with *T. regenti*, a new species record for Belgium and by extension the Benelux. Moreover, it is the most compelling case to date of the link between this neurotropic parasite and cercarial dermatitis. Additionally, an Echinostomatidae sp. and *Notocotylus* sp. were isolated from two other specimens of *A. balthica*. However, the lack of reference DNA sequences for these groups in the online repositories prevented genus- and species-level identification, respectively.

Conclusions The presence of *T. regenti* in Belgium might have severe clinical implications and its finding highlights the need for increased vigilance and diagnostic awareness among medical professionals. The lack of species-level identification of the other two parasite species showcases the barcoding void for trematodes. Overall, these findings demonstrate the need for a Belgian framework to rapidly detect and monitor zoonotic outbreaks of trematode parasites within the One Health context.

Keywords Trematode infections, Schistosomatidae, Zoonoses, *Trichobilharzia*, Dermatitis, Snail-borne diseases, Vector-borne diseases, Climate change, DNA barcoding

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Background

Cercarial dermatitis, or swimmer's itch, is caused by mammalian and avian schistosomes (Trematoda: Schistosomatidae) [1]. The most important schistosome species belong to the genus *Schistosoma*, affecting more than 200 million people worldwide, with the largest burden in Sub-Saharan Africa [2]. Nevertheless, cercarial dermatitis outbreaks across the world are rarely attributed to *Schistosoma* spp. [3]. Instead, outbreaks in temperate climate regions, such as the USA or Europe, are usually attributed to species belonging to the closely related *Trichobilharzia* genus [3]. This genus is one of seven genera of bird schistosomes described in Europe, which mostly parasitize ducks, geese, or swans [4, 5].

Bird schistosomes of the genus *Trichobilharzia* are ubiquitous trematode flatworms that infect pulmonate freshwater snails as intermediate host and waterfowl as final hosts (Fig. 1). *Radix* spp. and *Lymnaea stagnalis* Linnaeus, 1758 are most frequently reported as suitable intermediate snail hosts for *Trichobilharzia* species, yet a plethora of other snails have been described to transmit avian schistosomes [3, 4]. The many snail hosts, some with a high abundance and wide distribution, contribute

to the near-global geographic range of bird schistosomes [1]. Adult *Trichobilharzia* parasites inhabit either the visceral venous system or nasal tissue of waterfowl, depending on the species. Firstly, visceral species such as *Trichobilharzia szidati* Neuhaus, 1952 and *T. franki* Müller & Kimmig, 1994 are located in the intestinal and other veins and produce eggs that enter the freshwater environment through feces. Upon contact with fresh water, these eggs hatch into miracidia and start swimming to localize a suitable snail host. Secondly, adults of the nasal species, such as *Trichobilharzia regenti* Horák, Kolářová & Dvořák, 1998 are located in the nasal veins and lay eggs that hatch into miracidia in the nasal tissue, which can leave the final host in search of an intermediate snail host when it feeds or drinks. Upon snail infection, the miracidia will further develop and reproduce asexually, after which the cercarial stage will leave the snail. Hundreds to thousands of cercariae are released on a daily basis [6, 7], which in turn infect the final bird host, where they can develop into adults and start egg production. However, when encountering a human, the cercariae attach to the skin and preferentially attempt to penetrate through wrinkles or hair follicles [1]. Cercarial dermatitis arises

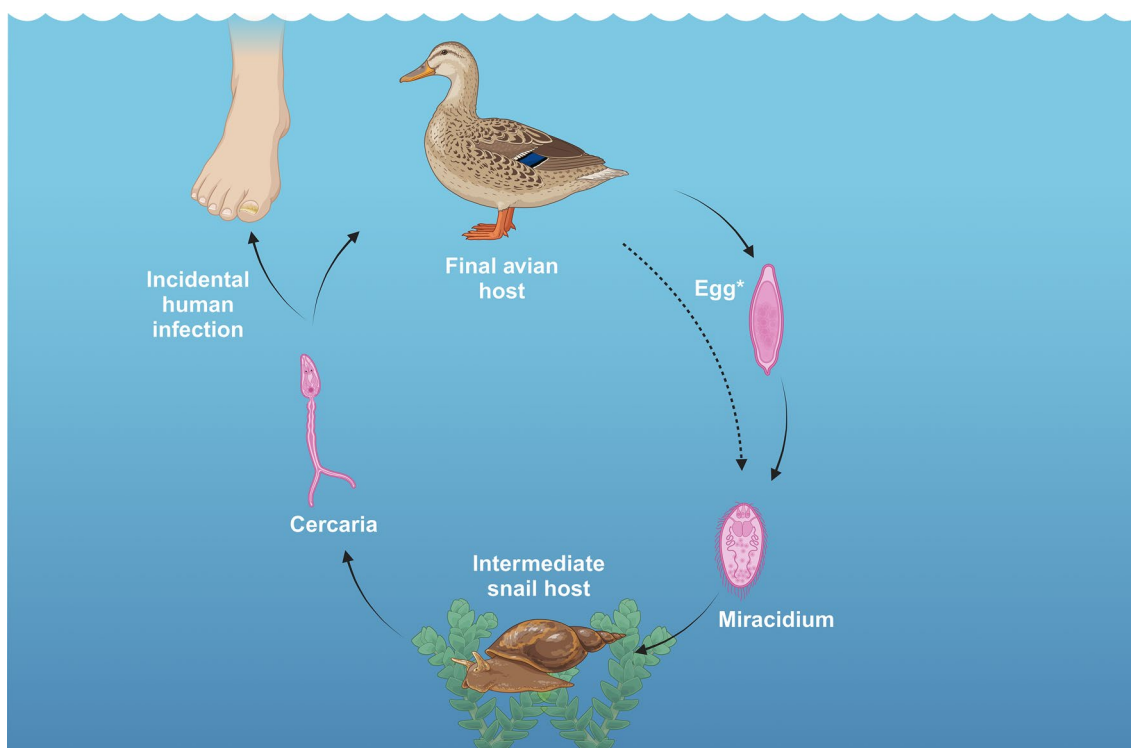


Fig. 1 The *Trichobilharzia* spp. lifecycle. The lifecycle usually involves pulmonate freshwater snails as intermediate hosts and waterfowl as final hosts. Human infections occur when cercariae incidentally penetrate human skin during water-bound activities, potentially causing swimmer's itch. *The eggs of nasal *Trichobilharzia*, such as *Trichobilharzia regenti*, already hatch into miracidia in the nasal tissue of the final host, leaving the nasal cavity while the host drinks or feeds (dotted line). This is in contrast to the lifecycle of visceral *Trichobilharzia*, whereby miracidia emerge from eggs following contact with water. Created with BioRender.com

due to an immune response that worsens with repeated exposure, effectively blocking further migration into the human body [1, 8]. However, upon first exposure of immunocompetent or in primary and challenge infections in immunodeficient murine model systems, some schistosomula survive and manage to migrate further in the body [9, 10]. The inability to stop these parasites from migrating throughout the mouse's body is especially impactful for the neurotropic *T. regenti*, whereby 10% of the infecting larvae may reach the peripheral nervous system [9, 10].

Swimmer's itch is considered an emerging cosmopolitan condition affecting human health and leading to economic losses due to the closing of recreational areas [4, 11, 12]. Soldánová et al. [4] review a broad range of ecological factors that can drastically affect swimmer's itch incidence. Increased nutrient concentrations boost snail abundance, snail growth, and bird visits, and consequently increase the trematode parasite biomass. In addition, higher temperatures, such as summer heatwaves, are often linked to cercarial dermatitis outbreaks, as swimming time, parasite development, and cercarial emergence all increase. Coinfections may also affect swimmer's itch incidence as intramolluscan competition can drive trematode community composition [4, 13, 14].

The genus *Trichobilharzia* consists of more than 35 species [15], and two have been linked to outbreaks of cercarial dermatitis across Europe: the visceral *T. szidati* and *T. franki* [8, 16–18]. Five additional *Trichobilharzia* species have been identified as potential agents of cercarial dermatitis in Europe: *T. physellae* Talbot, 1936 [19], *T. mergi* Kolářová, Skírnisson, Ferté, and Jouet, 2013 [20], *T. anseri* Jouet, Kolářová, Patrelle, Ferté, and Skírnisson, 2015 [21], *T. salmanticensis* Simon-Vicente and Simon-Martin, 1999 [22], and *T. regenti* [23]. The first scientifically recorded outbreak of swimmer's itch in Belgium occurred in 2012 and was attributed to *T. franki* infections [16]. The present study focuses on a private pond located in central Belgium following complaints of extremely painful and itchy skin lesions after swimming on the weekend of 17 June 2022. We set out to (1) map snail and trematode diversity within the respective water systems and (2) ascertain the possible cause of the reported swimmer's itch.

Methods

Snail collection

Snails were collected on 29 June 2022, 11 days after the onset of swimmer's itch symptoms, from two sites in the Flemish part of Belgium (pond coordinates: Lat.: 50.932121, Long.: 4.567706; creek coordinates: Lat.: 50.931657, Long.: 4.567487; Fig. 2) using a scooping net and protective gloves. The sampling was restricted to the

margins of the pond due to the steep bottom inclination: up to two meters from the water's edge. The content of the scooping net, aquatic vegetation, and any substrate were inspected for the presence of freshwater snails. Captured snails were transported alive in containers with water from the sites of origin to the Royal Museum for Central Africa (RMCA) in Tervuren (Belgium) for further analyses.

Shedding experiment

Upon arrival at RMCA, all snails were sorted per morphotype and per site and placed individually in multi-well cell culture plates with water from the site of origin. A subset of the *Lymnaea stagnalis* specimens had to be pooled in a larger container due to their large size and high abundance. Each well was inspected 1-h post-isolation at 4 pm to ascertain afternoon cercarial shedding. If only one cercaria was found, we attributed it to contamination of the source water, but none were found. The snails were then kept in the dark at room temperature until the next day. In the early morning, all plates were visually inspected with a stereo microscope to verify whether nocturnal shedding had taken place. Subsequently, bright artificial light exposure started at 7 am and blinds were opened to allow natural light to illuminate the room further. All wells were inspected at 8 am, 10 am, and 12 pm for cercariae. All snails were sacrificed at 12 pm. Released cercariae were stored in a separate tube with 70% ethanol for optimal preservation. All shedding snails were further stored individually, while uninfected snails were pooled per morphotype per site. All samples were stored in 70% ethanol at +4 °C until further processing.

Morphological identification of snails and larval trematodes (cercariae)

Snails were morphologically identified on the basis of external shell characteristics using the Field Guide to Slugs and Mussels [24]. Isolated cercariae were morphologically classified to cercarial type level on the basis of the identification guide of Frandsen and Christensen [25].

Digitalization

Following the methodology described in Brecko et al. [26], high-resolution photographs were captured for all snail morphotypes using the ZereneR stacker software (T2019-10-07-1410) in a focus stacking system. Subsequently, the resulting images underwent post-processing in Adobe Acrobat Photoshop (version 23.1.1). This involved several modifications, such as eliminating the support structure on which the snail was mounted, merging the forward and reverse orientations into a single image, and including an appropriate scale bar.



Fig. 2 Sampling area in Kampenhout (Flemish Brabant province; Belgium). The dots indicate the location of Kampenhout within Belgium, as well as the creek and pond sites on the second inset. The top right and bottom right pictures illustrate the creek and pond site, respectively

Cercariae isolated from each snail were poured into a watch glass with 70% ethanol and visually inspected with a stereomicroscope for different morphotypes. Four specimens per morphotype per snail were isolated for DNA extraction, one of which was first placed on a microscope glass in sterile milli-Q water with a cover glass. The BMS camera (model no.: XFCAM-1080PHD) and associated BMS_pix3 software (version 3.7.8942.20170412) were then used to photograph each specimen at all focal depths while manually adjusting the specimen's position. The resulting images were stacked with the ZereneR stacker software and post-edited with Adobe Acrobat Photoshop (version 23.1.1)

for the removal of background noise and the addition of an appropriate scale bar.

DNA extraction and Sanger sequencing

DNA was extracted from the internal organs of the snails (excluding the head and foot) with the E.Z.N.A. Mollusc DNA Kit (OMEGA bio-tek, Inc.), according to the manufacturer's guidelines and following Schols et al. [27], with an elution volume of two times 75 μ l. DNA was extracted from the entire individual cercaria using a lysis buffer containing proteinase *K* following the methodology outlined in Zietara et al. [28], resulting in a final volume of 20 μ l. The remaining soft tissue parts (head and

foot) of the snail specimens together with their respective shells and dried DNA extracts were stored at the Royal Belgian Institute of Natural Sciences (collection codes: RBINS:IG34767:INV311000).

Two snail and three trematode genetic markers were amplified using specific primer combinations. Part of the mitochondrial cytochrome *c* oxidase subunit I (COI) [29] for all snails, and of the rDNA region spanning the Internal Transcribed Spacer 2 (ITS2) marker for the *Stagnicola* snails [30, 31], were targeted for species identification using the primer combinations as displayed in Additional file 1: Tables S1, S2. Trematode-specific primers were used for the amplification of partial regions of the COI [32, 33], the 18S ribosomal RNA (18S) [32, 34], and the Internal Transcribed Spacer 2 (ITS2) [33] (Additional file 1: Tables S1, S2).

All amplifications, except for the ITS2 in *Stagnicola* snails, were performed in 20 µL reaction mixtures, including 2 µL of DNA template, 2 µL of 10X buffer, 1.5 mM MgCl₂, 0.2 mM dNTP, 0.4 µM of each primer, and 0.03 units/µL of Platinum™ Taq DNA Polymerase (Invitrogen™, Waltham, MA, USA). The cycling conditions were as displayed in Additional file 1: Tables S1, S2. Furthermore, the ITS2 region in *Stagnicola* sp. specimens was amplified following Schols et al. [35], using the Qiagen™ Taq DNA polymerase kit and the cycling conditions as displayed in Additional file 1: Tables S1, S2. Polymerase chain reaction (PCR) products and negative controls were checked on a 2% agarose gel using a UV transilluminator and the MidoriGreen™ Direct (NIPPON Genetics Europe, Dueren, Germany) method. Positive amplifications were subsequently purified using the ExoSAP-IT™ protocol (following manufacturer's instructions) and sequenced in both directions using the BigDye® chemistry (Macrogen™).

Analyses

Assembled bidirectional nucleotide strands were trimmed, corrected, and translated into amino acids to check for stop codons (if coding region) using Geneious Prime® 2019.2.3 (Biomatters Ltd., Auckland, New Zealand). A consensus sequence was generated for each DNA sequence and each specimen. Subsequently, consensus sequences were compared using the BOLD Identification System, with the Species Level Barcode Records option (www.boldsystems.org), and the Basic Local Alignment Search Tool (BLAST) of GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

From the 159 snail specimens collected across the sampling area, six different snail species were identified: *Gyraulus albus* O. F. Müller, 1774; *Planorbarius corneus*

Linnaeus, 1758; *Physella acuta* (Draparnaud, 1805); *Lymnaea stagnalis*, *Ampullaceana balthica* (Linnaeus, 1758); and *Stagnicola fuscus* Pfeiffer, 1821 (Fig. 3). The latter species was only retrieved from the creek site, together with *P. corneus*. The other four species occurred in the main pond (Table 1).

All morphological snail species identifications were validated using COI barcodes, except for *S. fuscus*, for which the investigation of the ITS2 marker was necessary to discriminate pure *S. fuscus* from *S. fuscus* × *S. palustris* hybrids [31] (GenBank pairwise identity ranges displayed in Table 1). ITS2 sequencing was attempted on both COI-barcoded *Stagnicola* specimens, one of which was eventually of sufficient quality for identification. This *Stagnicola* specimen was identified as pure *S. fuscus* on the basis of the ITS2 marker. All generated snail-specific sequences were deposited on GenBank, with accession numbers: COI: PP203049- PP203072; ITS2: PP203073.

Three specimens of *A. balthica* released cercariae during the shedding experiment, each one of them releasing one unique cercarial morphotype (Fig. 4). Details on the BLAST results are listed for all three markers in Table 2. Cercariae of the specimen V_RAD_4 were of the brevifurcate-apharyngeate distome type; the DNA sequences (18S, ITS, and COI) were 99.9–100% identical to GenBank reference sequences of *Trichobilharzia regenti* from the Czech Republic [ITS: 99.9% (OX235267); 18S: 100% (AY157218)] and France [COI: 100% (HM439500)] (Fig. 4A, Table 2). Snail specimen V_RAD_1 showed an infection with monostome type cercariae identified as *Notocotylus* sp. Diesing, 1839 on the basis of all three DNA markers (Fig. 4B, Table 2). For V_RAD_3, echinostome type cercariae were collected. The genetic identification results were ambiguous as the nuclear markers 18S and the ITS region seem to indicate either an *Echinoparyphium recurvatum* (von Linstow, 1873) Lühe, 1909 or an *Echinostoma revolutum* (Fröhlich, 1802) Looss, 1899 infection, while the COI sequence had the highest pairwise identity of 87.8% with *Echinoparyphium* sp., which dropped to 82% for *Echinostoma revolutum* (Table 2). Consequentially, the identification of this species can only be ascertained to the family level, being the Echinostomatidae (Looss, 1899) (Fig. 4C, Table 2). All generated trematode sequences were deposited in GenBank, with accession numbers: COI: PP232100- PP232102; ITS: PP274989- PP274991; 18S: PP232103- PP232105.

Discussion

This study reports on the first record of *Trichobilharzia regenti* in Belgium, while also investigating the local pulmonate freshwater snail and trematode biodiversity in two bodies of water located in Kampenhout (Belgium).

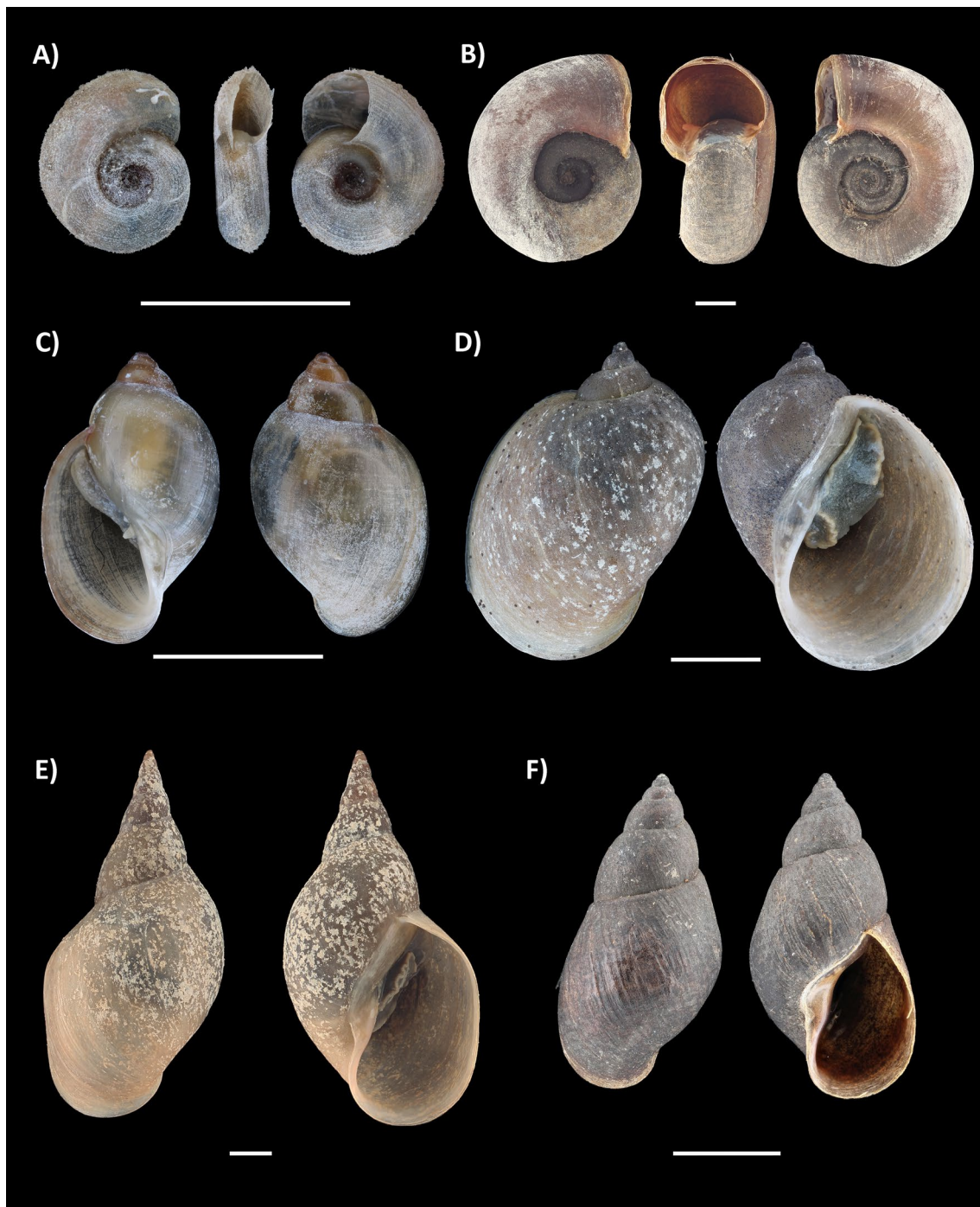


Fig. 3 Different snail species collected at the pond and creek sites in Kampenhout (Flemish Brabant province; Belgium). **A** *Gyraulus albus*, **B** *Planorbarius corneus*, **C** *Physella acuta*, **D** *Ampullaceana balthica*, **E** *Lymnaea stagnalis*, and **F** *Stagnicola fuscus*. Scale bar represents 5 mm in each picture

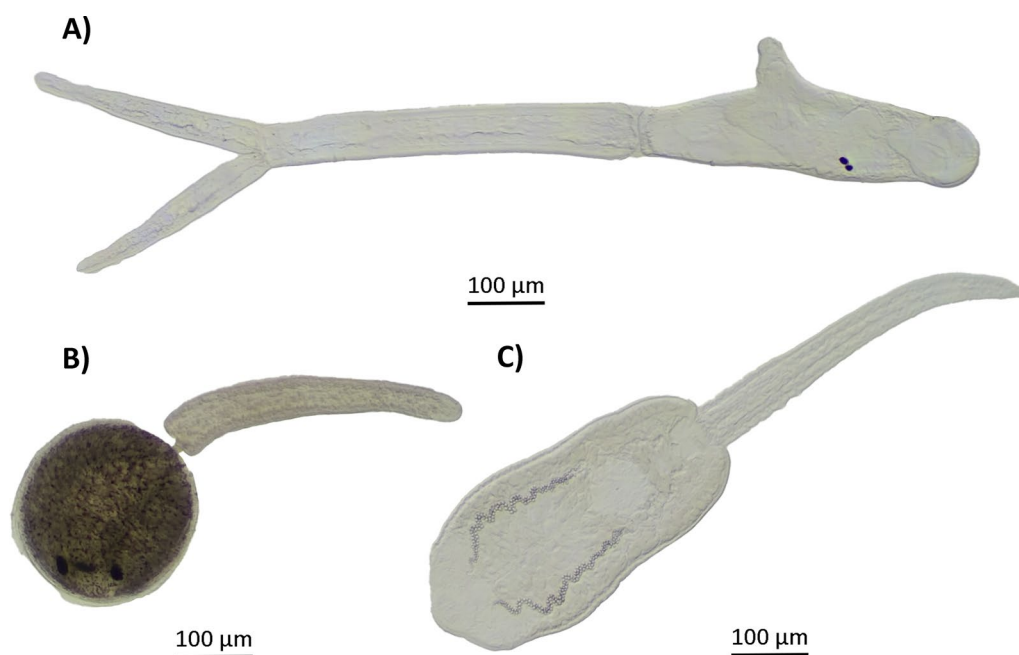
All but one snail species collected in this study are native to Belgium. *Physella acuta* is an invasive snail originating from North America that has been reported in Belgium since 1869 [36]. It is a globally invasive species

with records ranging from Argentina and South Africa to Australia and the Netherlands [37]. No larval trematodes were released from this snail species, which is consistent with the enemy-release hypothesis that describes

Table 1 Snail species and counts for both sampling sites, as well as the GenBank pairwise identity ranges expressed in percentages based on the COI sequences, with an exception for *Stagnicola fuscus*, for which ITS2 was also used

Snail species	Pond site (N)	Creek site (N)	Sequenced specimens (N)	GenBank pairwise identity (accession numbers)
<i>Ampullaceana balthica</i>	11 (3)	0	11	COI: 100–99.8% (MW801451, KP242705)
<i>Lymnaea stagnalis</i>	108	0	4	COI: 100% (ON653340)
<i>Stagnicola fuscus</i>	0	3	2 (COI); 1 (ITS2)	ITS2: 100% (HG931948)
<i>Physella acuta</i>	30	0	2	COI: 100% (OW485645, LC582932)
<i>Planorbium corneum</i>	2	4	4	COI: 100–99.4% (MT862415, AY227370)
<i>Gyraulus albus</i>	1	0	1	COI: 99.4% (KC495835)

The number of snails releasing cercariae is listed in between parentheses

**Fig. 4** High-resolution pictures of cercariae released by three *Ampullaceana balthica* specimens during the shedding experiment. **A** *Trichobilharzia regenti*, **B** *Notocotylus* sp., and **C** Echinostomatidae sp.**Table 2** Cercariae trematode identification results based on the investigation of the nuclear (18S rDNA and ITS region) and mitochondrial (COI) DNA sequences

Trematode sample	18S rDNA	ITS region	COI mtDNA	Species identification conclusion
V_RAD_1	1043 bp—100% (AJ287547)— <i>Notocotylus</i> sp.	1240 bp—97.7% (JQ766940)— <i>Notocotylus malhamensis</i>	419 bp—99.5% (MH369327)— <i>Notocotylus</i> sp.	<i>Notocotylus</i> sp. (Fig. 4B)
V_RAD_3	1050 bp—100% (OP627676)— <i>Echinostoma revolutum</i>	950 bp—100% (OP627676)— <i>Echinostoma revolutum</i> / 100% (AY168931)— <i>Echinoparyphium</i> <i>recurvatum</i>	890 bp—87.8% (MH369225)— <i>Echinoparyphium</i> sp. (82% (OR030109)— <i>Echinostoma</i> <i>revolutum</i>)	Echinostomatidae sp. (Fig. 4C)
V_RAD_4	1040 bp—100% (AY157218)— <i>Trichobilharzia regenti</i>	1070 bp—99.9% (OX235267)— <i>Trichobilharzia regenti</i>	800 bp—100% (HM439500)— <i>Trichobilharzia regenti</i>	<i>Trichobilharzia regenti</i> (Fig. 4A)

The table presents the amplicon length (bp), BLAST pairwise identity (%), and best match together with the GenBank accession number per sample and per sequence. The last column reports the resulting identification on the basis of the compilation of all BLAST outputs

the observation that many exotic species have a lower parasite load outside their native range [37]. In contrast, cercariae of three trematode species were released from the native *A. balthica*. The trematode species of the family Echinostomatidae infect its final vertebrate host through trophic interactions, i.e., when infected secondary intermediate hosts such as snails, fish, or amphibians are consumed by the final host. It has been linked to human infections in Africa, Asia, and Europe, although most case reports stem from Asia due to the routine consumption of raw fish [38]. The second species we encountered, *Notocotylus* sp., belongs to the monostome type cercariae, which rapidly encyst to form metacercariae on nearby substrates [39]. The exact lifecycle of most *Notocotylus* sp. is not fully known but final avian or rodent hosts become infected upon ingestion of these metacercariae during foraging [39]. Finally, the third trematode species was identified as *T. regenti*. At present, *T. regenti* is the second *Trichobilharzia* species reported in Belgium, after the swimmer's itch outbreak caused by *T. franki* in 2012 in the Plate Taille Lake (approximately 90 km south of our sampling location) [16].

Trichobilharzia regenti has already been described from snails and birds in several European countries (See [11, 23, 40–46]), and has been suggested as a potential cause of swimmer's itch. However, until now, *T. regenti* always co-occurred with other agents of swimmer's itch in the studied transmission sites. As a result, it was difficult to determine which of the *Trichobilharzia* species was responsible for the reported dermatitis. Since we only found *T. regenti* in our study, it more strongly supports *T. regenti* as a causative agent of swimmer's itch. *Trichobilharzia regenti* may potentially be the most dangerous cause of swimmer's itch in Europe, especially for immunocompromised people [10, 47]. As one of the nasal avian schistosomes, *T. regenti* feeds on nervous tissue while migrating through the central nervous system, frequently causing leg paralysis in avian and murine models [48, 49]. Experimental infections in mice show that the immune response in the skin can halt 90% of the schistosome, but the remaining 10% can reach the spinal cord [9, 50]. Thus far no human neuropathogenic cases linked to *T. regenti* infections have been reported, but a better understanding among medical professionals about this possibility is desirable [47].

Improved public awareness and a centralized contact point to report such cases could aid in the identification of transmission sites. Suspected transmission areas could then be investigated through targeted malacological and parasitological surveys. The use of an integrative taxonomic approach would lead to improved resolution at the species level [51] and thus more precise diagnosis and possibly also better control measures [52]. At the

same time, this integrative taxonomic approach would map biodiversity and feed the incomplete reference sequence database of trematodes, a grossly neglected group in barcoding databases [53–55]. Given the paucity of information for trematodes, it is not surprising that our results showcase the troublesome barcoding efforts for Belgian representatives, especially since there is little to no monitoring of trematode diversity in the country. Such a barcoding gap is problematic as it inhibits fast and accurate epidemiological studies due to incomplete disease transmission patterns [53]. To do so, a parasite's lifecycle should be fully known, linking intermediate snail hosts to final hosts, a piece of the puzzle still lacking for several *Trichobilharzia* species [3, 55]. Since cercarial traits frequently do not provide taxonomic information beyond the family level, molecular tools are imperative to identify both the snail intermediate host and the parasite infecting them [3, 55]. Ideally, this includes the study of adult parasites from the final bird species. Natural history collections could provide a more sustainable alternative, although curated helminth samples have frequently been stored in formaldehyde, a medium complicating DNA sequencing [27, 56, 57]. There are also protocols and even commercial kits for environmental DNA detection of *Trichobilharzia* spp. in water bodies [58–61], which could complement or replace the rather time-consuming malacological and parasitological surveys.

Historic and current reports underline the effect of human environmental changes on trematodiasis outbreaks [27, 62–65]. We argue that the sudden appearance of *T. regenti* in the study area may be attributable to global change and local environmental change. Global change affects snail–trematode interactions [66], bird migration patterns [67], the introduction of invasive species [68], and snail ranges [69], all potentially influencing swimmer's itch epidemiology. As thoroughly discussed by Soldánová et al. [4] and Mas-Coma et al. [12], climate change can drive the emergence and severity of trematode transmission foci since increased water temperatures result in increased parasite development and cercarial emergence while also increasing human water contact frequency and duration. Water contact intensity has already been linked to increased cercarial dermatitis incidence in the Netherlands [58] and beyond, including the USA [70] and Switzerland [71].

Conclusions

With the projected future temperature increase, swimmer's itch incidence will most likely also continue to increase [3, 4, 11, 12, 72]. We suggest that an improved patient reporting system combined with transmission site localization and monitoring efforts, such as in the Netherlands [36, 73] and Canada [74], will prove invaluable

assets to prepare for potential snail-borne parasite outbreaks in Belgium.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13071-024-06218-4>.

Additional file 1: Table S1. Table reporting the primers involved for the amplification of the five DNA regions (two and three genes within the snails and the trematodes, respectively). **Table S2.** PCR cycling conditions used for the amplification of the five DNA regions. *The elongation time for our protocol was increased to 60 s compared with the 45 s of Schols et al. [37].

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Author contributions

RS conceptualised the study and collected and analysed the data. NS collected and analysed the data. AV collected the data. TH conceptualised the study. All authors reviewed and approved the paper.

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Availability of data and materials

The resulting sequences of the study are deposited on NCBI GenBank, with accession codes PP203049–PP203073; PP232101–PP232105; and PP274989–PP274991. Tissue, shell, and DNA vouchers are stored in the collection of RBINS with accession codes RBINS:IG34767:INV311000.

Declarations

Ethics approval and consent to participate

No ethics approval or consent to participate was required for this study.

Consent for publication

Consent for publication was obtained from the owner of the sampling area.

Competing interests

The authors declare that they have no competing interests.

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