UNIWERSYTET
MIKOŁAJA KOPERNIKA
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WYDZIAŁ NAUK BIOLOGICZNYCH I WETERYNARYJNYCH

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Ptasie schistosomy – zagrożenie świądem pływaków na obszarach kąpieliskowych i biologiczne metody prewencji

Rozprawa na stopień naukowy doktora

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Podziękowania

Pragnę złożyć serdeczne podziękowania mojemu promotorowi Pani prof. dr hab. Elżbiecie Żbikowskiej, bez pomocy której ta praca nigdy by nie powstała. Dziękuję za nieocenioną pomoc udzieloną w trakcie przygotowywania pracy doktorskiej, cierpliwość i wyrozumiałość oraz motywację i inspirację do prowadzenia badań.

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Wykaz publikacji wchodzących w skład rozprawy doktorskiej

KOMENTARZ AUTORSKI

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Wykaz publikacji wchodzących w skład rozprawy doktorskiej

1.	**Marszewska, A.**, Cichy, A., Heese, T., & Żbikowska, E. (2016). The real threat of swimmers' itch in anthropogenic recreational water body of the Polish Lowland. *Parasitology Research*, *115*(8), 3049-3056. Współudział w zaplanowaniu pracy, wykonanie badań laboratoryjnych, wykonanie analiz i interpretacja wyników, przygotowanie publikacji do druku – **Udział 55 %**	**IF – 2,067 MNiSW – 30/70 (rok 2016/2020)**
2.	**Marszewska, A.**, Strzała, T., Cichy, A., Dąbrowska, G. B., & Żbikowska, E. (2018). Agents of swimmer's itch—dangerous minority in the Digenea invasion of Lymnaeidae in water bodies and the first report of *Trichobilharzia regenti* in Poland. *Parasitology Research*, *117*(12), 3695-3704. Współudział w zaplanowaniu pracy, wykonanie badań terenowych i autopsji mięczaków, współudział w badaniach molekularnych, wykonanie analiz i interpretacja wyników, przygotowanie publikacji do druku – **Udział 55 %**	**IF – 2,067 MNiSW – 30/70 (rok 2018/2020)**
3.	**Marszewska, A.**, Cichy, A., Bulantová, J., Horák, P., & Żbikowska, E. (2018). *Potamopyrgus antipodarum* as a potential defender against swimmer's itch in European recreational water bodies—experimental study. *PeerJ*, *6*, e5045. Współudział w zaplanowaniu pracy, współudział w wykonaniu eksperymentów, analiza i interpretacji wyników, przygotowanie publikacji do druku – **Udział 55 %**	**IF – 2,353 MNiSW – 35/100 (rok 2018/2020)**
4.	**Marszewska, A.**, Cichy, A., Bulantová, J., Horák, P., & Żbikowska, E. (2020). The chemotactic swimming behavior of bird schistosome miracidia in the presence of compatible and incompatible snail hosts *PeerJ*, DOI: 10.7717/peerj.9487 Współudział w zaplanowaniu pracy, współudział w wykonaniu eksperymentów, analiza i interpretacja wyników, przygotowanie publikacji do druku – **Udział 55 %**	**IF – 2,353 MNiSW – 35/100 (rok 2018/2020)**

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KOMENTARZ AUTORSKI

1. WPROWADZENIE

Ptasie schistosomy (Digenea: Schistosomatidae) to pasożyty o złożonym, dwużywicielskim cyklu życiowym. Rolę żywicieli ostatecznych pełni ptactwo wodno-błotne, natomiast żywicielami pośrednimi są płucodyszne ślimaki wodne (Horák i in. 2015). Inwazyjne dla kręgowców larwy – cerkarie, aktywnie opuszczają ciało mięczaków i poszukują żywicieli wykorzystując zdolności chemorecepcyjne (Haas i van de Roemer 1998). Zainteresowanie naukowe tą grupą pasożytów wynika nie tylko ze względów czysto poznawczych, ale w dużej mierze z przyczyn natury praktycznej. Ptasie schistosomy stanowią obok typowych czynników etiologicznych tzw. ludzkich schistosomatoz takich jak *Schistosoma haematobium, S. mansoni, S. japonicum* czy *S. mekongi* (Digenea: Schistosomatidae) zagrożenie dla zdrowia ludzi (Neal 2004). Podobieństwo pewnych komponentów lipidowych (m.in. kwasów tłuszczowych, cholesterolu czy ceramidów) pokrycia ciała ptaków i ssaków powoduje, że cerkarie ptasich pasożytów wnikają w skórę osób brodzących, pływających czy pracujących w wodzie (Haas i van de Roemer 1998). Pierwszym i najczęstszym symptomem inwazji jest uporczywie swędząca wysypka znana, jako świąd pływaków – „*swimmer's itch*", lub cerkariowe zapalenie skóry – „*cercarial dermatitis*". Parazytozie tej może towarzyszyć powiększenie węzłów chłonnych, nudności, biegunka, gorączka, bezsenność (Horák i in. 2002; Żbikowska 2003), a w ekstremalnych przypadkach nawet zaburzenie pracy układu oddechowego i/lub wstrząs anafilaktyczny (Bayssade-Dufour i in. 2001).

Podczas badań eksperymentalnych z wykorzystaniem ssaczych modeli cerkarie ptasich schistosom pokonywały barierę ich skóry i docierały do narządów wewnętrznych – płuc, serca, nerek, wątroby, jelit, rdzenia kręgowego, przekształcając się w kolejne stadium – schistosomule (Haas i Pietsch 1991; Horák i Kolářová 2001; Lichtenbergová i in. 2011). Olivier (1953), w pracy, do której odwołuje się większość współczesnych badaczy ptasich schistosom, odnotował obecność schistosomul w płucach eksperymentalnie zarażonych makaków – najbardziej zbliżonego modelu badawczego do człowieka. Niebezpiecznym aspektem przekraczania bariery gatunkowej w stosunku do kręgowych żywicieli przez ptasie schistosomy jest fakt występowania tzw. neurotopowych pasożytów tej grupy. Charakteryzują się one wysokim powinowactwem do centralnego układu nerwowego gospodarzy (Hrádková i Horák 2002) i w odróżnieniu od klasycznych przywr krwi („*blood flukes*") odbywają w żywicielu ostatecznym wędrówkę szlakiem nerwowym, powodując jego dysfunkcję (Horák i in. 2015). Przykładem takiej ptasiej schistosomy jest *Trichobilharzia regenti*, która nie tylko u ptactwa, ale także u nietypowych eksperymentalnie zarażonych żywicieli (ssaków) powoduje zaburzenie lokomocji (Lichtenbergová i in. 2011). Wymienione wyżej cechy biologii ptasich schistosom w opinii badaczy stanowią istotne powody do rozpatrywania tych pasożytów, jako czynników o medycznym znaczeniu (Horák i Kolářová 2001).

Świąd pływaków o etiologii ptasich schistosom jest chorobą o globalnym zasięgu (Horák i in. 2015). Szczególną uwagę poświęca się występowaniu wysypek „wodnego pochodzenia" na obszarach wodnej rekreacji (Selbach i in. 2016; Caron i in. 2017; Liberato i in. 2019). Brak jednoznacznych regulacji w zakresie prewencji powoduje, że ewentualne działania zmniejszające zagrożenie wprowadza się dopiero w odpowiedzi na nagłośnione przypadki symptomatycznego „*swimmer's itch*" i tylko w lokalizacjach ognisk inwazji (Lévesque i in. 2002; Caumes i in. 2003; Verbrugge i in. 2004; Jouet i in. 2008).

W zapobieganiu świądu pływaków postuluje się przede wszystkim ograniczanie liczebności żywicielskich gatunków ślimaków w zbiornikach wodnych poprzez ich zbieranie i/lub niszczenie siedlisk (Lévesque i in. 2002; Jouet i in. 2008). Mechaniczne usuwanie ślimaków z kąpielisk przynosi jednak tylko ograniczone pozytywne efekty (Jouet i in. 2008). W eksterminacji żywicielskich mięczaków od lat stosuje się również moluskocydy, które nie są obojętne dla lokalnej fauny (Blankespoor i Reimink 1991; McCullough 1992), wykazując działanie toksyczne, a nawet karcinogenne (Hemed 2010). Alternatywę dla stosowanych mechanicznych i chemicznych metod kontroli świądu pływaków stanowią działania oparte na wiedzy z zakresu biologii i ekologii tak żywicieli jak pasożytów (Toledo i Fried 2011). **Dlatego poznanie rozprzestrzenienia ptasich schistosom w populacjach żywicielskich ślimaków, z uwzględnieniem gatunkowego składu pasożytów, zbadanie produktywności inwazyjnych larw oraz ich przeżywalności, a w końcu możliwości biologicznej kontroli zagrożenia w celu jej ewentualnego zastosowania na terenach rekreacyjnych stanowiło główne zadania badawcze postawione w ramach przygotowanej rozprawy.**

2. OPIS MERYTORYCZNY

Ze względu na kosmopolityczny zasięg „*swimmer's itch*", a także potencjalne poważne następstwa inwazji ptasich schistosom dla zdrowia ludzi konieczne jest prowadzenie badań podstawowych dotyczących rozprzestrzenienia tych pasożytów na obszarach rekreacyjnych, jak również podjęcie prób skutecznej kontroli zagrożenia. Weryfikacja stawianych w niniejszej rozprawie doktorskiej hipotez ma posłużyć planom prewencji świądu pływaków na terenach związanych z wodną rekreacją, a w dalszej perspektywie do włączenia badań żywicielskich mięczaków do standardowej procedury kontroli bezpieczeństwa w miejscach kąpieliskowych.

2.1. Ptasie schistosomy w polskich zbiornikach wodnych – zagrożenie świądem pływaków

Dotychczas, w europejskich zbiornikach wodnych notowano ptasie schistosomy należące przede wszystkim do dwóch rodzajów – Trichobilharzia i Bilharziella, które jako pierwszych żywicieli pośrednich wykorzystują pospolite gatunki ślimaków słodkowodnych (Horák i in. 2015). Do żywicieli *Trichobilharzia* spp. zaliczamy m.in. *Lymnaea stagnalis* (Linnaeus, 1758), *Stagnicola palustris* (Müller, 1774), *Radix auricularia* (Linnaeus, 1758) i *R. balthica* (Linnaeus, 1758) z rodziny Lymnaeidae, natomiast gatunkiem żywicielskim dla *Bilharziella* sp. jest *Planorbarius corneus* (Linnaeus, 1758) należący do rodziny Planorbidae (Horák i in. 2002; Żbikowska 2004). W odniesieniu do żywiciela ostatecznego pasożyty te charakteryzują się znacznie niższą specyficznością i są notowane u ptactwa wodno-błotnego z różnych rodzin (Horák i in. 2002), np. Ardeidae, Podicipedidae, Ciconiidae, czy Anatidae (Sulgowska i Czaplińska 1987). Ptactwo jako żywiciele dorosłych form pasożytów zapewnia im transmisję nawet na tereny bardzo od siebie oddalone (Gordy i in. 2018). Jednak kluczowy dla sukcesu ptasich schistosom na określonym obszarze jest związek z kompatybilnym żywicielem pośrednim, w którym powstają liczne kohorty cerkarii sukcesywnie uwalniane do środowiska (Galaktionov i Dobrovolskij 2011). Według najnowszych badań w niewielkim zbiorniku eutroficznym biomasa cerkarii *T. szidati* emitowanych przez żywicielskie ślimaki może osiągnąć wartości do 4,65 ton rocznie (Soldánová i in. 2016). Ze względu na powszechność występowania na terenie Polski zarówno żywicieli pośrednich, jak i ostatecznych, odnotowywane przypadki dermatozy – zwłaszcza u dzieci oraz nadal nieznany los larw ptasich schistosom u pacjentów z objawami świądu pływaków, niezbędne jest stałe monitorowanie obecności tych pasożytów w środowisku. **W odpowiedzi na wyżej zaprezentowany problem w ramach dwóch pierwszych publikacji stanowiących części rozprawy doktorskiej przeprowadzone zostały badania, w których główna hipoteza zakładała, że istnieje realne zagrożenie inwazją ptasich schistosom na terenie Polski.**

2.1.1. Przykład realnego zagrożenia świądu pływaków w antropogenicznym zbiorniku wodnym zmodernizowanym do celów rekreacyjnych (publikacja nr 1)

„Wodna Dolina" w Koszalinie utworzona na rzece Dzierżęcinka to jeden z przykładów ingerencji człowieka w środowisko w celu wykorzystania naturalnych zasobów do zaspokojenia potrzeb społecznych. Sztuczne zbiorniki wodne budowane na rzekach stanowią bardzo istotny obiekt zainteresowania ekologów. Tworzą nowe warunki dla fauny i flory, powodując szybkie zmiany jakościowe i ilościowe w środowisku (Żbikowski i in. 2009). Mają zwiększoną powierzchnię w porównaniu do poprzedniego obszaru wodnego, charakteryzują się wolniejszym przepływem wody i lepiej rozwiniętą linią brzegową co w efekcie prowadzi do powstania wielu nowych nisz dla biocenozy (Poznańska i in. 2009). Zmiany te dotyczą nie tylko wolno żyjących organizmów wodnych, ale i związanych z nimi pasożytów (Morley 2007). Niezamierzone skutki wprowadzanych zmian mogą stanowić zagrożenie dla zdrowia ludzi. Jednym z takich nieprzewidzianych efektów ingerencji człowieka w przyrodę było masowe wystąpienie świądu pływaków u osób korzystających z rekreacji w Wodnej Dolinie w pierwszym roku jej funkcjonowania. **Udokumentowane przypadki przedstawiono w pierwszej pracy, w ramach, której przeprowadzono badania mające na celu przeanalizowanie koincydencji pomiędzy wybuchem świądu pływaków a zarażeniem żywicieli pośrednich ptasimi schistosomami na obszarze antropogenicznego zbiornika wodnego.**

2.1.2. Molekularna diagnostyka ptasich schistosom i ich udział w inwazjach przywr u przedstawicieli Lymnaeidae (publikacja nr 2)

Przebieg inwazji ptasich schistosom u żywicieli ostatecznych lub eksperymentalnie zarażonych nieżywicielskich kręgowców jest zdeterminowany przez gatunek pasożyta (Horák i in. 2002). Identyfikacja gatunków ptasich schistosom występujących u ślimaków stanowi ważne źródło wiedzy o lokalnym zagrożeniu. Morfologiczno-anatomiczna identyfikacja cerkarii nie jest jednak wystarczająca (Dvorák i in. 2002; Rudolfová i in. 2007; Podhorský i in. 2009), dlatego ocena zagrożenia inwazją tych pasożytów u ludzi powinna opierać się na diagnostyce molekularnej. Za główny czynnik sprawczy świądu pływaków w Europie uznaje się gatunki należące do rodzaju Trichobliharzia, które jak wspomniano związane są z Lymnaeidae (Horák i in. 2015). Oprócz gatunkowego zróżnicowania ptasich schistosom transmitowanych przez ślimaki, kluczowe dla oceny zagrożenia ze strony pasożytów jest ustalenie okresu ich najwyższej prewalencji u żywicieli pośrednich. Produkcja cerkarii różnych gatunków przywr jest stymulowana przez temperaturę, co tłumaczy wysoką emisję inwazjnych cerkarii w miesiącach najcieplejszych (Żbikowska 2004). Ponadto większe żywicielskie ślimaki zapewniają odpowiednie zasoby energii i/lub więcej miejsca do produkcji inwazyjnych cerkarii (Graham 2003). **Celem kolejnej publikacji składającej się na rozprawę doktorską było zbadanie różnorodności ptasich schistosom z rodzaju Trichobilharzia u żywicielskich Lymnaeidae w wybranych jeziorach Niżu Polskiego, prewalencji i sezonowej fluktuacji zarażenia ślimaków w okresie aktywnej wegetacji oraz związku inwazji ptasich schistosom z wielkością mięczaka, jako parametrów oceny ryzyka zagrożenia świądem pływaków.**

2.2. *Potamopyrgus antipodarum* (Gray, 1843) jako potencjalny czynnik biologicznej kontroli świądu pływaków

Z doświadczeń nad biologiczną kontrolą pasożytów wynika, że prewalencja przywr digenicznych u żywicieli pośrednich może zostać istotnie zredukowana poprzez obecność niekompatybilnego organizmu pomiędzy żywicielem docelowym a wolno żyjącymi larwami pasożytów (Kalbe i in. 1997). Zmniejszenie ryzyka choroby poprzez zwiększenie bioróżnorodności potencjalnych żywicieli znane jest jako hipoteza rozcieńczenia – „*dilution effect*” i posiada zarówno zwolenników jak i przeciwników (Keesing i in. 2006; Wood i in. 2014; Cichy i in. 2015). Zwolennicy przytaczają kilka sposobów wykorzystania nieżywicielskiego gatunku w celu zakłócenia transmisji pasożytów, m.in. poprzez tworzenie fizycznej bariery, drapieżnictwo, czy tzw. efekt wabika – „*decoy effect*” (Thieltges i in. 2008). Jednakże nie wszystkie współwystępujące nieżywicielskie gatunki w równym stopniu biorą udział w zakłóceniu transmisji pasożytów (Hopper i in. 2008). Interesującą sugestię wysunęli Kopp i Jokela (2007), zgodnie z którą w efekcie rozcieńczenia szczególne zastosowanie mogą mieć obce gatunki – zazwyczaj wolne od naturalnych wrogów na nierodzimych obszarach. W ramach prezentowanej rozprawy doktorskiej podjęto badania nad przetestowaniem nowozelandzkiego przybysza – *Potamopyrgus antipodarum* (Gray, 1843) (Gastropoda: Tateidae), jako potencjalnej „tarczy” chroniącej żywicielskie ślimaki przed inwazją miracydiów ptasich schistosom. Decyzja o wyborze *P. antipodarum* wynikała między innymi z faktu, że ten obcy w Europie gatunek od lat sympatrycznie występuje z żywicielskimi Lymnaeidae w zbiornikach wodnych (Piechocki 2009), a ponadto charakteryzuje się wieloma cechami sprzyjającymi planom biologicznej manipulacji z jego udziałem (Kopp i Jokela 2007). **W kolejnych włączonych do rozprawy doktorskiej publikacjach postawiono hipotezę, że obecność sympatrycznie występującego *P. antipodarum* z żywicielskimi gatunkami mięczaków ptasich schistosom zakłóca transmisję tych pasożytów, co w dalszej konsekwencji mogłoby pomóc zmniejszeniu ryzyka świądu pływaków.**

2.2.1. Sukces inwazji *Trichobilharzia regenti* u żywicielskich mięczaków w konfrontacji z współwystępowaniem w sąsiedztwie żywicieli grup *Potamopyrgus antipodarum* o różnej liczebności – badania eksperymentalne (publikacja nr 3)

Uwolnione przez żywicieli ostatecznych jaja ptasich schistosom muszą trafić do środowiska wodnego (Horák i in. 2015). W wodzie z jaja wykluwa się miracydium – inwazyjna dla żywiciela pośredniego postać larwalna (Gryseels i in. 2006). Brak kompatybilności między ślimakiem i przywrą nie wyklucza możliwości penetracji larw pasożyta przez powłoki gospodarza (Sapp i Loker 2000). Już w pracach wykonywanych w 70 latach ubiegłego stulecia wskazywano, iż miracydia przywr digenicznych mogą wnikać do różnych, także nieżywicielskich gatunków mięczaków, chociaż ich dalszy prawidłowy rozwój jest możliwy wyłącznie w kompatybilnym gospodarzu (Basch 1976).

Wysokie zagęszczenie w jakim występują populacje *P. antipodarum* w środowisku (Piechocki i Wawrzyniak-Wydrowska 2016) czynią go atrakcyjnym organizmem do wykorzystania jako fizyczną barierę, jak również jako tzw. żywiciela „*dead-end*", czyli uniemożliwiającego pasożytowi dalszy prawidłowy rozwój (Prüter i in. 2017). **Praca, stanowiąca część prezentowanej rozprawy doktorskiej miała na celu sprawdzenie czy sympatrycznie występujący *P. antipodarum* przyczynia się do zmniejszenia obecności inwazji ptasich schistosom u żywicielskich mięczaków w warunkach eksperymentalnych, jednocześnie nie stanowiąc źródła inwazyjnych dla kręgowych żywicieli cerkarii.**

2.2.2. Chemotaksja miracydiów *Trichobilharzia* spp. w obecności kompatybilnych i niekompatybilnych żywicieli (publikacja nr 4)

Wolno żyjące stadia larwalne Digenea charakteryzują się ograniczonym czasem życia w środowisku zewnętrznym (Anderson i in. 1982). Miracydia wyczerpują rezerwy energetyczne w czasie zależnym od temperatury wody, natężenia światła czy związków chemicznych rozpuszczonych w wodzie (Gryseels i in. 2006). W poszukiwaniu żywicielskiego mięczaka miracydia wielu gatunków przywr digenicznych wykorzystują chemorecepcję (Haas 2003). W środowisku wodnym larwy odbierają sygnały chemiczne pochodzące od różnych organizmów, co może powodować zaburzenie orientacji. Odpowiedzią na rozproszenie bodźców, jak postulują Hertel i in. (2005), może być specyficzny behawior larw uwolnionych do środowiska, polegający początkowo na bezładnej lokomocji, która w miarę zbliżania się larw do źródła chemicznego sygnału staje się bardziej uporządkowana. Większość gatunków przywr digenicznych wykazuje specyficzność w stosunku do pierwszych żywicieli pośrednich (Sapp i Loker 2000). Zjawisko to obserwuje się również w przypadku gatunków należących do rodzaju Trichobilharzia (Kock 2001). Już w latach 90 XX wieku Kalbe i in. (1997) wykazali, że miracydia ptasich schistosom najsilniej reagują na składniki znajdujące się w wodzie kondycjonowanej żywicielskimi mięczakami oraz prezentują słabą reakcję lub całkowity jej brak na sygnały pochodzące od innych mięczaków, pijawek, kijanek czy ryb. **Celem czwartej pracy stanowiącej część rozprawy doktorskiej było zweryfikowanie reakcji miracydiów ptasich schistosom notowanych w polskich zbiornikach wodnych na bodźce chemiczne wysyłane przez żywicielskie i nieżywicielskie ślimaki ze szczególnym uwzględnieniem *P. antipodarum*.**

2.3. Najważniejsze wyniki i wnioski

Wobec słabo udokumentowanego w Polsce medycznego zagrożenia ze strony ptasich schistosom w ramach prezentowanej rozprawy doktorskiej **pozytywnie zweryfikowano hipotezę o istnieniu potencjalnego jak i realnego problemu świądu pływaków**. Badane zbiorniki, w których wykryto obecność ślimaków zarażonych larwami *Trichobilharzia* spp. posiadały oficjalne lub nieoficjalne miejsca kąpieliskowe, na których wyznaczono stanowiska poboru prób. Ustalono, że ślimaki wysiewające inwazyjne larwy ptasich schistosom notowane są głównie w miesiącach intensywnej letniej rekreacji, a zarażone osobniki to mięczaki o dużych rozmiarach, których wysokość muszli przekracza 4,9 cm. Sytuację zagrożenia potęguje fakt wykrycia wśród gatunków ptasich schistosom neurotopowej *T. regenti*, a także wysoka intensywność inwazji ptasich schistosom u zbadanych żywicielskich ślimaków z rodzaju Radix. Dane te jednoznacznie wskazują na konieczność podjęcia działań w celu opracowania skutecznego programu ochrony kąpielisk przed zagrożeniem świądem pływaków. Przeprowadzono badania eksperymentalne, w których pozytywnie zweryfikowano hipotezę, że obcy w Europie gatunek ślimaków *P. antipodarum* może być wykorzystany, jako czynnik ograniczający cerkariogenezę i transmisję ptasich schistosom w środowisku. Z badań laboratoryjnych, które zostały przeprowadzone w ramach rozprawy doktorskiej wynika, że **ślimaki te mogą stanowić skuteczną mechaniczną tarczę dla żywicielskich ślimaków przed miracydiami ptasich schistosom** zwłaszcza, że chemotaksja miracydiów, z powodu obecności w środowisku sygnałów chemicznych pochodzących od *P. antipodarum,* ulega zaburzeniu. Należy też podkreślić, że z przeprowadzonych eksperymentów jasno wynika brak możliwości dalszego rozwoju ptasich schistosom u *P. antipodarum*. Uzyskane wyniki prac nad biologiczną kontrolą „*swimmer's itch*" są wprawdzie efektem badań prowadzonych w kontrolowanych warunkach laboratoryjnych, ale stanowią obiecujący materiał do kontynuacji w warunkach polowych.

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4.STRESZCZENIE

Zainteresowanie naukowców ptasimi schistosomami (Digenea: Schistosomatidae) wynika nie tylko ze względów czysto poznawczych, ale posiada duży potencjał aplikacyjny. Inwazyjne dla kręgowych żywicieli larwy mogą wnikać w skórę przebywających w wodzie ludzi. Symptomem takiej inwazji są zmiany dermatologiczne znane, jako świąd pływaków – „*swimmers' itch*”. Ze względu na częste u ludzi przypadki wysypek wodnego pochodzenia, a także nieznany los larw ptasich schistosom w organizmie człowieka, konieczne jest prowadzenie badań podstawowych nad rozprzestrzenieniem tych pasożytów na obszarach rekreacyjnych oraz podjęcie prób skutecznej kontroli zagrożenia. W ramach prezentowanej rozprawy zweryfikowano hipotezę o istnieniu potencjalnego i realnego problemu „swimmer's itch” na terenie Niżu Polskiego, a uzyskane wyniki stały się punktem wyjścia do badań mieszczących się w ramach planu biologicznej ochrony kąpielisk przed świądem pływaków. Postawiono zatem drugą hipotezę, że nowozelandzki przybysz – *Potamopyrgus antipodarum* (Gray, 1843) (Gastropoda: Tateidae) może pełnić rolę „tarczy” chroniącej żywicielskie ślimaki przed inwazją miracydiów ptasich schistosom. Zrealizowano dwa zadania badawcze, których wyniki wskazują na możliwość przerwania transmisji ptasich schistosom w zbiornikach, gdzie żywicielskim gatunkom ślimaków towarzyszą odpowiednio liczebne populacje *P. antipodarum*. Pozytywne zweryfikowanie kolejnej hipotezy prezentowanej rozprawy na poziomie badań w kontrolowanych warunkach laboratoryjnych stanowi obiecujący fundament do ich kontynuacji w warunkach polowych.

ABSTRACT

Scientists' interest in bird schistosomes (Digenea: Schistosomatidae) is not only due to purely cognitive reasons but has a large application potential. Infective larvae for vertebrate hosts can penetrate into the skin of people in the water. The symptom of this infection is dermatological changes known as swimmers’ itch. Due to the frequent causes of aquatic origin rashes for humans, and the unknown fate of bird schistosomes larvae inside the human body, it is necessary to conduct basic research on the spread of these parasites in recreational water bodies and attempts to effectively control the threat. In the presented dissertation, the hypothesis about the presence of potential and real swimmer's itch problem in the Polish Lowlands was verified, and the obtained results became the starting point for research on the biological plan for the protection of bathing areas against swimmer’s itch. Therefore, a second hypothesis was put forward that the New Zealand newcomer – *Potamopyrgus antipodarum* (Gray, 1843) (Gastropoda: Tateidae) may play a role as a "shield" protecting snail hosts against the infection of bird schistosomes. Two research tasks were carried out, which results indicate the possibility of interrupting the transmission of bird schistosomes in water reservoirs, where the host species of snails are accompanied by adequately numerous populations of *P. antipodarum*. Positive verification of the above hypothesis of the presented dissertation in controlled laboratory conditions is a promising foundation for their continuation in field studies.

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ORIGINAL PAPER

The real threat of swimmers' itch in anthropogenic recreational water body of the Polish Lowland

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Abstract After numerous reports the local press about the "stinging water" in created on the Dzierżęcinka River—Water Valley reservoir and recognizing in bathers the symptoms of swimmers' itch, environmental study on the presence of bird schistosome larvae in snail hosts was conducted. Snails belonging to Lymnaeidae and Planorbidae were collected at two sites: (i) part of anthropogenic reservoir (192 individuals) and (ii) the river part (37 individuals). Higher prevalence of Digenea was observed in snail populations living in Water Valley (29.8 %) compared to Dzierżęcinka River (21.3 %). The larvae of bird schistosomes were recorded in both localities in 1.8 % of collected snails. The prevalence of bird schistosomes reached 2.9 % in *Planorbarius corneus*, 2.8 % in *Radix auricularia*, and 5.9 % in *Radix balthica/labiata*. Laboratory tests have shown that at 19 °C the number of bird schistosome cercariae released from snail hosts significantly exceeded the number of cercariae of other identified Digenea species. It is worth underlining that despite the low prevalence of bird schistosomes, the high number of released cercariae was sufficient to create a real threat of swimmers' itch in bathers. As indicated by the example presented, anthropogenic reservoirs create excellent conditions for Digenea species including bird schistosomes. In view of the real risk of people using the waters, tests on presence of the parasites in snail hosts should be included to the standard procedure of security control in bathing places.

Keywords Anthropogenic reservoir · Swimmers' itch · Bird schistosome · Cercariae · *Planorbarius corneus* · *Radix* spp.

Introduction

Cercarial dermatitis called swimmers' itch has been listed for several years in human populations around the world (Cort 1936; Hunter et al. 1949; Jarcho and van Burkalow 1952; Macy 1952; Hoeffler 1974; Leedom and Short 1981; Eklu–Natey et al. 1985; Blankespoor and Reimink 1988; Loken et al. 1995; Pilz et al. 1995; Lindblade 1998; Kolářová et al. 1999). It reveals an allergic skin reaction (Kolářová et al. 2010) in the form of painful, then itchy lumpy rash (Żbikowska et al. 2002). It occurs in people bathing or wading in ponds populated by snails infected with bird schistosome larvae. Lumpy skin lesions resembling early stage of chickenpox, is a result of penetration by cercariae of bird schistosomes, and the number and size of bubbles depend on the number of penetrating larvae (Żbikowska 2003).

Snails play a key role as first intermediate hosts in the life cycle of bird schistosomes. Miracydium enters the mollusk, then in hepatopancreas transforms into sporocyst, inside which after 6–7 weeks numerous furcocercariae invasive for vertebrates emmerge (Amen and Meuleman 1992). Cercariae abandon host snail and penetrate the webbed feet of waterfowl, transforming into schistosomulae. In ventral organs of bird host, parasites mature and reproduce sexually (Soldánová et al. 2013). However, bird schistosomes are not capable of sexual maturity in man, some authors suspect the possibility of schistosomulae occurrence inside human organs (Horák and Kolářová 2001; Olivier 1953). This point of view is based

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on facts that appearing dermatitis in a person infected can be accompanied by further symptoms such as nausea, diarrhea, swollen glands, insomnia, fever (Horák et al. 2002; Żbikowska et al. 2002), or even anaphylactic shock and disorders of the respiratory system (Bayssade–Dufour et al. 2001). Additionally, the results of studies conducted on experimentally invaded mammals showed presence of schistosomulae in their lungs (Horák and Kolářová 2001; Haas and Pietsch 1991; Appleton and Brock 1986), liver, kidney, heart or intestine (Haas and Pietsch 1991; Horák and Kolářová 2000). A particularly interesting finding of bird schistosome larvae in the lungs of rhesus monkeys (Olivier 1953)—most similar research model for *Homo sapiens*—should be emphasized.

The potential risk of human invasion is increased by the fact that bird schistosomes quoted around the world including several taxa of hosts (Liu 2012). Some species belonging to the so-called "nasal schistosomes" are considered to be particularly dangerous because they have a high affinity for the central nervous system (brain and spinal cord) (Horák et al. 1999; Kolářová et al. 2001; Hrádková and Horák 2002). Kouřilová et al. (2004) and Lichtenbergová et al. (2011) observed a locomotion disorder in mice experimentally invaded by nasal bird schistosome *Trichobilharzia regenti*, very similar to those in typical bird host. No conclusive data on the fate of penetrating human skin cercariae, along with the growing number of documented invasion by bird schistosomes in people around the world (Marie et al. 2015; Gohardehi et al. 2013; Valdovinos and Balboa 2008; Rao et al. 2007), may negatively affect the local tourism economy (Horák et al. 2015), and although for that reason, the problem of these parasites invasion needs further study.

Swimmers' itch in Europe is mostly recorded in anthropogenic basins and eutrophic lakes (Soldánová et al. 2013). Risk assessment and preventive measures are usually introduced where the problem swimmers' itch is the fact (Jouet et al. 2008; Verbrugge et al. 2004; Caumes et al. 2003; Lévesque et al. 2002; Chamot et al. 1998). One of the examples—French lake Annecy—indicates the need for the earlier prevention preceding the planned changes in environment. This attractively landscaped downtown area has become the place of numerous cases of dermatitis caused by bird schistosomes (Caumes et al. 2003). The high number of reported cases in humans was associated with the increase in population of *Radix* sp. and the creation of an artificial bird-island in the lake (Jouet et al. 2008). Human intervention in the ecosystem of the Lake Annecy caused unforeseen consequences, the manifestation of which was increasing cases of swimmers' itch.

In Poland, in response to the needs of local communities, authorities started to create inner-city baths, being an interesting alternative to indoor swimming pools and water parks in the summer. Expanding river and streams beds and damming in the areas without earlier bathing places, creates good conditions for penetration both—the elements of wild ecosystem and human habitat. One of the adverse effects of changes in the environment may be the emergence of swimmers' itch in people using new recreational waters. In this paper, we present an analysis of coincidence between water-derivate dermatitis and etiological factors of swimmers' itch in the area of anthropogenic reservoir in the Polish Lowland. Our thesis concerns potential health risks accompanied by water ecosystem transformation and addresses the need to take those risks into account in future hydro plans.

Material and methods

Description of the area

Multifunctional reservoir on the Dzierżęcinka River was opened on October 14th, 2013, in the place of the retention basin functioning before World War II, much smaller than the current one. Covering the area of 6.05–7.08 ha, the reservoir is 800-m long and reaches 150 m in its widest point. It is 2 to 2.5-m deep and may take from 118 000 to 152 000 m^3 of water. In addition to the role of water storage and fire protection, it is a recreational attraction for locals and tourists, called the Water Valley. The hydro project is one of five steps implemented within the framework: "Protection of the Jamno Lake flood basin and revitalization of the Dzierżęcinka River – protection areas of Koszalin."

The river with a length of 29.3 km has its source in the west of the village Kliszno. In the initial run, below the village Manowo, it flows in artificially created riverbed, connecting with the Lubiatowskie Lake. From the outflow of the lake the river flows in the XIII century canal, then continues through the city of Koszalin in a deep glacial valley, supplying the Pond Castle (1.5 ha) in the Park of the Pomeranian Dukes. After leaving the northern part of the city the river flows into the coastal lake Jamno.

Six years ago, before the launch of a revitalization plan, the Dzierżęcinka River carried waters of very low quality. The main reason was illegal sewage pollution of the river. The actions taken by the local authorities have contributed not only to the improvement of esthetic qualities of the river but also to some positive changes in the development of biocenosis. Nowadays, fishing sources report the increase in biological diversity of fish populations—occurrence of tench, crucian carp, roach, ide, and also pike and eels. Additionally, damming and the creation of the reservoir have caused some waterfowl of the Park of Pomeranian Dukes move into the area of the Water Valley. The current swimming pool is a picturesque place where the processes within the biocenosis intersect with the needs of active rest of people. A safe area has been separated for children and young people to play in, comprising a small canoe dock, a beach area, and a walking zone along the banks of the reservoir.

Medical case description

In the summer 2015, on June 27th a 3-year girl went to the doctor's surgery because of a sore throat, with body temperature elevated up to 38 °C and the symptoms of swimmers' itch on the skin of both legs (Fig. 1a) and forearms (Fig. 1c) continuing for 2 weeks. The dermatitis consisted of minor lumps, resembling the ones in chickenpox. In the treatment, fenistil and pimafucort cream to the local lubrication was applied for weeks—the child went to the doctor again, because the itching intensified, and the changes were not reversible; the bumps on the forearms were still noticed (Fig. 1d). In addition, the child developed catarrh and catarrhal changes of the nasal mucosa. After dermatological consulting, the treatment was maintained for the next weeks and then the lesions gradually disappeared.

Medical interview shows that skin symptoms in a child appeared a few hours after bathing in the Water Valley reservoir. At the same time, a similar rash appeared in an adult caregiver, bathing with the child in the basin described above. In the case of the adult, the same treatment was used, the effectiveness of which was already observed after 2 weeks of application of medication. The changes in the skin gradually underwent exfoliation (Fig. 1b).

During the summer season of 2015, more than ten cases of similar rashes caused by the "stinging water" in the Water Valley was reported (personal information). Local authorities and sanitary service decided to examine water samples for the presence of toxic bacteria and blue-green algae. The tests carried out by the sanitary station and the Inspectorate of Environmental Protection did not reveal the presence of the toxic microorganisms in water (Rejestr Stacji Sanitarno–Epidemiologicznej w Koszalinie 2015).

Parasitological diagnosis of potential intermediate hosts of bird schistosomes

Material for parasitological study were pulmonate snails: *Lymnaea stagnalis*, *Radix auricularia*, *Radix balthica/labiata*, and *Planorbarius corneus*, which can play a role of intermediate hosts of bird schistosomes. Snails were collected in August 2015, in two localities—(i) the Water Valley (54°10′45″N, 16°12′31″E), and for comparison in (ii) parts of the Dzierżęcinka River running through the Park of Pomeranian Dukes (54°11′13″N, 16°11′15″E). Snails were collected from plants and the bottom of reservoir and the river by hand or by using metal sieves.

Taxonomic affiliation of collected snails was verified on the basis of morphological data (Piechocki 1979), and anatomical features of the reproductive system—in the case of the genus Radix (Jackiewicz 2000). All collected snails were individually kept in culture beakers with conditioned tap water in the incubator (SANYO) at 19 °C and natural photoperiod. The culture temperature was determined based on the results

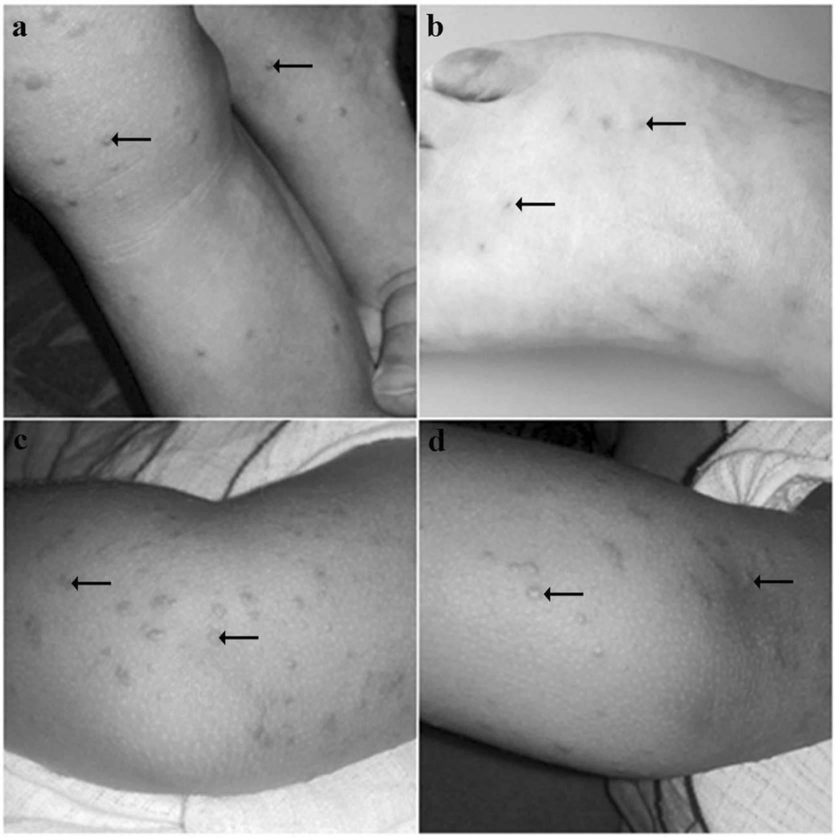

Fig. 1 Swimmers' itch on: **a** The legs of 3-year girl 2 weeks after infection, **b** The leg of adult man 2 weeks after infection, **c** The forearms of 3-year girl 2 weeks after infection, **d** The forearms of 3-year girl 4 weeks after infection

of previous studies on thermal preferences of snails (Żbikowska 2005). Diagnostics of parasite presence was carried out by a non-invasive method, and Digenea species were recognized on cercariae morphology according to Faltýnková et al. (2007, 2008). Every day, each snail was placed for 1 h in a beaker with a small amount of water under a light source. Under these conditions, snails with patent invasion released cercariae. Snails were replaced into culture beakers filled with fresh water, and cercariae in small beakers were fixed with 75 % ethanol then counted by quantitative method in a Sedgwick-Rafter chamber. The survey was repeated until the death of all snails. Dead animals were necropsied for checking their invasion status. At the end of the experiment, the obtained data were divided into the groups on the basis of invasion status of snails—non-infected and infected with different parasite species. Snails' lifespan duration and number of cercariae released from naturally invaded hosts were analyzed.

Statistical analysis

A Chi-square test of contingency table was used to compare the number of infected and non-infected snails collected in both sampled localities. For further analysis, concerning lifespan of snails and the number of released cercariae only individuals of the same snail genus were used—naturally invaded with bird schistosomes, hosts of other Strigeida species and non-infected snails. Only the groups that reached at least three specimens were compared.

The average number of Strigeida species cercariae (furcocercariae per snail host) was calculated, and the results were analyzed by one-way ANOVA (factor: parasite species), followed by post-hoc Tukey test. The same type of analysis was used to compare an average lifespan duration of the studied snail groups.

The term prevalence (%) was used for the description of one snail species invaded by bird schistosomes, whereas the term infection (%)—as a proportion of hosts infected by specific parasite species in relation to all invaded snails.

Results

In total, 229 snails were sampled: 106 individuals of *Lymnaea stagnalis*, 17 *Radix balthica/labiata*, 71 *Radix auricularia*, and 35 *Planorbarius corneus*. In the Park area of the Dzierżęcinka River only 37, while in the Water Valley the remaining 192 individuals were collected. On both studied localities, the same snail species were found. In nearly half of all the collected animals, 94 revealed the presence of Digenea larvae. Among the snails collected in the Water Valley, 29.8 % were naturally invaded by parasites and in the samples from park part of the Dierżęcinka River only 21.3 %. The difference was statistically significant ($\chi 2 = 100.25$, df = 1, $P < 0.001$). Also the number of diagnosed parasite species found in the snails sampled in the Water Valley was higher. Among the total 14 identified Digenea species, 12 were noted in the Water Valley and only 6 in park locality. Throughout the parasites developing inside the studied snails dominated fluke species producing furcocercariae (Fig. 2).

Larvae of bird schistosomes were recorded in both studied localities (Fig. 2). The snails naturally infected with bird schistosomes accounted for 1.8 % of all collected specimens. In the Water Valley bird schistosome larvae were detected in *P. corneus* (*Bilharziella polonica*) and in *R. auricularia* (*Trichobilharzia* sp.) while in park locality, only *Trichobilharzia* sp. was found but inside two lymnaeids snail species—*R. auricularia* and *R. balthica/labiata*. The prevalence of bird schistosomes in snails' populations was low and amounted to 2.9 % (in *P. corneus*), 2.8 % (in *R. auricularia*) and 5.9 % (in *R. balthica/labiata*).

There was a statistically significant difference in the lifespan between four groups of *Radix* sp. individuals kept at controlled temperature conditions 19 °C (one-way ANOVA $F_{3,8} = 33.17$, $p < 0.001$) (Table 1). Those snails at experimental conditions released different, parasite species dependent number of cercariae (one-way ANOVA $F_{2,6} = 62.12$, $p < 0.001$). Post-hoc tests indicated that amount three furcocercous Digenea species invading *Radix* sp. the most productive were bird schistosomes from complex species *T. ocellata* (Table 1).

Discussion

The lowering of groundwater level and the need for rational management of water resources are the reasons for using the artificial reservoirs in summer season for recreational or educational purpose. The latter function may take an unexpected course of "education on the living organism," as evidenced by the above case. The occurrence of swimmers' itch in the child and adult bathers in the artificial reservoir is the first well-documented case in this region, although another one associated with anthropogenic reservoir used for recreation in the world (Chamot et al. 1998; Lindblade 1998; Lévesque et al. 2002; Caumes et al. 2003; Soleng and Mehl 2011).

A medical case presented in this paper fully corresponds to the characteristics of swimmers' itch (Żbikowska et al. 2002). The location of dermatitis—on the parts of the body that come into contact with the water of basin, the rash, resembling the initial stage of chickenpox, and the time elapsed since the contact with the lake water to the appearance of first symptoms (Liu 2012) indicate the related cercariae etiology of illness. This point of view is additionally underlined by negative results of bacterial and blue-algae tests made in Sanitary Station (Rejestr Stacji Sanitarno–Epidemiologicznej w Koszalinie 2015).

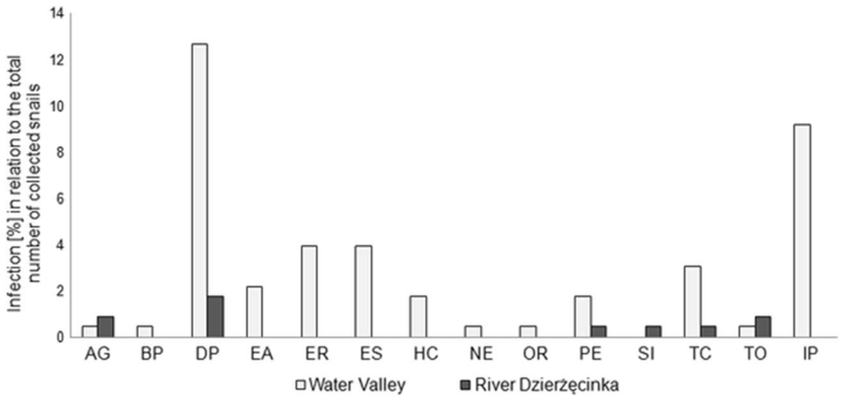

Fig. 2 Infection of snails by Digenea larvae in collected samples. Parasites—Srigeida: *AG Apatemon gracilis*, *BP Bilharziella polonica*, *DP Diplostomum pseudospathaceum*, *SI Sanguinicola inermis*, *TC Tylodelphys clavata*, *TO Trichobilharzia ocellata*; Echinostomida: *EA Echinoparyphium aconiatum*, *ER Echinostoma revolutum*, *ES Echinostoma spiniferum*, *HC Hypoderaeum conoideum*, *NE Notocotylus ephemera*; Plagiorchiida: *OR Opisthoglyphe ranae*, *PE Plagiorchis elegans*; undiagnosed pre-patent invasion—PI

It is particularly noteworthy that most severe symptoms of disease were observed in the child compared with the adult, which is consistent with the reports of other authors who indicated even bronchial reactions in the course of the attack of bird schistosomes in children (Bayssade–Dufour et al. 2001). Moreover, the differences in the course of the reported cases, confirm the individual susceptible of host (depending on the activity of the immune system) (Kolářová et al. 2013).

As follows from the case, a rash in the child and the adult appeared a few hours after the bath in anthropogenic basin. Creating such reservoirs, according to some authors, has a dramatic impact on the environment (Morley 2007). Researchers agree that not all the consequences of the changes can be predicted and therefore they postulate the need for constant monitoring of the environment (Morley 2007; Żbikowski et al. 2007). Artificial reservoirs built on the rivers require special attention. They create new conditions for biota, causing rapid qualitative and quantitative changes in the environment (Poznańska et al. 2009; Żbikowski et al. 2007). Such reservoirs are characterized by increased surface compared to the previous one, attracting numerous species of waterfowl (Morley 2007). The created lagoon is characterized by a slower flow of water and a better developed coastline, thus creating more favorable conditions for many invertebrates e.g. molluscs (Poznańska et al. 2009; Żbikowski et al. 2007). These changes concern both—free living organisms and the accompanying parasites (Morley 2007). The effects of such changes unintentionally may turn against human posing a serious threat to health, affecting children in particular.

Such unforeseen result was the appearance of the real problem of swimmers' itch in the basin described. Statistical analysis of the data shows that both the intermediate Digenea hosts and the parasites have gained better living conditions in parts of the reservoir as compared to the river. Shallow depth of the river flowing in the park, a high water transparency, and mass colonization by feeding waterfowl were the factors effectively limiting the development of molluscs (van Leeuwen et al. 2012). The number of snails collected in the river accounted for only the sixth part of the whole sample. Despite the similar snail host species composition on both studied localities, there were significant differences concerning diversity and prevalence of parasites.

Good living conditions for the snail development in the reservoir, the presence of waterfowl freely moving between both parts, and recreational use of the Water Valley allowed the invasion of bird schistosome cercariae to the people. A similar coincidence effect of these factors was observed in the reservoirs created on the River Ruhr (Germany), where

Table 1 Snail survival and cercariae production in experimental condition: constant temperature and natural photoperiod

Radix sp.	Temperature (°C)	Survival avg. (days) (± SE)	Number of emerged cercariae/snail (± SE)
Non–infected	19	23 (±1)	–
With *T. ocellata*	19	12 (±1)*	1657 (±54)a
With *D. pseudospathaceum*	19	11 (±1)*	990 (±110)b
With *T. clavata*	19	8 (±1)*	503 (±35)c

Different letters indicate statistically significant difference between compared values (a/b, $p < 0.01$, b/c, $p < 0.01$, a/c, $p < 0.001$)

*Statistically different from non-infected ones (Tukey test $p < 0.001$)

the prevalence of infection *R. auricularia* by *T. franki* for one of the sampling sites was up 27 % (Soldánová et al. 2010).

In the samples, larvae of bird schistosomes were found in three species of snails, which confirms the favorable conditions for the transmission of these parasites in the environment. Most of the infected snails belonged to the family Lymneidae, considered by Horák et al. (2002) as the source of the main etiological factors of swimmers' itch in Europe—the larvae of the genus Trichobilharzia. Preliminary studies carried out in parts of the park have allowed a note of the presence of cercariae of complex species *T. ocellata* in individuals *R. balthica/labiata* (=*R. ovata/peregra*) (Gloger and Meier–Brook 2003), which is a host for nasal schistosome *T. regenti* (Lichtenbergová and Horák 2012). After skin penetration, nasal schistosomes migrate through the central nervous system of vertebrate host. This migration causes tissue damage, and also locomotion or orientation disorders in bird or mammals hosts (Horák et al. 1999; Kolářová et al. 2001; Kouřilová et al. 2004; Lichtenbergová et al. 2011). The mere suspicion of the presence of nasal schistosomes in the study area indicates the need for monitoring of parasites in the environment, and determination of the taxonomic affiliation, which requires molecular diagnostics.

The real threat of swimmers' itch in the basin lies in a dissonance with a low prevalence of bird schistosomes in snail host populations, however the recorded values (2.8–5.9 %) did not differ from the results of other authors conducting research in the regions of frequent occurrence of this kind of dermatosis (Skírnisson and Kolárová 2005; Farahnak and Essalat 2003; Lévesque et al. 2002; Picard and Jousson 2001). The real danger of the invasion can be affected by three important factors: (i) high productivity of cercariae inside snail host, (ii) extended life of the infected snails, and (iii) high survival of invasive cercariae in an environment. This hypothesis was positively verified by the result of Soldánová et al. (2016) and also of current observation (Table 1)—the number of produced bird schistosome cercariae by the same snail species was significantly higher in comparison to other Digenea or our previous laboratory studies (Żbikowska 2004a, b, 2005, 2012)—$LT_{50\%}$ of the host survival dependent on temperature and was 32 to 79 days, and cercariae of *T. ocellata* under culture conditions alive even more than 30 h.

Temperature has a significant and direct influence on a life cycle of trematodes (Mas–Coma et al. 2009). In physiological limits, temperature increase accelerates the development of parasites inside ectothermic animals (Kendall and McCullough 1951; Lo and Lee 1996; Poulin 2006), but may also cause an accelerated death of hosts (Żbikowska 2005; Lee and Cheng 1971). These observations were also confirmed in our study, it has been demonstrated that naturally infected snails lived significantly shorter than non-infected ones (Table 1). On the other hand, the increase in ambient temperature can facilitate the transmission of free-living stages of the parasites to next hosts (Poulin 2006), but beyond a certain value their invasiveness can dramatically decrease (Morley and Lewis 2013; Morley et al. 2010). Taking into account the complexity of the effect of temperature on the biology and survival of bird schistosomes and their snail hosts, it can be assumed that in the summer, the density of invasive cercariae in the basin of the Water Valley reached a critical value.

Conclusion

Not clearly explained the ultimate fate of bird schistosome larvae penetrating human skin, additional symptoms like fever, catarrhal changes in the mucosa and bronchial obstruction in people, especially in infected children, and common presence of these parasites in environment are a strong argument for the inclusion of snail testing for the presence of bird schistosome larvae to the standard control procedure of security in bathing places. Interesting postulate in this field has been put forward by the Danish researchers Christiansen et al. (2016), suggesting the use of molecular diagnostics of pre-patent phase of the bird schistosomes development in snails.

Environmental monitoring and molecular diagnostics of bird schistosome larvae in snail host population from bathing places will be conducted in the coming period of active vegetation.

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Publikacja nr 2

Agents of swimmer's itch—dangerous minority in the Digenea invasion of Lymnaeidae in water bodies and the first report of *Trichobilharzia regenti* in Poland.

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ORIGINAL PAPER

Agents of swimmer's itch—dangerous minority in the Digenea invasion of Lymnaeidae in water bodies and the first report of *Trichobilharzia regenti* in Poland

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Abstract

Trichobilharzia spp. have been identified as a causative agent of swimmers' itch, a skin disease provoked by contact with these digenean trematodes in water. These parasites have developed a number of strategies to invade vertebrates. Since we have little understanding of the behavior of these parasites inside the human body, the monitoring of their invasion in snail host populations is highly recommended. In our research, lymnaeid snails were collected from several Polish lakes for two vegetation seasons. The prevalence of bird schistosomes in snail host populations was significantly lower than that of other digenean species. We were the first to detect the presence of the snails emitted *Trichobilharzia regenti* (potentially the most dangerous nasal schistosome) in Poland. In addition, by sequencing partial rDNA genes, we confirmed the presence of the snails positive with *Trichobilharzia szidati* in Polish water bodies, showing that swimmer's itch is more frequent during summer months and that large snails are more often infected with bird schistosomes than small ones.

Keywords Trichobilharzia · Lymnaeidae · Molecular identification · Prevalence · Seasonality · Shell size

Introduction

Digenea include parasites with a complex life cycle. Generally, they use snails as their first intermediate hosts, and vertebrates, as final (Cheng 1986; Cichy et al. 2011). Many digenetic trematodes pose threat to animal and human health (Cheng 1986). These parasites include bird schistosomes, whose invasion on humans has been recently reported in many countries (Rao et al. 2007; Valdovinos and Balboa 2008; Gohardehi et al. 2013; Marie et al. 2015; Marszewska et al. 2016; Caron et al. 2017). The disease caused by cercariae of these trematodes occurs globally and is considered to be re-emerging (Kolářová et al. 2010).

The first intermediate hosts of widely spread *Trichobilharzia* spp. include freshwater snail species of the family Lymnaeidae (Horák et al. 2002). Their final hosts include waterfowl of the families Ardeidae, Podicipedidae, Ciconiidae, and Anatidae (Sulgostowska and Czaplińska 1987; Rudolfová et al. 2007; Jouet et al. 2009). The specificity of bird schistosomes is much lower in relation to the final than to the intermediate host. According to Horák et al. (2002), the same species can develop and sexually reproduce in representatives of different bird families. Cercariae of the parasites are released from snails and seek their final vertebrate hosts using chemoreceptors. They respond to external stimuli (e.g., shadow) and signals from a potential host (e.g., fatty acids) (Horák et al. 2008). When the search is complete, larvae penetrate the host through the skin. In this process, the products of parasite's penetration glands are activated (Horák et al. 1997; Mikeš et al. 2005). The similarity between

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some lipid components in the integument of humans and birds makes the parasites attack people wading, swimming, or working in water (Haas and van de Roemer 1998). The consequence of such invasions is a skin disease known as swimmers' itch (Kolářová et al. 2010), whose first symptoms appear within 2 h after the exposure to cercariae. Within the next 2 days, the symptoms worsen. The rash, which initially causes only redness and itching, develops into small, red bumps (Żbikowska et al. 2002). The severity of symptoms may vary, depending on the number of parasites attacking the skin (Żbikowska 2003). The affected area is warm, swollen, and painful. A pricking, tingling, and sometimes burning sensation leads to discomfort and even insomnia (Żbikowska et al. 2002). The condition may be accompanied by other symptoms including swollen lymph nodes, diarrhea, nausea, or fever (Horák et al. 2002; Żbikowska et al. 2002). Occasionally, anaphylactic shocks or respiratory system disorders may also be observed (Bayssade–Dufour et al. 2001). The disease intensity depends on the individual susceptibility of the host (Kolářová et al. 2013a).

The swimmer's itch is a recurrent disease listed also in Poland. Therefore, there are several reasons for monitoring the prevalence of these parasites *Trichobilharzia* spp. in snail populations: (i) the abundance of snails releasing cercariae of *Trichobilharzia* spp., (ii) the abundance of water birds, (iii) frequent cases of itchy rashes (with a range of symptoms), especially in children, (iv) the lack of data on the behavior of cercariae invading humans through the skin. It is recommended that monitoring should be conducted in two ways: (i) using molecular method to identify particularly dangerous nasal schistosomes and (ii) conducting environmental inspection of recreational water bodies. The present study was aimed at investigating the diversity of Trichobilharzia species in Poland and highlighting the risk of swimmer's itch based on the spread of bird schistosomes in intermediate snail host populations (prevalence and seasonal fluctuations of parasite invasion, shell size of snail hosts) and in comparison with overall risk of Digenea invasion.

Material and methods

Field sampling

Lymnaeid snails (intermediate hosts of *Tricholilharzia* spp.), namely *Lymnaea stagnalis*, *Radix* spp., and *Stagnicola palustris* were collected monthly from May to September in 2016 and 2017. In 2016, they were collected from seven lakes of central and northern Poland: Głuszyńskie (52° 29′ 8″ N, 18° 38′ 13″ E), Ostrowąskie (52° 49′ 46″ N, 18° 42′ 3″ E), Służewskie (52° 51′ 14″ N, 18° 38′ 38″ E), Skulskie (52° 28′ 0″ N, 18° 19′ 18″ E) (Kuyavian-Pomeranian Voivodeship), Skulska Wieś (52° 28′ 58″ N, 18° 19′ 34″ E) (Greater Poland Voivodeship), Szymbarskie (53° 36′ 52″ N, 19° 30′ 39″ E) (Warmian-MasurianVoivodeship), and Wodna Dolina (Water Valley) (54° 10′ 45″ N, 16° 11′ 15″ E) (West Pomeranian Voivodeship), while in 2017, from three lakes: Głuszyńskie, Skulskie, and Skulska Wieś. Research sites were selected based on a combination of factors such as preliminary parasitological tests of snails in 2015, the presence of waterfowl, and previous reports of swimmer's itch episodes.

The snails, collected from the littoral zone (depth of ca. 0.5–1.5 m) of each lake by two researchers within the span of 1 h and under stable weather conditions, were transported to the laboratory in containers with lake water and examined for Digenea invasion.

Snail/cercaria examination

Piechocki's and Wawrzyniak-Wydrowska's (2016) and Jackiewicz's (2000) keys were used for morphological and anatomical identification of snails. Shell sizes (shell lengths) were measured using an electronic caliper (accuracy of 0.1 mm). Snails were placed individually in beakers with a small amount of conditioned tap water and exposed to artificial light for 3 h to stimulate the release of cercariae. Larval species were preliminarily determined using a light microscope (Primostar Carl Zeiss) and available keys, descriptions, and pictures from numerous publications on these parasites (Combes 1980; Našincová 1992; Faltýnková et al. 2007, 2008; Cichy and Żbikowska 2016). When no cercariae were released into water, an autopsy of snail hepatopancreas and gonads was carried out. The digenetic species were identified from fully developed cercariae. Morphologically classified larvae of bird schistosomes were subjected to molecular identification.

DNA extraction, PCR amplification, sequencing, and phylogenetic analyses

The suspension of bird schistosome cercariae was centrifuged. The isolated larvae were preserved in ethanol (96%) and frozen (at − 20 °C) for subsequent molecular identification (Jouet et al. 2008). Several dozens of cercariae were used for DNA extraction. Prior to DNA extraction, cercariae were centrifuged at 5000*g* for 5 min and washed three times in PBS buffer (pH 7.4). Total genomic DNA was isolated with Sherlock AX (A&A Biotechnology, Gdynia, Poland), according to the manufacturer's instruction. The quality and quantity of the isolated DNA was assessed in gel electrophoresis (1% agarose gel). The partial nuclear ribosomal 28S rDNA (D1–D3) gene (28SrDNA) of cercariae released from *Radix* spp. was amplified using the forward primer DLS1 (5′-ACCCGCTGAACTTAAGCATATCACTAAGC-3′) (Laskowski and Rocka 2014) and the reverse primer 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′) (Tkach et al. 2003). A fragment of the ribosomal DNA of bird schistosomes invading *L. stagnalis*, spanning the sequences of internal transcribed spacers 1, 2, and 5.8S (ITS), was amplified using the forward primer its5Trem (5′-GGAAGTAAAAGTCG TAACAAGG-3′) and the reverse primer its4Trem (5′-TCCT CCGCTTATTGATATGC-3′) (Dvorák et al. 2002) according to PCR conditions described by Dvorák et al. (2002).The amplified products were purified with Clean-Up (A&A Biotechnology, Gdynia, Poland) according to the producer's manual. DNA product sequencing in both directions was carried out by Genomed S. A., Warsaw. From the obtained genetic material, one sample from each research site for each snail species was used for sequencing. Furthermore, species membership of the analyzed individuals was identified with phylogenetic approach within two datasets (28rDNA and ITS). Newly sequenced haplotypes, along with homological DNA sequences from GenBank (Supplementary Material 1, 2), were first aligned using Muscle algorithm (Edgar 2004) implemented in Seaview software (Gouy et al. 2010). After the alignment, the sequences were cut to obtain a uniform block of sequences and a model of nucleotide substitution was chosen for each dataset using jModelTest 2.1.10 (Darriba et al. 2012). Next, both datasets were analyzed with MrBayes 3.2.6 (Ronquist et al. 2012) using GTR +G as the best-fit model. Two independent runs of four chains starting from different random trees were used. The trees were sampled every 100th generation for 25,000,000 generations of Markov chain steps and all trees making up the final tree were probed when the average standard deviation between the runs was much lower than 0.01.

Statistical analysis

Seasonal infection fluctuations were calculated and the results were analyzed using Friedman rank test followed by post-hoc Wilcoxon signed-rank test. A chi-square test of contingency table was used to determine statistical differences in the number of snails infected with bird schistosomes and uninfected ones between four shell size classes. The same analysis was used to compare the numbers of snails infected and uninfected with Digenea. Next, post hoc test based on standardized residuals was used. Standardized residuals presented the degree to which an observed value deviates from the expected value in terms of a z score (Sidanius et al. 2008). A standardized residual (SR) of plus or minus 1.96 presented a significant deviation from 0 at the $p = 0.05$ level (Sidanius et al. 2008). Statistical analysis was prepared based only on data on bird schistosomes from *L. stagnalis*. The term prevalence was used for the description of one snail species invaded by one parasite species.

Results

Larval trematode infection in Lymnaeidae

We collected a total of 3456 snails (2484 in the first and 972 in the second year of study): 2325 individuals of *L. stagnalis*, 890 *Radix* spp., and 240 *S. palustris*. Over 30% of the collected Lymneaidae were infected with Digenea. The infection was most frequent among *L. stagnalis* (36.34%) (Table S1), followed by *S. palustris* (21.25%) (Table S2), and *Radix* spp. (18.08%) (Table S3). The following species were most frequent in *L. stagnalis*: *Diplostomum pseudospathaceum* Niewiadomska, 1984 (in 27.3% of infected snails), *Opisthioglyphe ranae* (Frolich, 1791) (20%), *Plagiorchis elegans* (Rudolphi, 1802) (12.5%), and *Echinoparyphium aconiatum* (Dietz, 1909) (11.5%). *Radix* spp. were most frequently invaded by *O. ranae* (19.9%), *Cotylurus* sp. (13.7%), *Echinoparyphium recurvatum* (Linstow, 1873) (12.4%), and *P. elegans* (8.1%), while *S. palustris,* by *D. pseudospathaceum* (27.5%), *E. aconiatum* (9.8%), *Hypoderaeum conoideum* (Bloch, 1782) (9.8%), *Molinella anceps* (Molin, 1859) (9.8%), and *O. ranae* (9.8%). Bird schistosomes were recorded in 1.24% of all examined snails. *L. stagnalis* were the most common host of bird schistosomes (1.68%), followed by *Radix* spp. (0.44%). *S. palustris* were not infected with these parasites (Fig. 1). *L. stagnalis* were found in all seven studied lakes, while *Radix* spp., only in two (Fig. 1).

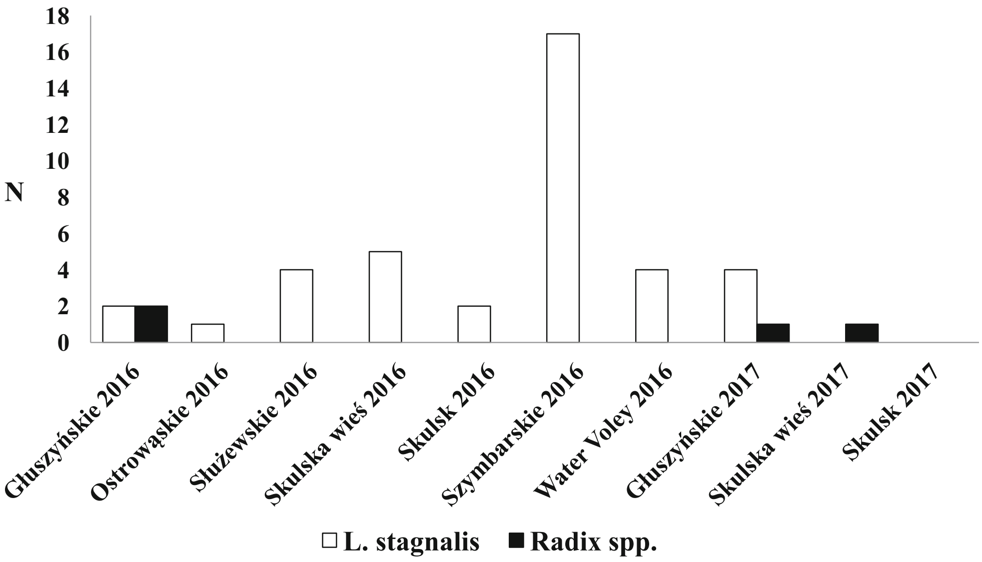
Molecular analyses of cercariae of bird schistosomes

As a result of sequencing, we revealed four haplotypes for the ITS and one haplotype for the 28SrDNA (GenBank accession numbers: MH190224, MH190225, MH190226, MH190227, MH190228). Phylogenetic tree created with 28S rDNA (Fig. 2) (Table S4) showed that the one haplotype presented in this study belonged to *Trichobiharzia regenti*. Haplotype from our study, along with sequence of *T. regenti* possessed from GenBank NCBI, created one clade with the highest possible probability (100%) of the common node. Similarly, phylogenetic tree created for ITS (Fig. 3) (Table S5) allowed to determine species belonging of four revealed in this study haplotypes as *Trichobiharzia szidati.* All four haplotypes from this study connected with five sequences of *T. szidati* from GenBank and formed very well-supported (100% posterior probability) and distinct genetic clade.

Seasonal fluctuations of Digenea larvae invasion in *L. stagnalis*

Snails infected with bird schistosomes were found in all research months in 2016. Statistically significant seasonal fluctuations of schistosome infection were observed ($N = 7$, $\chi^2 = 12.036$, $\mathrm{df} = 4$, $p = 0.017$) (Fig. 4a). A similar trend (Fig. 4b) is reflected in statistically significant differences for seasonal infection of all Digenea species found inside *L. stagnalis* individuals ($\mathrm{N} = 7$, $\chi^2 = 14.857$, $\mathrm{df} = 4$, $p = 0.005$). The post hoc test indicated that *L. stagnalis* infected with Digenea larvae was most frequently recorded in July and August.

Fig. 1 The number of Lymnaeidae infected with bird schistosomes at all research sites



Infection of *L. stagnalis* with Digenea larvae related to four shell size classes

Bird schistosomes were found in snails whose shell length ranged from 30.9 to 63.1 mm. We identified four shell size classes: class size I < 40.0 mm of shell length, class size II 40.1–44.9 mm, class size III 45.0–49.9 mm, and class size IV ≥ 50.0 mm. The number of bird schistosome hosts from individual size classes was significantly different ($\chi^2 = 9.42$, df = 3, $p = 0.02$). The post hoc test indicated that infected individuals of the highest shell size class were statistically significant. Similarly, the number of *L. stagnalis* infected with all Digenea (Fig. 5) was significantly dependent on correlated with shell size classes ($\chi^2 = 119.83$, df = 3, $p < 0.001$). Moreover, the post hoc test indicated that individuals from the highest and also lowest classes were statistically significant (Fig. 5).

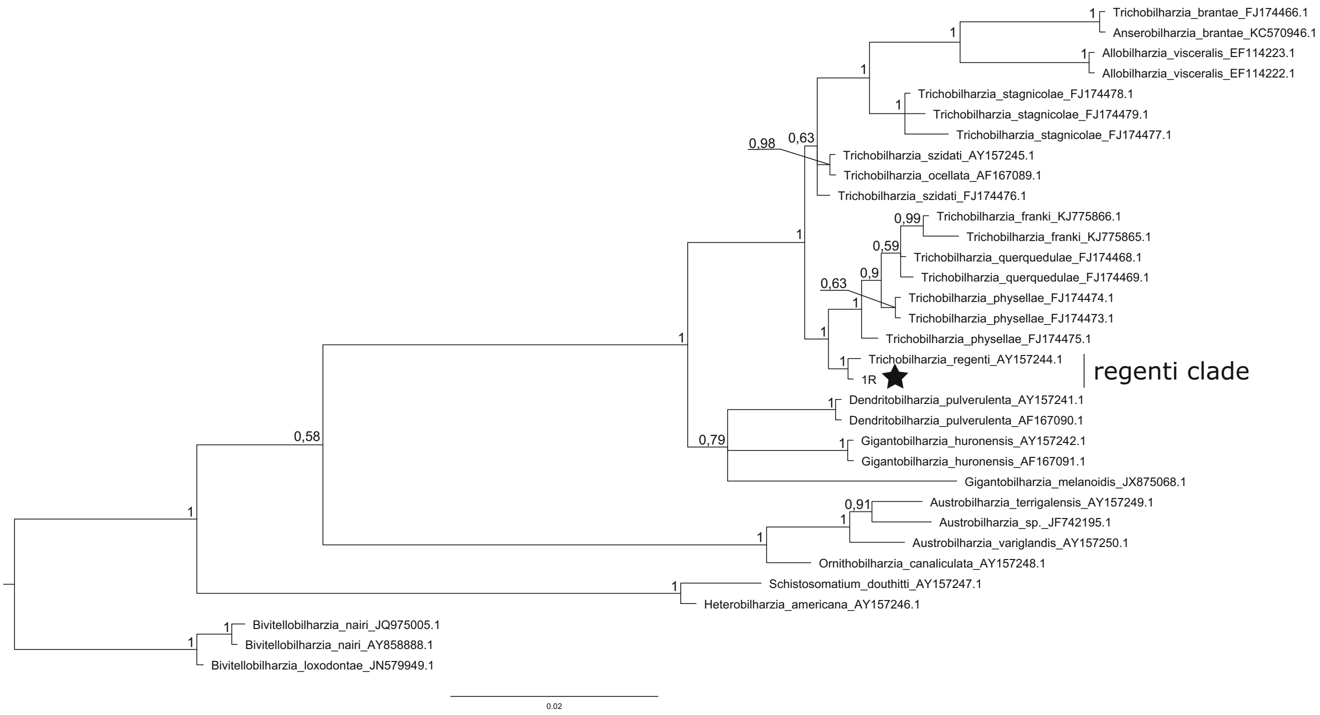


Fig. 2 Bayesian phylogenetic tree of dataset X consisted of 33 sequences of bird schistosomes. Three sequences from representatives of *Bivitellobriharzia nairi* were used as an outgroup. Numbers along the nodes are posterior probability of the node. DNA sequence revealed in this study is marked with a star

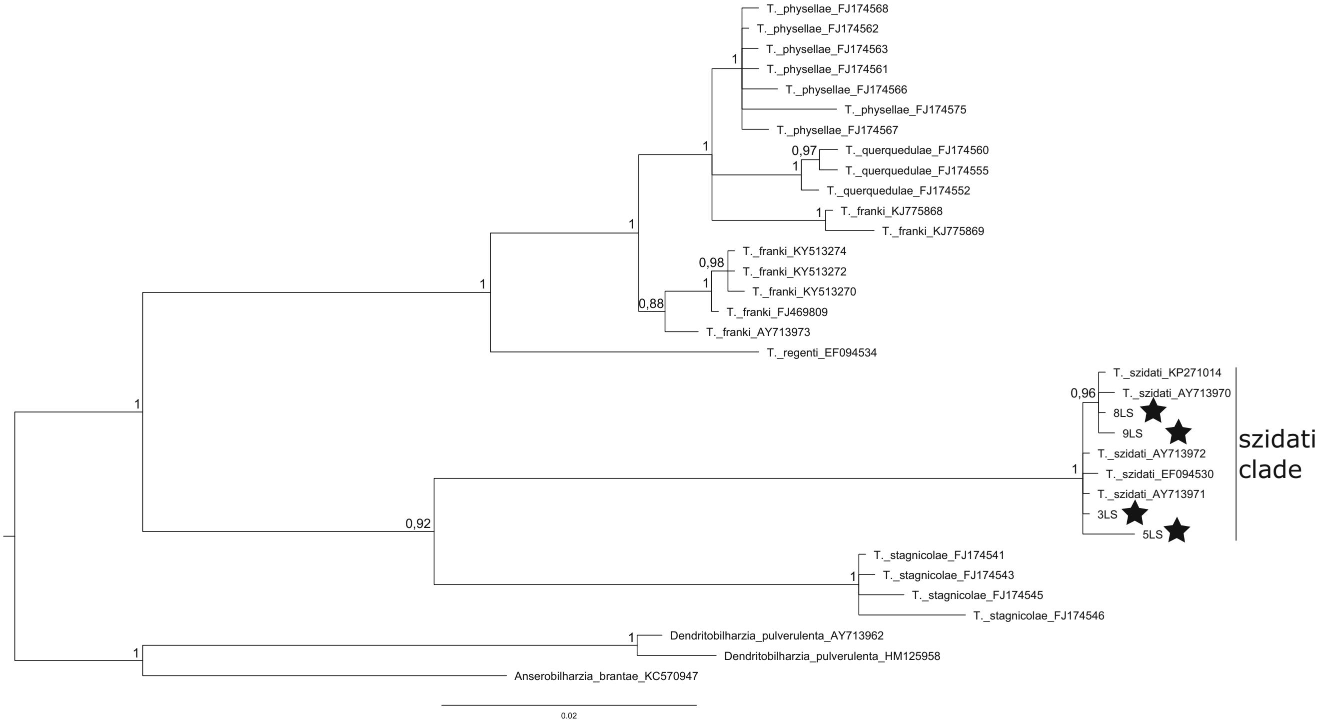


Fig. 3 Bayesian phylogenetic tree of dataset X consisted of 34 sequences of bird schistosomes. Three sequences from representatives of *Dendrirobiharzia pulverulenta* and *Anserobiharzia brantae* were used as an outgroup. Numbers along the nodes are posterior probability of the node. DNA sequence revealed in this study is marked with a star

Discussion

Symptoms of swimmer's itch caused by bird schistosomes depend greatly on their species. Since morphological and anatomical identifications of cercariae are unreliable (Dvorák et al. 2002; Horák et al. 2002; Rudolfová et al. 2005; Podhorský et al. 2009), human health risk assessment should be based on a molecular analysis of these larvae. Recent reports (Jouet et al. 2008, 2015; Christiansen et al. 2016) show much higher species diversity within the genus *Trichobilharzia* in Europe than previously thought. The following species have been recorded: *Trichobilharzia anseri* (Jouet et al. 2015), *Trichobilharzia franki* (Müller and Kimmig 1994), *Trichobilharzia mergi* (Kolářová et al. 2013b), *Trichobilharzia salmanticensis* (Simon-Martin and Simon-Vicente 1999), *T. regenti* (Horák et al. 1998), and *T. szidati* (Neuhaus 1952). Our study is the first to report the presence of *T. regenti* in Polish water bodies. The results also confirm widespread occurrence of *T. szidati* in Polish freshwater snails. What is surprising, despite the examination of nearly 1000 *Radix* sp. individuals, none of them was infected with *T. franki*—a bird schistosome widely distributed in the European populations of snail and waterfowl (Jouet et al. 2010). Both recorded species, as well as mentioned *T. franki* are widely recognized as causal agents of swimmer's itch (Müller and Kimmig 1994; Żbikowska 2004). Bird schistosomes have been found in the majority of Polish lymnaeid species, but many reports have been limited to giving their complex name *T. ocellata* (Żbikowska 2004; Żbikowska et al. 2006; Cichy 2013). Only isolated cases of the presence of *T. szidati* in *L. stagnalis* (Żbikowska 2005) and *S. palustris* (Cichy 2013) as well as *T. franki* in *R. auricularia* (Żbikowska 2004) have been reported. In our previous study (Marszewska et al. 2016), we made an assumption that cercariae found in *R. balthica* belonged to the nasal bird schistosome species *T. regenti*. Molecular analysis performed in the present study confirmed this assumption. Also, the successful experimental infection of *R. balthica* by miracidia of *T. regenti* confirms the developing of this bird schistosome inside this species of *Radix* snails (Marszewska et al. 2018). However, the lack of molecular diagnosis of the *Radix* individuals naturally infected with *T. regenti* larvae does not allow to give the species name of hosts, that the conchological and anatomical data indicate that they were not snails belonging to the *R. auricularia* (Jackiewicz 2000; Piechocki and Wawrzyniak-Wydrowska 2016). For humans, the invasion of *T. regenti* may be more dangerous than that of other bird schistosomes. Nasal schistosomes migrate inside the final host through the nervous system (Kolářová et al. 2001; Leontovyč et al. 2016). Experimental studies have indicated that the movement of *T. regenti* within the nervous system of unusual mammalian hosts led to leg paralysis (Kouřilová et al. 2004; Lichtenbergová et al. 2011; Horák and Kolářová 2001). Although so far bird schistosomes have not been found inside

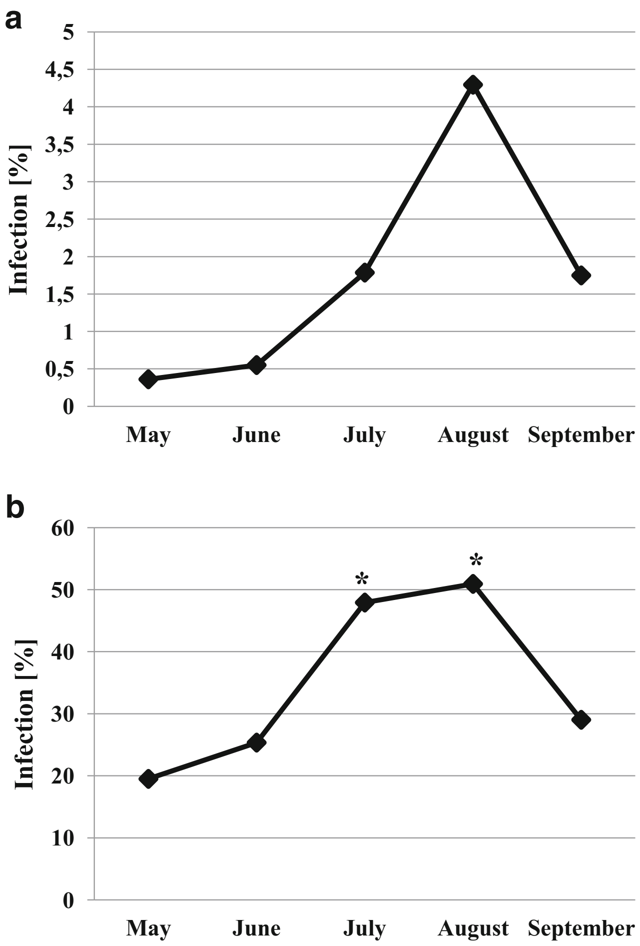


Fig. 4 Seasonal infection of *Lymnaea stagnalis* with **a** *Trichobilharzia szidati* and **b** all digenean species in the growing season 2016. *Statistically different from May and June (post hoc Wilcoxon signed-rank test, $p < 0.05$)

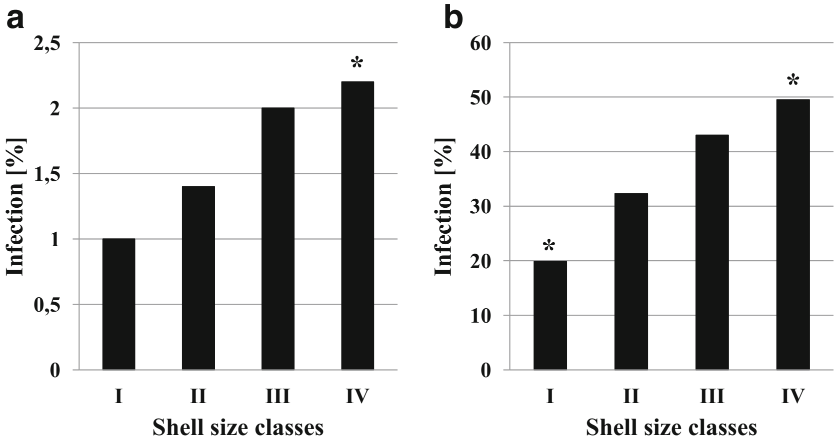
the human body (Horák et al. 2015), it should be emphasized that in laboratory larvae of *Trichobilharzia* sp. invaded mammals through the skin and migrated to their internal organs (Horák and Kolářová 2001). In an experiment using mice, schistosomules of bird schistosomes were found in the lungs (Appleton and Brock 1986; Haas and Pietsch 1991; Horák and Kolářová 2000), heart, kidneys, liver, and intestines of these rodents (Haas and Pietsch 1991). According to Olivier (1953), they have also been found in the lungs of other mammalian hosts including hamsters, guinea pigs, rabbits, and even rhesus monkeys.

In Polish lakes, the prevalence of bird schistosomes was very low (Fig. 1), which corresponds to the observations of other European water bodies. The prevalence of *Trichobilharzia* spp. in intermediate hosts ranges from 0.05 to 5% (Soldánová et al. 2013) and is far lower than that of other Digenea. It should be noted that low prevalence of snails infected with these parasites does not exclude high risk of swimmer's itch (Chamot et al. 1998; Lévesque et al. 2002; Farahnak and Essalat 2003; Skírnisson and Kolárová 2005; Jouet et al. 2008). This can be explained by very high bird schistosome cercarial emission (significantly higher than of other Digenean species) (Żbikowska 2005). Even low prevalence of invaded snails is sufficiently balanced by high intensity of cercarial release.

On the other hand, high risk of swimmer's itch in European lakes results from seasonal fluctuations of the invasion. The results of our present and previous studies (Żbikowska 2004) confirm bird schistosome infection in snails collected from May to September, with the highest prevalence in the peak of the summer season (Fig. 4a). Therefore, risk assessment and preventive measures (e.g., removing snails from lakes) are extremely important (Chamot et al. 1998; Lévesque et al. 2002; Caumes et al. 2003; Verbrugge et al. 2004; Jouet et al. 2008).

Many authors have described seasonal changes in the prevalence of bird schistosomes and other Digenean species in snail hosts, indicating the highest rate during the warmest and the lowest during the coldest months (Loy and Haas 2001; Żbikowska et al. 2006; Żbikowska and Nowak 2009; Brown et al. 2010). This results from the fact that temperature has a huge impact on the life cycle of trematodes (Mas-Coma et al. 2009; Żbikowska and Cichy 2012). High temperature facilitates the transmission of parasites in the environment (Poulin 2006; Cichy et al. 2016) and stimulates the production of cercariae

Fig. 5 Infection of *Lymnaea stagnalis* of different shell size classes with **a** *Trichobilharzia szidati* and **b** all digenean species in the growing season 2016. Asterisks (*) indicate groups significantly contributing to the differences in the parasite presence (*z*-scores of standardized residuals)



inside molluscs (Kendall and McCullough 1951; Lo and Lee 1996; Poulin 2006).

Finally, bird schistosome invasion inside host snails is an important factor affecting swimmer's itch risk level. Our research shows that large snails are more often infected with flukes than small ones (Fig. 5), which is in line with the observations of other researchers (Loker 1983; Brown et al. 1988; Sorensen and Minchella 1998; Graham 2003; Sichun et al. 2005). According to Sichun et al. (2005), this correlation is beneficial for the parasite. Larger host snails provide greater energy resources and/or more space for the production of invasive cercariae (Graham 2003; Sichun et al. 2005). More intensive invasion of Digenea in snail hosts results in a bigger number of cercariae released into water and therefore with a higher risk of swimmer's itch. The fact that larger snails are more often infected with these trematodes depends on many factors (Sturrock 1966; Baudoin 1975; Wilson and Denison 1980; McCarthy et al. 2004; Żbikowska et al. 2006; Miura and Chiba 2007). First of all, it may be connected to the preferences of parasites, which choose larger host snails over small ones (Baudoin 1975). On the other hand, it is well-know that the parasite may affect the host's phenotypic traits, for example as the size of the shell (Miura and Chiba 2007). Scientists postulate that digenetic trematodes contribute to the abnormally large shells of host molluscs, known as parasitic gigantism (Wilson and Denison 1980; McCarthy et al. 2004; Żbikowska et al. 2006). However, when the snails are not yet infected, bigger snails are usually older snails and have more time to meet potentially a larger number of invasive larvae (Graham 2003; Sichun et al. 2005). Finally, smaller (younger) host snails are characterized by greater mortality because of the parasite (Sturrock 1966; Baudoin M; 1975) and as a result, smaller infected individuals are harder to find in the environment. This point of view is supported by our observation that large specimens of *L. stagnalis* invaded with bird schistosomes were collected in early May. Taking into account that the development of bird schistosomes from miracidia to cercariae takes about 7 weeks (Amen and Meuleman 1992), we can assume that snails releasing cercariae in May were invaded in autumn (McMullen and Beaver 1945; Jarcho and van Burkalow 1952) and survived winter (Horák et al. 2002). This situation is beneficial for bird schistosomes and increases the risk of swimmer's itch, especially in the situation of recent climate change, and the earlier beginning of the recreational season in the temperate zone.

In conclusion, there are numerous indicators of real risk of swimmer's itch including the following: (i) recent detection of the presence of potentially most dangerous nasal schistosome *T. regenti* in Poland, (ii) widespread presence of snails infected with *T. szidati* in Polish water bodies, (iii) widespread presence of these parasites during the summer season, (iv) frequent presence of these parasites in larger (more resistant) hosts. In view of these facts, we believe that it is necessary to develop effective methods of protection against cercarial dermatitis.

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

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Potamopyrgus antipodarum as a potential defender against swimmer's itch in European recreational water bodies - experimental study.

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Potamopyrgus antipodarum as a potential defender against swimmer's itch in European recreational water bodies—experimental study

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ABSTRACT

Swimmer's itch is a re-emerging human disease caused by bird schistosome cercariae, which can infect bathing or working people in water bodies. Even if cercariae fail after penetrating the human skin, they can cause dangerous symptoms in atypical mammal hosts. One of the natural methods to reduce the presence of cercariae in the environment could lie in the introduction of non–host snail species to the ecosystem, which is known as the "dilution" or "decoy" effect. The caenogastropod *Potamopyrgus antipodarum*—an alien in Europe—could be a good candidate against swimmer's itch because of its apparent resistance to invasion by European bird schistosome species and its high population density. As a pilot study on this topic, we have carried out a laboratory experiment on how *P. antipodarum* influences the infestation of the intermediate host *Radix balthica* (a native lymnaeid) by the bird schistosome *Trichobilharzia regenti*. We found that the co–exposure of 200 *P. antipodarum* individuals per one *R. balthica* to the *T. regenti* miracidia under experimental conditions makes the infestation ineffective. Our results show that a non–host snail population has the potential to interfere with the transmission of a trematode via suitable snail hosts.

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INTRODUCTION

Biodiversity loss and disease emergence have become two of the most challenging issues confronting science and society (*Johnson et al., 2009*). Different authors indicate the strong correlation between parasite success in ecosystems and the biodiversity of ecological communities (*Johnson et al., 2012*; *Lagrue & Poulin, 2015*). *Mitchell et al. (2003)*, *Begon (2008)*, *Allan et al. (2009)* as well as many others underlined that rapid loss of populations significantly increase disease emergence. Studies concerning the causal relationship between biodiversity and disease emergence in an environment are focused on testing the "dilution effect", which parasitologists refer to as a "decoy-effect" hypothesis (*Combes & Moné, 1987*; *Johnson & Thieltges, 2010*). According to these authors, the "decoy effect"

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mechanisms, observed in the case of high biodiversity of ecological communities, concern: (i) degeneration of invasive parasite stages penetrating the non-target host, (ii) exhausting of these stages by trying to penetrate the non-target host and (iii) stimulation of defense mechanisms of the non-target host against invasive stages of parasite. Regardless of the mechanism, the non-target host becomes the dead-end host, that is the real factor reducing the parasitic disease emergence (*Mehlhorn, 2008*).

One of the re-emerging worldwide medical problems connected to parasites of complex life cycle is cercarial dermatitis, also known as swimmer's itch (*Cort, 1936*; *Hunter et al., 1949*; *Jarcho & Van Burkalow, 1952*; *Macy, 1952*; *Hoeffler, 1974*; *Leedom & Short, 1981*; *Eklu-Natey et al., 1985*; *Blankespoor & Reimink, 1991*; *Loken, Spencer & Granath Jr, 1995*; *Pilz, Eisels & Disko, 1995*; *Lindblade, 1998*; *Kolářová, Skírnisson & Horák, 1999*; *Horák et al., 2015*). *Marszewska et al. (2016)* observed this medical problem in many bathing localities in Polish Lowland Lakes during the last two years. The dermatitis appears as an itchy, lumpy rash on the skin that persists for several weeks (*Żbikowska, Wójcik & Grygon-Franckiewicz, 2002*). The skin lesions resemble the early stage of chickenpox, and are a result of penetration by cercariae of bird schistosomes (*Żbikowska, 2003*). Normally, cercariae of bird schistosomes develop inside the host snail for six to seven weeks (*Amen & Meuleman, 1992*). Cercariae then abandon the mollusk, swim in the water environment seeking to penetrate the skin of an avian final host; once in the skin they transform to schistosomulae, then they migrate through the blood or nervous pathway, mature, and reproduce sexually (*Soldánová et al., 2013*). If a human becomes the accidental target of a cercariae attack, an allergic skin reaction may follow, but the worms do not mature in humans (*Kolářová, Horák & Skírnisson, 2010*; *Horák et al., 2015*).

The current increase in the number of swimmer's itch cases in temperate climate might be a consequence of both: (i) climate change accompanied by the extension of the period of active vegetation in freshwater ecosystems, linked with abundant populations of host snails releasing bird schistosome cercariae, and (ii) people spending more time in recreational activities (*Angilletta Jr, 2006*; *Rempfer et al., 2010*). Biomass of cercariae of the bird schistosome *Trichobilharzia szidati* can even reach 4.65 tons per year for a small eutrophic reservoir (*Soldánová, Selbach & Sures, 2016*).

The above factors limit safe water recreation (*Chamot, Toscani & Rougemont, 1998*; *Lévesque et al., 2002*; *Farahnak & Essalat, 2003*; *Skírnisson & Kolárová, 2005*; *Jouet et al., 2008*). Efforts to reduce human cercarial dermatitis have been made by using some trematode species or by lowering the density of first intermediate host snail populations; however, such efforts were not always successful (Chapter 1; *Loker & Hofkin, 2015*). The mechanical removal of potential intermediate hosts of bird schistosomes brings only limited positive effects (*Dubois, 2003*), and the use of molluscicides, however successful for a short period, has a clear limitation (see as review: *King & Bertsch, 2015*) or even a negative impact on local fauna (*McCullough, 1992*).

The increasing number of cases of human cercarial dermatitis together with our knowledge on migration of bird schistosomes in mammalian hosts (*Horák & Kolářová, 2001*; *Horák et al., 2008*; *Horák et al., 2015*) foster research on natural methods that may decrease the risk. As for human schistosomes, biological control has been tested in some areas (see review: *Pointier, David & Jarne, 2011*) and promising results have been obtained for the use of applied alien or even invasive snail species for reduction of parasite prevalence in the snail hosts. The "decoy effect" described by *Combes & Moné (1987)* can be another mode of resolving the problem. *Combes & Moné (1987)* indicate that *Schistosoma mansoni* miracidia can fail to actively penetrate non-host snails. We suspect that the same variant of biological control can be useful in the case of bird schistosomes, especially when using the planned alien species for human cercarial dermatitis control—*Potamopyrgus antipodarum*, which has been present in European waters for years (*Boycott, 1936*; *Walter, 1980*; *Dorgelo, 1987*; *Ponder, 1988*; *Simoes, 1988*; *Hinz, Boeters & Guenther, 1994*; *Berg et al., 1997*; *Carlsson, 2000*; *Wagner, 2000*; *Mouthon & Dubois, 2001*). The presence of this New Zealand native species has been recorded in several European countries (*Gérard & Le Lannic, 2003*; *Zettler & Richard, 2004*; *Sousa, Guilhermino & Antunes, 2005*; *Alonso, 2006*; *Lewin & Smolinski, 2006*; *Soler, 2006*; *Cianfanelli, Lori & Bodon, 2007*; *Múrria, Bonada & Prat, 2008*; *Son, Nabozhenko & Shokhin, 2008*; *Zieritz & Waringer, 2008*; *Radea, Louvrou & Economou-Amilli, 2008*; *Arle & Wagner, 2013*), but only a few reports have given it the status of invasive species (*Brzeziński & Kołodziejczyk, 2001*; *Gaino et al., 2008*; *Thomsen et al., 2009*). *P. antipodarum* spreads easily thanks to its wide tolerance to environmental factors and its parthenogenetic reproduction, so a population can start from a single female. In some non–native regions even up to six generations per year can develop (*Piechocki & Wawrzyniak-Wydrowska, 2016*). *P. antipodarum* can create populations with densities reaching thousands of individuals per square meter under favorable conditions (*Richards, Cazier & Lester, 2001*; *Hall Jr, Tank & Dybdahl, 2003*), but densities may undergo a drastic collapse in a few months (*Extence, 1981*; *Moffitt & James, 2012*) or in a longer term (*Moore et al., 2012*; *Gérard, Hervé & Hechinger, 2017*). These top-down and bottom-up changes in invaded ecosystems can be extremely temporally dynamic and connected to environmental factors (*Moore et al., 2012*). Among the reasons for a collapse, an impact of acquired parasites was postulated. Even if parasites cannot complete the life cycle in *P. antipodarum* due to host–parasite incompatibility (*Żbikowski & Żbikowska, 2009*), the penetration of miracidia or cercariae through the tegument can be devastating for snails. The expansion of *P. antipodarum* in European waters and scarce cases of its stable association with a trematode species (*Gérard & Le Lannic, 2003*; *Morley, 2008*; *Gérard, Hervé & Hechinger, 2017*; *Żbikowska & Nowak, 2009*) resulted in the hypothesis that the introduction of *P. antipodarum* to the European bathing localities may help eliminate the risk of dermatitis in a safe way.

Our pilot laboratory experiments aimed at evaluating the potential impact of *P. antipodarum* on the effectiveness of *T. regenti* (an avian schistosome) miracidia to infect the natural, native host snail *Radix balthica*.

MATERIALS AND METHODS

Snails

Radix balthica (Linnaeus, 1758) (Pulmonata: Basommatophora: Lymnaeidae) is one of the most common pond snails in Poland (*Piechocki & Wawrzyniak-Wydrowska, 2016*).

Based on external morphology, these snails are similar to *R. labiata* (Rossmassler, 1835). Therefore, the species-level taxonomy within the *Radix* genus was verified on the basis of anatomical features of the reproductive system (*Schniebs et al., 2011*). *R. balthica* is the intermediate host for many digenean species, such as bird schistosomes, including *T. regenti* (*Horák, Kolářová & Dvořák, 1998*; *Cichy, Faltynkova & Żbikowska, 2011*). In the experiment, 40 *R. balthica* individuals with shell height of 8–10 mm (mean size: 9.0 ± 0.1) and shell width of 4–6 mm (mean size: 5.1 ± 0.1) (very susceptible to parasitic invasion) were used. All *R. balthica* individuals came from laboratory breeding cultures of the Department of Invertebrate Zoology at *Nicolaus Copernicus University* in Toruń, Poland.

Potamopyrgus antipodarum (Gray, 1843) (Caenogastropoda, Hydrobioidea, Tateidae) is a mud snail species introduced from New Zealand to Europe in the mid 1850s (*Hubendick, 1950*). In Poland it was first found in Lake Trląg (*Urbański, 1938*). Nowadays it is common in Pomerania, Greater Poland, Masurian Lakeland and Upper Silesia (*Cichy, Faltynkova & Żbikowska, 2011*). In the experiment, parthenogenetic females with shell height of 4 mm (most prevalent during summer season in Poland) were used. The snails were collected from Sosno Lake (53°20′15″N, 19°20′55″E) in May 2016.

Bird schistosome

Trichobilharzia regenti (Schistosomatidae, Bilharziellinae) was described by *Horák, Kolářová & Dvořák (1998)*. As for the maintenance of parasites in the laboratory, the intermediate host snails of *Radix lagotis* were kept in aquaria with sponge filters, fed on lettuce leaves, and repeatedly collected and placed in glass beakers to stimulate release of cercariae after lighting. The definitive hosts, ducks (*Anas platyrhynchos* f. dom.), were kept in cages approved for this purpose (accreditation no. 13060/2014-MZE-17214). Their infection with cercariae was performed as described by *Meuleman, Huyer & Mooij (1984)*. After 22 days, the ducks were sacrificed by decapitation to obtain eggs with developing miracidia, and adult trematodes living in the nasal mucosa. The maintenance care and sacrificing of experimental animals was carried out in accordance with European Directive 2010/63/EU and Czech law (246/1992 and 359/2012) for biomedical research involving animals. Experiments have been performed under legal consent of the Expert Committee of the Section of Biology, Faculty of Science, Charles University, Prague, Czech Republic, and the Ministry of Education, Youth and Sports of the Czech Republic under ref. no. MSMT-31114/2013-9.

Four ducks in the patent period (22 days post infection) were sacrificed, and their heads immediately (within 10 h) transported to the Polish laboratory at the temperature of 8 °C. In the laboratory nasal conchae were removed from the duck beaks and torn apart in Petri dish with conditioned tap water to release eggs and hatched miracidia, which were then placed in a dark flask with conditioned tap water at 20 °C. The flask was placed under artificial light. After a few minutes, hatched miracidia were concentrated under illuminated water surface. The larvae were then individually collected with a micropipette.

Experiment I: the infection of *P. antipodarum* snails with miracidia of *T. regenti*

P. antipodarum individuals were experimentally infected in Petri dishes (50 mm in diameter) with conditioned tap water at 20 °C. In the experiment two variants were applied—one miracidium per one snail, and five miracidia per one snail. The experiment was performed in 25 replicates. The time of exposure was 20 h, and was adjusted to the duration of the miracidia life span (*Horák et al., 2015*). Then snails were carefully placed into beakers with conditioned tap water at 20 °C, and water in Petri dishes was checked under a stereomicroscope for presence of living or dead miracidia.

Twice a week, the water in incubation beakers was changed, and the *P. antipodarum* individuals were fed. Every day the activity of snails was tested. Dead individuals were immediately checked for parasite infestation. After 60 days all surviving snails were killed and autopsied.

Experiment II: the infection of *R. balthica* in the presence of *P. antipodarum* with miracidia of *T. regenti*

During this experiment snails were exposed to miracidia in Petri dishes (50 mm in diameter) filled with conditioned tap water at 20 °C. In the experiment, three variants of non–host snail density were applied: 50, 100 and 200 individuals of *P. antipodarum* per one individual of *R. balthica*. The size of experimental *P. antipodarum* groups was determined according to the ratio of number specimens of both snail species per square meter in different Polish water bodies (*Żbikowski & Żbikowska, 2009*; *Strzelec, Krodkiewska & Królczyk, 2014*). *R. balthica* were placed individually in the central part of the dish, whereas *P. antipodarum* specimens were arranged around them. For each dish with snails, three newly hatched miracidia of *T. regenti* were added, according to a common laboratory procedure (*Lichtenbergová et al., 2011*). The dishes were covered and placed in the incubator (SANYO, Osaka, Japan) at 20 °C and natural photoperiod for 24 h (adjusting the time to the maximum survival of larvae). The experiment was performed in 10 replicates. The three control groups of snails constituted (i) separately placed individual of *R. balthica* with three miracidia (without *P. antipodarum*), and (ii) separately placed individual of *P. antipodarum* with three miracidia (without *R. balthica*)—both in 10 replicates. The additional, third control consisted of only one Petri dish with fifty *P. antipodarum* snails incubated together with fifty miracidia (Table 1). After 24 h, the control snails were rinsed with water and placed separately in beakers with conditioned tap water at 20 °C. Similarly to experiment I, the water was changed and the snails were fed twice a week. Every day their activity was observed. Dead individuals were immediately checked for parasite infestation. After 60 days all remaining (living) snails were killed and autopsied.

Statistical analysis

The prevalence of *T. regenti* in both snail species populations was counted as percent of specimens of *R. balthica* or *P. antipodarum* with bird schistosome larvae (sporocysts and/or cercariae). In order to verify if the density of *P. antipodarum* accompanying *R. balthica* can affect *T. regenti* miracidia infectivity, logistic regression was used, with the *Potamopyrgus antipodarum* abundance as a predictor variable and infection status of *Radix balthica* as

Table 1 The exposure of host and/or non-host snails on *Trichobilharzia regenti* miracidia at 20 °C—Experiment II.

Number of snails exposed to parasitic larvae[a]	Number of miracidia	Number of replicates	Experimental condition
1 *R. balthica* + 50 *P. antipodarum*	3	10	Experimental
1 *R. balthica* + 100 *P. antipodarum*	3	10	Experimental
1 *R. balthica* + 200 *P. antipodarum*	3	10	Experimental
1 *R. balthica*	3	10	Control
1 *P. antipodarum*	3	10	Control
50 *P. antipodarum*	50	1	Control

Notes.

[a] Animals were placed in Petri dishes of 50 mm diameter.

a dependent variable. Snail life time since exposure to miracidia, expressed in number of days, was analyzed by one–way ANOVA, followed by post–hoc Tukey test. Significant differences in the survival rates between infected and uninfected *R. balthica* were tested using Mann–Whitney U test.

RESULTS

None of the *P. antipodarum* individuals exposed to *T. regenti* miracidia in Experiment I or Experiment II were found to be infected. During Experiment I, after 20 h of incubation we did not find parasitic larvae in the water of the Petri dishes, where *P. antipodarum* snails were individually exposed to three miracidia. Also, no patent infection (with fully developed cercariae) was found in *R. balthica* specimens experimentally exposed to *T. regenti* miracidia. Inside the infected snails only sporocysts or sporocysts with immature cercariae were noticed. The effective infestation of *R. balthica* by *T. regenti* (Table S1) depended on the number of accompanying *P. antipodarum* individuals during exposure to miracidia (Table S2). Abundance of *Potamopyrgus antipodarum* significantly decreased the probability of infection of *Radix balthica* (logistic regression: Wald statistic = 9.5, $df = 1$, $p = 0.002$) (Fig. 1). Almost all control *R. balthica* individuals (90%), and all *R. balthica* snails co-incubated with 50 specimens of *P. antipodarum* exposed to miracidia had parasite sporocysts. The infestation of *R. balthica* was completely ineffective in the density combination of 200 *P. antipodarum* per one *R. balthica* specimen ($p < 0.0001$). When 100 *P. antipodarum* individuals were co–exposed to miracidia, up to 40% *R. balthica* snails were non–infected, however, the difference was not statistically significant if compared with the *P. antipodarum*—absent control ($p = 0.0867$).

Experimental conditions had an impact on the life span of snails. All *P. antipodarum* individuals survived until the end of the experiment (60 days), whereas the life span for *R. balthica* varied (Table S3). The average survival of *R. balthica* ranged from 35 to 58 days, and depended on the presence of non–host *P. antipodarum* snails during exposure to miracidia (one–way ANOVA $F_{3,36} = 16.85$, $p < 0.001$)). Post–hoc tests indicated that the longest survival rate occurred in the case of *R. balthica* exposed to miracidia in the presence of 200 individuals of *P. antipodarum* (Fig. 2). Additionally, the Mann–Whitney U test ($p < 0.001$) indicated that infected *R. balthica* lived shorter than non–infected ones

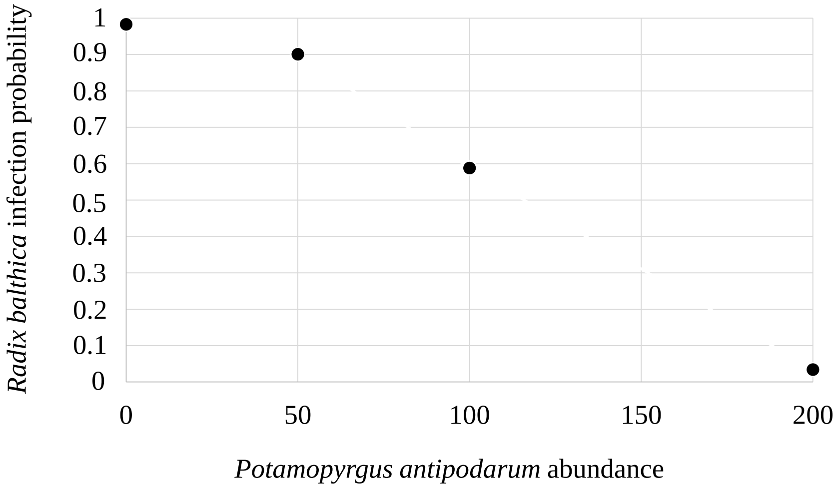

Figure 1 **Infection probability of *Radix balthica* predicted by the logistic regression model on the basis of the abundance of *Potamopyrgus antipodarum*.**

Full-size DOI: 10.7717/peerj.5045/fig-1

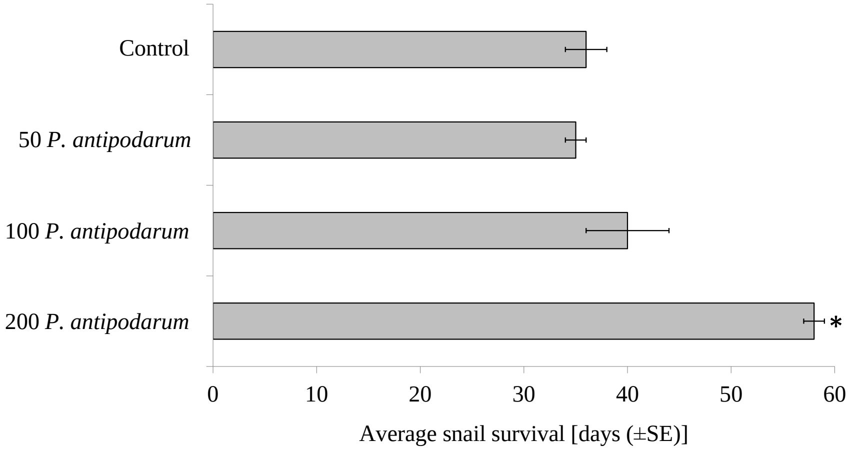

Figure 2 **Survival of *Radix balthica* exposed to *Trichobilharzia regenti* miracidia in the presence of different number of *Potamopyrgus antipodarum* specimens.** (*) the Mann–Whitney U test: $p < 0.001$.

Full-size DOI: 10.7717/peerj.5045/fig-2

regardless of experimental condition (avg. 34 ±1 and 57 ±1 days respectively) (Table S4, Fig. 2). Infected *R. balthica* survived for 18–40 days (range) after exposure to miracidia, while most non-infected *R. balthica* lived until the end of the 60 day experiment.

DISCUSSION

Our study indicates that *T. regenti* larvae cannot use *P. antipodarum* as an intermediate host succesfully. This is not surprising because all known *Trichobilharzia* species use only Lymnaeidae and Physidae as intermediate hosts (Horák et al., 2015). On the other hand, the lack of miracidia in water after 20 h exposure to snails is extremely interesting. It could be the effect of parasitic larvae entering the snail shell or even possible attachment to non-host

P. antipodarum body. *Sapp & Loker (2000)* observed miracidia which tended to adhere to incompatible snails, but these larvae could not develop inside a non-host mollusk. It should be noted that according to *King, Jokela & Lively (2011)* trematodes have only one chance when they attach to a snail body—succesful infection or death. *Combes & Moné (1987)* described the impact of non-target hosts on parasite success as a "decoy effect" and suggested the "decoy effect" as potentially useful in schistosomiasis control. Unfortunately, the protocol of our experiment did not allow us to track the fate of *T. regenti* miracidia, because the snails were stored in the incubator during the exposure period. Observations after the end of exposure revealed the lack of invasive larvae on Petri dishes, which could be the result of them being swallowed by snails or the effect of the degeneration of the unsuccessful larvae that died during the experiment. The only certainty is that the presence of non–host snails (*P. antipodarum*) of bird schistosome (*T. regenti*) in the neighborhood of native host (*R. balthica*) can affect the parasite transmission success of miracidia. The hatched larvae respond to different environmental stimuli, such as light or gravity, and various chemical compounds released by potential host species (*Hertel et al., 2006*). *Smyth & Halton (1983)* when using the choice–chamber to study miracidial chemo–orientation indicated that nearly half of the tested larvae were attracted by chemical attractants other than those released by their specific host snail. *P. antipodarum* individuals co–exposed to miracidia may have successfully disturbed the access of parasitic larvae to the specific host. However, the lack of data on the chemical composition of *P. antipodarum* mucus does not allow a clear conclusion that the lack of miracidia in water after 20 h exposure to individuals of this species during Experiment I could support our hypothesis.

According to *Sullivan & Yeung (2011)*, miracidia that were experimentally injected into snails were encapsulated inside incompatible hosts, but survived and developed in the compatible ones only. The result shows that real recognition of the intruder by the immune system of the host depends on the internal milieu of the snail. This fact allows us to understand why the imprecise identification of the host by miracidia does not result in snail—Digenea compatibility (*Combes & Moné, 1987*). We suggest that the probable lack of precision in *T. regenti* miracidia orientation could be used for the biological control of this trematode invasion in the environment.

The introduction of an alien snail species into the environment, even to protect people against parasites, may raise doubts concerning long-term consequences of manipulation in the environment: (i) the influence on populations of native snail species, and (ii) the danger of a new parasite–snail association. As for the first consequence, data on the displacement of native European snail species by *P. antipodarum* seem to be exaggerated. Some statistical analysis has shown the coincidence between the appearance of *P. antipodarum* in water bodies and a drop in Simpson's diversity index. Such an analysis was presented by *Strzelec, Spyra & Krodkiewska (2006)* who used number of individuals as currency in the Simpson's diversity index. It should be emphasized that the large numbers of the small *P. antipodarum* could easily drive down the Simpson index (or any other abundance-based diversity index) if numbers are used as currency. In our opinion the biomass would be more appropriate currency in such analysis.

The threat of new snail-parasite association seems to be more serious collateral damage to planned manipulation in the environment (*Morley, 2008*). However, it must be emphasized that *P. antipodarum* already occurs in European waters, and the possible introduction into recreational waters would only slightly increase its range (*Städler et al., 2005*). From New Zealand, where this mud snail plays the role of intermediate host for many avian parasite species, there are no reports of infection of *P. antipodarum* with *Trichobilharzia* species (*Hechinger, 2012*), even if *Trichobilharzia quequedulae* was noted in birds of the Southern Hemisphere (*Ebbs et al., 2016*). The facts above indicate potentially safe use of *P. antipodarum* against swimmer's itch.

The absence of patent infection in *R. balthica* individuals after an experimental exposure is also of interest. *Huňová et al. (2012)* underlined that the intramolluscan development of *T. regenti* needs several weeks. In our experiments, none of the successfully infected *R. balthica* lived longer than 40 days. The increased mortality of snails experimentally infected with trematodes is widely known (*Muñoz Antoli et al., 2007*; *Kalinda, Chimbari & Mukaratirwa, 2017*), especially when juvenile snails are exposed to miracidia. In our experiments, the *R. balthica* snails that remained uninfected after exposure to *T. regenti* lived longer than the infected ones (Fig. 2). Many of them survived until the end of the 60 day experiment. As the limiting factor for parasite invasion of *R. balthica* seems to be the presence of *P. antipodarum* individuals, it can be concluded that the presence of non–host snails during exposure to miracidia indirectly increased the survival of *R. balthica* hosts. Although the results do not show clear evidence of the non–invasive nature of *P. antipodarum*, they may suggest an additional, indirect effect of this snail species on native malacofauna in new areas. Our results highlight the additional aspect of the influence of alien snail species on native malacofauna. Ecologists emphasize the direct changes caused by newcomers (*Riley, Dybdahl & Hall Jr, 2008*). In our opinion, especially in the case of research on freshwater snails, the aspect of their association with trematodes should be taken into account in analyses concerning the impact of alien species introduction.

CONCLUSION

The data represent a pilot study that precedes a wide–planned series of field and laboratory studies focused on the influence of alien molluscan species, namely *P. antipodarum*, on possible reduction of swimmer's itch in European recreational water bodies. Our experimental work demonstrates that a high population density of *P. antipodarum* lowers the transmission of bird schistosomes miracidia to suitable snail hosts such as *R. balthica*. Further research will focus on the potential ability of *P. antipodarum* to limit native gastropod infections in natural conditions.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Anna Marszewska conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables.
- Anna Cichy analyzed the data.
- Jana Bulantova contributed reagents/materials/analysis tools.
- Petr Horak contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper.
- Elzbieta Zbikowska conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The maintenance and care of experimental animals were carried out in accordance with the European Directive 2010/63/EU and Czech law (246/1992 and 359/2012) for biomedical research involving animals. Experiments have been performed under legal consent of the Expert Commission of the Section of Biology, Faculty of Science, Charles University in

Prague and the Ministry of Education, Youth and Sports of the Czech Republic (ref. no. MSMT–31114/2013–9).

Data Availability

The following information was supplied regarding data availability:

The raw data are supplied in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5045#supplemental-information.

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Publikacja nr 4

The chemotactic swimming behavior of bird schistosome miracidia in the presence of compatible and incompatible snail hosts

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The chemotactic swimming behavior of bird schistosome miracidia in the presence of compatible and incompatible snail hosts

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ABSTRACT

No effective method has yet been developed to prevent the threat posed by the emerging disease—cercarial dermatitis (swimmer's itch), caused by infective cercariae of bird schistosomes (Digenea: Schistosomatidae). In our previous studies, the New Zealand mud snail—*Potamopyrgus antipodarum* (Gray, 1853; Gastropoda, Tateidae)—was used as a barrier between the miracidia of *Trichobilharzia regenti* and the target snails *Radix balthica*. Since the presence of non-indigenous snails reduced the parasite prevalence under laboratory conditions, we posed three new research questions: (1) Do bird schistosomes show totally perfect efficacy for chemotactic swimming behavior? (2) Do the larvae respond to substances emitted by incompatible snail species? (3) Do the excretory-secretory products of incompatible snail species interfere with the search for a compatible snail host? The experiments were carried out in choice-chambers for the miracidia of *T. regenti* and *T. szidati*. The arms of the chambers, depending on the variant, were filled with water conditioned by *P. antipodarum*, water conditioned by lymnaeid hosts, and dechlorinated tap water. Miracidia of both bird schistosome species chose more frequently the water conditioned by snails—including the water conditioned by the incompatible lymnaeid host and the alien species, *P. antipodarum*. However, species-specific differences were noticed in the behavior of miracidia. *T. regenti* remained more often inside the base arm rather than in the arm filled with water conditioned by *P. antipodarum* or the control arm. *T. szidati*, however, usually left the base arm and moved to the arm filled with water conditioned by *P. antipodarum*. In conclusion, the non-host snail excretory-secretory products may interfere with the snail host-finding behavior of bird schistosome miracidia and therefore they may reduce the risk of swimmer's itch.

Subjects Animal Behavior, Ecology, Parasitology, Zoology
Keywords *Trichobilharzia* spp., *Potamopyrgus antipodarum*, Lymnaeid hosts, Miracidia, Chemo-orientation

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INTRODUCTION

Cercarial dermatitis (swimmer's itch) caused by bird schistosomes (Digenea: Schistosomatidae) is considered an emerging disease in Europe (*Horák et al., 2015*; *Tracz et al., 2019*). These parasites have a complex life cycle. The intermediate hosts are

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freshwater snails, whereas the final hosts are predominantly waterfowl. Snails infected with bird schistosomes release huge numbers of infective cercariae (*Soldánová, Selbach & Sures, 2016*). Thus, even with a low prevalence of bird schistosomes in intermediate host populations, there is a real threat for final hosts (*Marszewska et al., 2016*). As a result of the similarity of some lipid components in the integument of humans and birds, bird schistosome cercariae can accidentally penetrate the skin of people swimming or wading in the water (*Haas & Van de Roemer, 1998*). An attack by many infective larvae may be accompanied by some additional reactions, such as diarrhea, nausea, limb and lymph node swelling, and/or fever (*Horák et al., 2015*). The removal of freshwater snails, the source of infective cercariae, and waterfowl, the source of miracidia infective for the snails, from the environment to reduce the risk of human swimmer's itch provides equivocal effects (*Lévesque et al., 2002*; *Jouet et al., 2008*). Also, chemical methods such as the use of molluscicides may have an adverse impact on local fauna (*McCullough, 1992*). An increasing number of cercarial dermatitis outbreaks from year to year (*Marszewska et al., 2016*; *Tracz et al., 2019*), as well as alarming data on bird schistosome migration inside the body of experimental mammals (*Horák & Kolářová, 2001*; *Horák et al., 2008*) stimulated our interest in the biological control that would be applicable, especially in recreational water bodies.

Prevalence of digeneans in snails may be significantly reduced when a non-host snail species occurs in sympatry with the target snails and miracidia—the first larval stages of the parasites (*Kalbe, Haberl & Haas, 1997*). In detail, snail-finding by miracidia is influenced by several factors. The larvae react to, for example, the chemical cues secreted by snails (*Hertel et al., 2006*; *Seppälä & Leicht, 2015*), and light stimuli (*Gryseels et al., 2006*) with changes in movement. The larvae have a limited lifespan (*Anderson et al., 1982*) and may exhaust their penetration enzymes and energy reserves during unsuccessful penetration attempts (*Combes & Moné, 1987*), or they can penetrate into incompatible snails whose plasma kills the unspecific species of digenean trematodes (*Sapp & Loker, 2000*).

Free-living larvae of parasites and their hosts live in the midst of a complex biocenosis; as a result, their transmission takes place within a diverse community of non-host organisms that can have a huge influence on the success or failure of parasite transmission (*Hopper, Poulin & Thieltges, 2008*). The phenomenon of the reduction of disease risk as a result of the increased diversity of co-occurring non-host species is commonly known as the "dilution effect" (*Keesing, Holt & Ostfeld, 2006*; *Kopp & Jokela, 2007*; *Johnson & Thieltges, 2010*; *Cichy et al., 2016*). Studies have shown several ways how these non-host organisms can disrupt parasite transmission, for example, by acting as physical barriers (*Christensen, 1979*), predation, that is, active feeding on larvae (*Thieltges et al., 2008*; *Thieltges, Jensen & Poulin, 2008*; *Vielma et al., 2019*), non-host filter-feeders (*Mouritsen & Poulin, 2003*; *Hopper, Poulin & Thieltges, 2008*; *Marszewska & Cichy, 2015*; *Selbach, Rosenkranz & Poulin, 2019*) or decoys attracting the parasites (*Thieltges, Jensen & Poulin, 2008*). However, not all non-host species are of equal importance as diluters (*Hopper, Poulin & Thieltges, 2008*). For example, *Hopper, Poulin & Thieltges (2008)* demonstrated that only selected filter-feeding species have a significant influence on the transmission of cercariae by feeding on these larvae, while other sympatric filter-feeders have no measurable effects on metacercariae formation in target second intermediate host. Kopp & Jokela (2007) showed that particularly alien species can be involved in the "dilution effect", that is, the snail species incompatible for an infectious agent can serve as a local protective shield for compatible hosts. For example, *Lymnaea stagnalis* (Linnaeus, 1758) (Gastropoda: Pulmonata), a species non-native to New Zealand, serves as a defender of the native *Potamopyrgus antipodarum* (Gray, 1843) (Gastropoda: Tateidae) against the trematode *Microphallus* sp. (Digenea: Microphallidae) (Kopp & Jokela, 2007). In our previous study (Marszewska et al., 2018), we made a successful attempt to experimentally use *P. antipodarum*, a snail species non-native to Europe, to interfere with the finding of a compatible lymnaeid host by bird schistosome miracidia.

On the other hand, it should be emphasized that nonindigenous animal species may become competent hosts for indigenous parasites (Kelly et al., 2009), or can be the source of pathogens with a possible/unpredictable effect on domestic fauna. In the native range, *P. antipodarum* is widely used by numerous digenean species, but there are no reports of bird schistosome infestation (Hechinger, 2012; Selbach, Rosenkranz & Poulin, 2019). However, *P. antipodarum* living in European waters is extremely rarely infected with a pre-patent and/or a patent invasion of digenean parasites (Gérard & Le Lannic, 2003; Żbikowski & Żbikowska, 2009; Gérard et al., 2017). In addition, *P. antipodarum* infection by some bird schistosome species failed under experimental conditions (Rind, 1989; Marszewska et al., 2018).

We hypothesize that any interference with snail host-finding by bird schistosome miracidia might influence the risk of swimmer's itch. Thus, the aim of the present research was to examine (1) whether the miracidia of bird schistosomes show totally perfect efficacy for chemotactic swimming behavior, (2) whether the larvae respond to substances emitted by incompatible snail species, (3) whether the excretory-secretory products of incompatible snail species disturb the search for a compatible snail host.

MATERIALS AND METHODS

Obtaining miracidia

Two specimens of definitive hosts—*Anas platyrhynchos* f. dom.—were experimentally infected by *Trichobilharzia regenti* (Digenea: Schistosomatidae), and two specimens of the same duck species were experimentally infected by *T. szidati* (Digenea: Schistosomatidae) according to the procedure described by Meuleman, Huyer & Mooij (1983).

In order to obtain *T. regenti* eggs, the host nasal conchae were isolated during necropsy 20 days post infection (dpi), while the bird droppings were collected 18 dpi to obtain *T. szidati* eggs. Experimental duck hosts were euthanized by inhalation of isoflurane followed by decapitation.

The collected biological material with eggs was placed in dark long-necked flasks with dechlorinated tap water at 20 °C. After a few minutes, having hatched the first larval stages (miracidia) were clustered under the illuminated water surface. The miracidia were then individually collected with a micropipette.

Animal ethics statement

Care and maintenance of experimental animals were carried out in accordance with European Directive 2010/63/EU and Czech law (246/1992 and 359/2012) for biomedical research involving animals. Experiments were performed with the legal consent of the Expert Committee of the Section of Biology, Faculty of Science, Charles University, Prague, Czech Republic, and the Ministry of Education, Youth and Sports of the Czechia under ref. no. MSMT-33740/2017-2

Water conditioning process

As to the snail species, we prepared 3 types of conditioned water: (1) young individuals of *Radix lagotis* (Gastropoda: Pulmonata) (compatible host to *T. regenti*)—shell height of 6 (SE 0.1) mm; (2) young individuals of *L. stagnalis* (compatible host to *T. szidati*)—shell height of 9 (SE 0.3) mm; (3) adult parthenogenetic females of *P. antipodarum*—shell height of 3 (SE 0.2) mm. We used younger (smaller) lymnaeid snail individuals to increase the number of snails for water conditioning to reduce the impact of individual characteristics of particular specimens. Lymnaeid snails came from our laboratory breeding, whereas *P. antipodarum* came from the natural environment—Sosno Lake, Poland (53°20′15′′N, 19°20′55′′E); before the experiments they were examined by non-invasive method for the presence of patent invasions (*Żbikowska et al., 2006*). So far, no pre-patent invasion of *P. antipodarum* has been reported in Sosno Lake (*Cichy et al., 2017*; *Marszewska et al., 2018*).

The water conditioning process was conducted in beakers at room temperature. Inside each beaker, 5 g of living snails (97 individuals of *R. lagotis*, 60 individuals of *L. stagnalis* or 116 individuals of *P. antipodarum*) in 100 ml of dechlorinated water were kept for 2 h. This concentration was used to assure enough quantity of an emitted substance in case it had a low effect. After the incubation, the snail-conditioned water was filtered through filter paper. Only freshly prepared substances no older than 24 h were used in the experiment.

Bird schistosome miracidial chemotactic swimming behavior

The experiment was carried out in choice-chambers (Fig. 1) designed by *Haberl et al. (1995)*. The larvae were released from the base arm (Fig. 1) with 1 ml of dechlorinated tap water to choose between two side arms. Depending on the variant (Table 1), the side arms were filled with 1 ml of water conditioned by *P. antipodarum*, water conditioned by lymnaeid hosts and/or dechlorinated tap water. Each variant of the experiment was performed in three series of 10 repetitions at 20 °C under standardized artificial even lighting. These series were conducted within a few minutes directly one after another.

In total, 30 replicates were performed for each parasite species per variant. One larva was placed in the base arm of the choice-chamber (Fig. 1) for each repetition. A new larva was used for each replicate. After 3 min, the junction between all the arms was closed and the location of the larvae was checked under a stereoscopic microscope. The entire arm including part of the migratory channel (Fig. 1) was considered the choice of the larva. The choice-chambers were thoroughly washed and dried before each use.

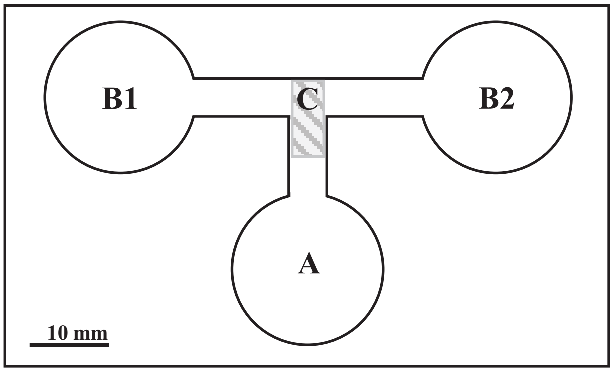

Figure 1 The choice-chamber for evaluation of bird schistosome miracidial chemotactic swimming behavior (channel depth: 3 mm). A, base arm; B1 and B2, side arms; C, closure.

Full-size DOI: 10.7717/peerj.9487/fig-1

Table 1 The experimental variables.

No	First arm	Second arm
I*	Dechlorinated tap water	Dechlorinated tap water
II	Compatible lymnaeid host**	Dechlorinated tap water
III	Incompatible lymnaeid host***	Dechlorinated tap water
IV	*Potamopyrgus antipodarum*	Dechlorinated tap water
V	Compatible lymnaeid host	Incompatible lymnaeid host
VI	Compatible lymnaeid host	*Potamopyrgus antipodarum*
VII	Incompatible lymnaeid host	*Potamopyrgus antipodarum*

Notes:

* Control experiment.

** *R. lagotis* for *T. regenti* and *L. stagnalis* for *T. szidati.*

*** *L. stagnalis* for *T. regenti* and *R. lagotis* for *T. szidati.*

Statistical analysis

We used a binomial test (*Zar, 1984*) to assess if the occupation of the side arms by larvae departed from the random distribution (assuming 50% probability of entering each arm) (Table 1). The tests were performed separately for each variant and species of the parasite, taking only the larvae present in the side arms (B1 and B2, Fig. 1) into account. Analyses were conducted in Microsoft Excel (version 2013).

RESULTS

In the control experiment (dechlorinated tap water vs dechlorinated tap water) the miracidia of both bird schistosomes, *T. regenti* and *T. szidati*, occupied arms of the choice-chamber in similar proportions (Tables S1 and S2), and there were no significant statistical differences (a binomial test: $p = 0.4$, $p = 0.5$, respectively) (Fig. 2, I).

In the experiments with lymnaeid snail-conditioned water vs dechlorinated tap water (variants: II, III (Table 1)) statistically significant differences were observed in the distribution of miracidia (a binomial test: *T. regenti*—variant II: $p < 0.001$, variant III: $p < 0.01$; *T. szidati*–variant II: $p < 0.001$, variant III: $p = 0.001$) (Fig. 2, II–III). In variant IV, a higher number of larvae moved to *P. antipodarum*-conditioned water than to

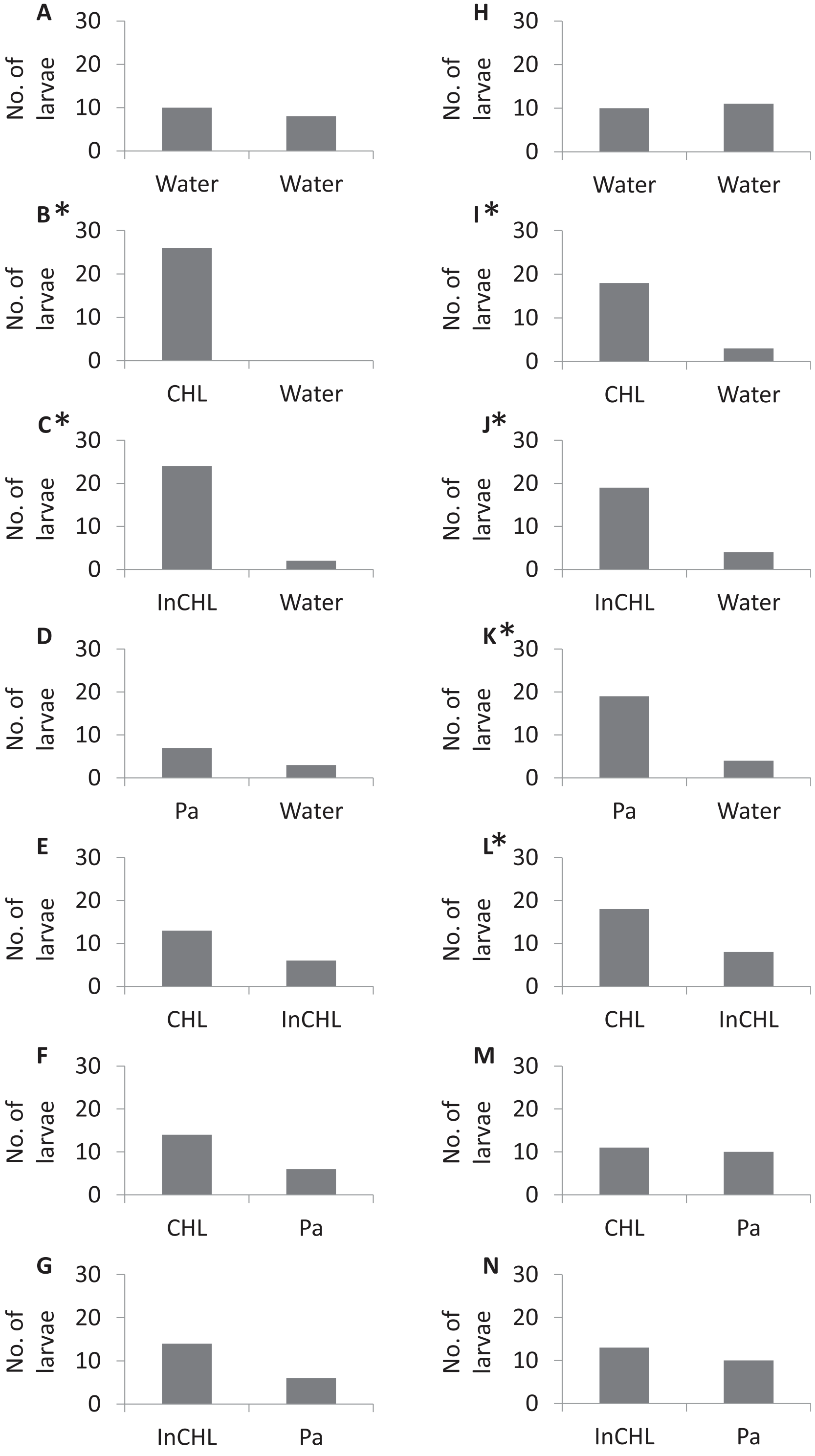

Figure 2 The number of miracidia in the side arms per serie. (A–G) Variants numbers from I to VII, respectively, for *T. regenti*; (H–N) variants numbers from I to VII, respectively, for *T. szidati*; Water, dechlorinated tap water; CLH, compatible lymnaeid host-conditioned water: *R. lagotis* for *T. regenti* and *L. stagnalis* for *T. szidati*; InCLH, incompatible lymnaeid host-conditioned water: *L. stagnalis* for *T. regenti* and *R. lagotis* for *T. szidati*; Pa, *P. antipodarum*; *Statistically significant a binomial test ($p < 0.05$).

Full-size DOI: 10.7717/peerj.9487/fig-2

dechlorinated tap water for both parasite species, but statistically significant differences were recorded only for *T. szidati* (a binomial test: $p = 0.001$) (*T. regenti*—a binomial test: $p = 0.2$). For the variant with *T. regenti*, only one-third of the larvae left the base arm. We were also able to observe statistically significant differences in variant V for *T. szidati* (a binomial test: $p = 0.04$). There was no significant statistical difference in variant V for *T. regenti* (a binomial test: $p = 0.08$). For both species of miracidia in variant V, we observed a higher number of the larvae in the arms filled by compatible lymnaeid host-conditioned water than those filled by incompatible lymnaeid host-conditioned water (Fig. 2, V).

There were no statistically significant differences in the variants VI and VII (lymnaeid snail-conditioned water vs *P. antipodarum*-conditioned water) (a binomial test: *T. regenti*—variant VI: $p = 0.06$, variant VII: $p = 0.06$; *T. szidati*—variant VI: $p = 0.5$, variant VII: $p = 0.3$). But, for miracidia of *T. regenti*, we observed a higher number of larvae in the arms filled by lymnaeid snail-conditioned water (compatible and incompatible) than by *P. antipodarum*-conditioned water, whereas for miracidia of *T. szidati*, the distribution of larvae in the arms was similar (Fig. 2, VI–VII).

DISCUSSION

The majority of digenean species show a high specificity for their first intermediate hosts (*Sapp & Loker, 2000*). The phenomenon is also observed for species belonging to the genus of *Trichobilharzia* (*Kock, 2001*). It is also well known that many digeneans use chemo-orientation to find suitable hosts in the water column (*Haas, 2003*). *Kalbe, Haberl & Haas (1997)* showed that the miracidia preferred water conditioned by their specific host snails compared to incompatible snail species, and did not respond to water conditioned by leeches, tadpoles, and fish. Our study supports these findings and shows that bird schistosome miracidia significantly more often choose water conditioned by host snails than unconditioned water (Fig. 2, II). However, our results indicate that bird schistosomes respond also to the excretory-secretory products of non-host snails (Fig. 2, III), leading to the interference with miracidial chemo-orientation towards the host and non-host snail-conditioned water (*T. regenti*—Fig. 2, V, VI; *T. szidati*—Fig. 2, VI). These observations coincide with the results of many authors (*Kalbe, Haberl & Haas, 1996*, *2000*; *Haberl et al., 2000*; *Haas, 2003*; *Hassan et al., 2003*; *Kalbe et al., 2004*), who showed some strains of *Schistosoma mansoni* could not distinguish between their specific and five incompatible host species. This behavior of miracidia indicates the adaptive plasticity of the parasite or the similarity of individual components present in the range of snail-derived stimulators.

Generally, our results present differences in the accuracy of chemotactic swimming behavior of the miracidia of bird schistosomes. The miracidia of *T. regenti* react to substrates from the host snails and from the snails closely related to the suitable hosts to such an extent that it interferes with finding the substrate from the target species. The larvae of *T. szidati*, despite reacting to non-host lymnaeid snails, in the vast majority cope with finding a suitable substrate in the variant consisting of non-host lymnaeid snail-conditioned water and host lymnaeid snail-conditioned water. It is well known that the used bird schistosome species migrate through the body of the final host in different ways, and also their eggs enter the external environment in other ways (*Horák et al., 2015*). Perhaps these differences then have their reflection in the accuracy of the first larval stages (hatched from the eggs) in search of a suitable host snail.

As to the species-specific reactions, *T. szidati* larvae more often choose water conditioned by *P. antipodarum* than dechlorinated tap water, while *T. regenti* larvae more often remain in the base arm (Fig. 2, IV). We know that the individual species of miracidia are adapted to the behavioral patterns of compatible snail species (*Behrens & Nollen, 1992*). *Lymnaea stagnalis* lives primarily at the water surface on aquatic plants, while young individuals of *Radix* spp. often form high-density populations covering the lake bottom in the shallow littoral zone, similar to *P. antipodarum* (*Piechocki & Wawrzyniak-Wydrowska, 2016*). We can only assume that the differences in the response of bird schistosome larvae to the *P. antipodarum*-conditioned water may be related to the above-mentioned difference between lymnaeid snail species.

Our observation is the first indication of these subtle differences in miracidial chemotactic swimming behavior, and the phenomenon merits further research.

CONCLUSIONS

Based on our previous (*Marszewska et al., 2018*) and present results it seems that the increased biodiversity of malacofauna may interfere with the life cycle of bird schistosomes (snail host-finding and penetration), and thus it can potentially reduce the risk of swimmer's itch. To prove this possibility, we plan to test in the future not only the chemotactic behavior/chemical cues, but also the ability of bird schistosome miracidia to penetrate the incompatible snail host (*P. antipodarum* in our case). Moreover, currently conducted large-scale field research should answer whether this non-native snail is occurring in lakes at sufficiently high densities to interfere with the spread of swimmer's itch.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Anna Marszewska conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Anna Cichy performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Jana Bulantová performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Petr Horák conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Elżbieta Żbikowska conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Experiments have been performed under the legal consent of the Expert Committee of the Section of Biology, Faculty of Science, Charles University, Prague, Czech Republic, and the Ministry of Education, Youth and Sports of the Czech Republic under ref. no. MSMT-33740/2017-2.

Data Availability

The following information was supplied regarding data availability:

The raw data are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.9487#supplemental-information.

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Toruń, 18.06.2020

OŚWIADCZENIE

Oświadczam, że mój udział w realizacji pracy:

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polegał na udziale w przeprowadzeniu badań i przygotowaniu manuskryptu w części dotyczącej molekularnej identyfikacji gatunkowej.

Udział swój oceniam na **20 %**.

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polegał na wykonaniu wywiadu środowiskowego dotyczącego zaprezentowanych przypadków „swimmers' itch" i wyznaczeniu terenu badań.

Udział swój oceniam na**10%**.

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To whom it may concern

I hereby declare that my contribution to the articles:

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was **10%** for each article (co-conducted the experiments, revised it critically for important content).

17. 6. 2020 in Prague, Czechia

Jana Bulantová

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