***Biomphalaria pfeifferi* and intestinal schistosomiasis in Lake Malawi**

Mohammad H. Alharbi1,2, Charlotte Condemine1, Rosie Christiansen1, E. James LaCourse1, Peter Makaula3, Michelle C. Stanton 4, Lazarus Juziwelo5, Seke Kayuni1,6 and J. Russell Stothard1,\*

1 Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK; 2 Ministry of Health, Qassim, Kingdom of Saudi Arabia; 3 Research for Health Environment and Development, P.O. Box 345, Mangochi, Malawi;4 Lancaster University Medical School, Lancaster, LA1 4YW, UK; 5 National Schistosomiasis and STH Control Programme, Ministry of Health, Lilongwe, Malawi; 6 Medi Clinic Limited, Medical Aid Society of Malawi (MASM), 22 Lower Sclatter Road, P.O. Box 1254, Blantyre, Malawi.

*\**Address for correspondence: Professor J.R. Stothard, Parasitology Department, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK. Tel: +44 (151)7053724. E-mail: russell.stothard@lstmed.ac.uk

**Abstract** Two malacological surveys have demonstrated *Biomphalaria pfeifferi* in Lake Malawi. The emergence of intestinal schistosomiasis was confirmed upon targeted epidemiological examination of 175 local children across three primary schools. Our findings highlight hitherto unknown autochthonous transmission of *Schistosoma mansoni* in Lake Malawi and the need to revise international travel advice.

Throughout sub-Saharan Africa, *Biomphalaria pfeifferi* is a keystone freshwater intermediate snail host for *Schistosoma mansoni*, the blood fluke responsible for intestinal schistosomiasis (*1*(, its geographical distribution broadly delineates actual or potential disease transmission zones (*2*). Save the historical report of a single *Biomphalaria* shell at Karonga in the far north of Lake Malawi by Smith (*3*), considered by Mandahl-Barth to be from a marginal swamp (*4*), *B. pfeifferi* does not occur within Lake Malawi (*5*). In November 2017, however, during malacological surveillance for intermediate hosts of schistosomiasis in Mangochi District at the southern-most tip of Lake Malawi, two discrete populations of *Biomphalaria* in submerged *Vallisneria* sp. beds were unexpectedly encountered (see Figure 1A). Upon DNA sequence analysis of the mitochondrial cytochrome oxidase subunit 1 (*cox*1) following (*6*), the obtained *cox*1 sequences (1,006 bp) differed from *B. pfeifferi* from Chiweshe, Zimbabwe (GenBank Accession numbers DQ084829, i.e. HCO/LCO region and DQ084872, i.e. Asmit1/2 region) in only 3 synonymous single nucleotide polymorphisms.

<*please insert Figure 1 near here*>

To clarify *B. pfeifferi* colonisation within the lake and suspected transmission risk of intestinal schistosomiasis, a conjoint malacological and parasitological survey was undertaken in May 2018 with ethical approvals from Liverpool School of Tropical Medicine, UK (application 17-018) and Ministry of Health and Population, Malawi (application 1805). All prior malacological sampling locations were re-visited with an additional 43 sites inspected. Further populations of *B. pfeifferi* were found (Figure 1A); its occurrence reaffirmed at site M9 in large numbers (n > 50) alongside innumerable dead shells. All *Biomphalaria* collected were inspected for shedding cercariae and although cercariae were seen by eye from M5 snails, identification by microscopy (x100) did not meet with success. Supplementary analysis of *cox*1 sequences from 9 snails from M2, M5, M7, M10 and M11 were identical.

The epidemiological survey examined 175 school children, aged 5-15 years of balanced gender, across three primary schools purposively selected closest to *B. pfeifferi* site M9 (Figure 1B). Mean prevalence of intestinal schistosomiasis by circulating cathodic antigen (CCA) urine-dipstick was 34.3% [95%CI 27.9-41.3] with prevalence by school variable: Samama 46.7% [95%CI 36.7-56.7], Mchoka 25.0% [95%CI 15.0-36.7] and Palm Beach 9.1% [95%CI 0.0-22.7]. A single stool sample was requested from each CCA positive child (n=60), with 46 children able to provide; upon duplicate Kato-Katz examinations, ova of *S. mansoni* were confirmed in 7 children and graded of light-infection intensity (< 100 eggs per gram of stool). All urine samples were inspected for ova of *Schistosoma haematobium* by filtration (10 ml) finding general prevalence of 14.9% [95%CI 9.8-20.1] with 52% of these also positive by CCA urine-dipstick indicative of *S. mansoni* co-infections. To further determine autochthonous transmission of *S. mansoni,* two egg-positive children from Samama and Mchoka pinpointed, by foot, their shoreline water collection/bathing site(s) and shown to match M10 and M11 locations (Figure 1B). Children either CCA-positive or *S. haematobium* egg-patent were provided with praziquantel (40 mg/kg) treatment.

The demonstration of successful colonisation of *B. pfeifferi* in Lake Malawi and in fringing water bodies is of concern, especially as active *S. mansoni* infectionswere found within local children. This highlights emergence of intestinal schistosomiasis, a disease not previously documented here (5, *7*, *8*) or detected within this vicinity by the most recent national survey (Fiona Fleming, SCI, UK personal communication). It has, however, been shown in children some 150 km away along the lower Shire River shoreline (*9*). Finding both snail and infected children here in Mangochi District is suggestive of recent ecological and epidemiological change. In May 2018, the lake level was some 75-80 cm higher than in November 2017 which perhaps favoured first detection of *Biomphalaria* in then more accessible *Vallisneria* beds. Seasonal dynamics, such as lake level fluctuations are well-known alongside more longer-term perturbations of the lake biota, either induced by climate change or mediated by anthropogenic activities; these have altered the transmission of urogenital schistosomiasis (*10*) with over-fishing, particularly of molluscivorous fish *Trematocranus placodon*, changing many freshwater snail distributions (*5*).

Local aquaculture of fish such as Chambo (*Oreochromis* sp.), using water pumped inland from the lake, has also created novel, permanent water bodies colonised by *B. pfeifferi* (e.g. M2-M7) which may now (re)seed snails into the lake to establish further. An absence of *cox*1 genetic diversity in *B. pfeifferi* sampled here implies a limited number or even single founder event but as conditions for autochthonous transmission became favourable, upon introduction of *S. mansoni*, emergence of intestinal schistosomiasis has occurred in local school children. This is of significant public health concern when set against current control with annual praziquantel distribution in schools (*7*, *8*) and we call for increased surveillance of snails and characterisation of schistosomes, with intensified control interventions to arrest further spread. Furthermore, health and travel advice given to shoreline communities and international tourists who make use of the lake here needs revision and updating.

Mr Mohammad Alharbi is a PhD student under the supervision of James LaCourse and Russell Stothard. He has specific interests in medical malacology and molecular epidemiology of schistosomiasis in Africa and Kingdom of Saudi Arabia.

**Acknowledgements**

We each thank Alexandra Shaw and Joanna Fawcett for assistance during the epidemiological survey in Mangochi District. We are grateful to the local health and education authorities of Malawi, district teachers, local community health workers (Flora Jumbe, Caroline Nthubula, Angelina Mwenyewe, Witness Mapira) and hosting communities for their enthusiasm and support. We are also indebted to Danie and Hazel Britz for assistance at Palm Beach School and to Paul and Stacey Kennedy for local boat hire, as well as, to Anthony Butterworth and Liz Corbett for their kind hospitality in Blantyre. MA and SK are funded by PhD scholarships from the Ministry of Health, Kingdom of Saudi Arabia and Commonwealth Scholarship Commission, respectively. Urine CCA-urine dipsticks were supplied by Rapid Medical Diagnostics, South Africa manufacturer lot number 171103130.

**References**

1. Brown DS. Freshwater snails of Africa and their medical importance*.* 1994,2nd Edition,

London: Taylor & Francis.

2. Stensgaard AS, Utzinger J, Vounatsou P, Hurlimann E., Schur N, Saarnak et al. Large-

scale determinants of intestinal schistosomiasis and intermediate host snail distribution across Africa: does climate matter? Acta Trop. 2013; 128: 378-390. doi: 10.1016/j.actatropica.2011.11.010.

3. Smith HE. On a collection of land and freshwater snails transmitted by Mr H.H. Johnston

C.B. from British Central Africa. Proc Zoo Soc (London). 1893; 632-641.

4. Mandahl-Barth G. The freshwater Mollusca of Lake Malawi. Revue Zoo Bot Afric. 1972;

86: 129-160.

5. Madsen H, Bloch P, Makaula P, Phiri H, Furu P, StaufferJR JR. Schistosomiasis in Lake

Malaŵi villages. Ecohealth, 2011:8;163-176. doi: 10.1007/s10393-011-0687-9.

6. Jorgensen A, Kristensen TK, Stothard JR. Phylogeny and biogeography of African

*Biomphalaria* (Gastropoda: Planorbidae), with emphasis on endemic species of the

great East African lakes. Zoo J Lin Soc, 2007:151; 337-349. doi.org/10.1111/j.1096-3642.2007.00330.x.

7. Mtethiwa AH, Nkwengulila G, Bakuza J, Sikawa D, Kazembe A. Extent of morbidity

associated with schistosomiasis infection in Malawi: a review paper. Inf Dis Pov.

2015; 4: 25. doi: 10.1186/s40249-015-0053-1.

8. Makaula P, Sadalaki JR, Muula AS, Kayuni S, Jemu S, Bloch P. Schistosomiasis in

Malawi: a systematic review. Parasites & Vectors. 2014: 7; 570. doi: 10.1186/s13071-014-0570-y.

9. Poole H, Terlouw DJ, Naunje A, Mzembe K, Stanton MC, Betson M, et al.

Schistosomiasis in pre-school-age children and their mothers in Chikhwawa district, Malawi with notes on characterization of schistosomes and snails. Parasites & Vectors, 2014: 7; 153.  doi: 10.1186/1756-3305-7-153.

10. Van Bocxlaer B, Albrecht C, StaufferJR, JR Growing population and ecosystem change

increase human schistosomiasis around Lake Malaŵi. Trends in Parasitol, 2014: 30, 217-220. doi: 10.1016/j.pt.2014.02.006.

**Figure 1A**. Diagrammatic map of all sampled locations in November 2017 (grey dots) and May 2018 (black dots) with presence(+)/absence(-) of *B. pfeifferi* in each survey with collected snail numbers indicated in white circles holding *B. pfeifferi* (2017, n=2; 2018 n = 10) versus those that did not (2017, n=12; 2018 n = 36). Over 50 *Biomphalaria* were collected at M9 on each sampling occasion. The GPS coordinates of *B. pfeifferi* positive sites M1 - M11 in decimal degrees are: M1: **S**14.27752°, **E**35.10419°; M2: **S**14.31371°, **E**35.14174°; M3: **S**14.31424°, **E**35.14383°; M4: **S**14.31354°, **E**35.14424°; M5: **S**14.31568°, **E**35.14030°; M6: **S**14.32033°, **E**35.13613°; M7: **S**14.32100°, **E**35.13072°; M8: **S**14.36919°, **E**35.17629°; M9: **S**14.39363°, **E**35.22104°; M10: **S**14.42708°, **E**35.23349° and M11: **S**14.44928°, **E**35.23890°. **Figure 1 B**. Outline position of the 3 sampled primary schools (Palm Beach School GPS: **S**14.391346°, **E**35.215137°; Samama School GPS: **S**14.417465°, **E**35.217580°; Mchoka School GPS: **S**14.439481°, **E**35.220644°) with local prevalence (%) and 95%CI of intestinal schistosomiasis indicated by CCA urine-dipstick. The water collection sites pinpointed by the two egg-patent children from Samama and Mchoka are indicated, respectively.