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Phylogenetic analysis of *Biomphalaria pfeifferi* from Nkalagu, Ebonyi State, Nigeria

Chiamah, O. C[1*], Akinwale, O. P.[2], Ubachukwu, P. O.[3], Udechukwu, B. E.[1], Ukaegbu, C. M.[1], Adeoye, O. O.[1], Idam, C. O.[1], Imakwu, C. A.[4], Mgbanyi, I. J.[1]

[1]Department of Biology, Faculty of Biological Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State.

[2]Department of Public Health and Epidemiology, Nigerian Institute of Medical Research (NIMR)

[3]Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka

[4] Department of Parasitology and Entomology, Faculty of Biosciences, Nnamdi Azikwe University, Awka, Anambra State, Nigeria

***Corresponding Author:** Ogochukwu Chiamah. caroline.okeke@funai.edu.ng

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Abstract

A previous report on the *B. pfeifferi* snail found in Nkalagu, southeastern Nigeria, indicated that the snail does not shed *Schistosoma* cercaria. However, in a recent study, we discovered that the snail does shed *Schistosoma* cercaria, prompting an investigation into its phylogeny. This study aimed to trace the evolutionary history of *B. pfeifferi* from Nkalagu. We retrieved ITS2 sequence data from eight samples of Nkalagu *B. pfeifferi* from GenBank NCBI, along with sequences from *B. pfeifferi* found in other regions of Africa. Using MEGA 11, we constructed a phylogenetic tree and an evolutionary divergence matrix. The resulting tree revealed four clusters. Notably, *B. pfeifferi* sample 6 from Nkalagu showed a close relationship with *B. pfeifferi* samples from Yaoundé, Cameroon, and other African regions. In contrast, Nkalagu samples 1 and 4 exhibited a connection with another population of *B. pfeifferi* from a location in Cameroon that is not Yaoundé. Meanwhile, Nkalagu samples 2, 3, 5, 7, and 8 clustered together, displaying high evolutionary divergence from samples 1, 4, and 6, as well as from *B. pfeifferi* found in other parts of Africa. These results suggest the presence of *B. pfeifferi* with varying levels of genetic differentiation in Nkalagu.

Introduction

Biomphalaria snails are freshwater pulmonate mollusks that have significant medical and veterinary importance, as they serve as intermediate hosts for the parasitic worm *Schistosoma*. *Schistosoma* is the genus of the causative agent of intestinal schistosomiasis, a neglected tropical disease that affects regions across Africa, the Middle East, the Caribbean, Brazil, Venezuela, and Suriname (WHO 2023; Magero *et al* 2025).

African *Biomphalaria* species are classified into four groups based on their shell and anatomical features: pfeifferi, choanomphala, alexandrina, and sudanica (Standley *et al* 2011). Of these, *B. pfeifferi* is the most widespread, found throughout the tropical regions of Africa, as well as in Madagascar and Arabia (Dejong *et al* 2003). Different geopolitical zones in Nigeria have recorded the presence of *B. pfeifferi* (Okeke and Ubachukwu 2017). In Nkalagu, a rural community in Ebonyi State, southeastern Nigeria, *B. pfeifferi* has been reported not to shed *Schistosoma* cercariae (Okafor and Ngang 2004). However, in our study (Okeke *et al* 2020), we found that *Biomphalaria* specimens from River Uzuru in Nkalagu did shed *Schistosoma* cercariae, and through molecular characterization, we identified the snail species as *B. pfeifferi*. This finding raises important questions about the phylogeny of *B. pfeifferi* from River Uzuru in Nkalagu, especially considering the differing results from previous studies.

Molecular phylogeny involves using gene sequences from organisms to gain insights into their evolutionary relationships (Philippe *et al* 2000). For *Biomphalaria* populations, molecular phylogenetic analysis is crucial for accurate species identification, understanding their role in schistosomiasis transmission, and guiding the development of effective, targeted control strategies (Habib *et al* 2018). Several molecular markers have been utilized in the phylogenetics of *Biomphalaria*, including allozyme electrophoresis (Bandoni *et al* 1995), the mitochondrial Cytochrome Oxidase subunit 1 gene (CO1), the 16S ribosomal RNA gene (Osman *et al* 2025), the NADH dehydrogenase subunit 1 gene (nad1) (Hammoud *et al* 2022), the 28S ribosomal RNA gene (He *et al* 2025), and the Internal Transcribed Spacer regions (ITS1 and ITS2).

The ITS1 and ITS2 regions are useful for phylogenetic analysis because they are part of the eukaryotic ribosomal RNA locus, known for its gene copy number, universality, and rapid, diverse evolution within

components and among subunits and spacers. The ITS regions are also effective for clear species identification and differentiation (Vidigal *et al* 2000, 2004). The ITS2 region has been employed in the phylogenetic analysis of *Biomphalaria* snails (Tuan and Santos 2007; Vidigal *et al* 2000, 2004; Tchami Mbagnia *et al* 2020). Therefore, in this study, the ITS2 gene sequence of *B. pfeifferi* collected from Nkalagu was compared with previously published sequences of *B. pfeifferi* from other African regions in NCBI GenBank. This comparison aims to elucidate the evolutionary relationships between the *B. pfeifferi* snails from Nkalagu and those from other parts of Africa.

Materials and methods

Snail collection and molecular analysis

The River Uzuru in Nkalagu, Ishielu Local Government Area (longitudes 7° 45' and 70° 50'E and latitudes 60° 31' and 60° 35 'N, Figure 1), Ebonyi State, were surveyed for the presence of *Biomphalaria* snails. The snails were transported to the Molecular Parasitology Research Laboratory at the Nigerian Institute of Medical Research in Lagos, Nigeria (NIMR), for molecular analysis following morphological identification. The snails were identified using the field guide to West African freshwater snails prepared by the Danish Bilharziasis Laboratory (Brown and Kristensen 1993). The *Biomphalaria* snails were screened by the crushing method for *Schistosoma* cercariae in the laboratory. Genomic DNA was extracted from the whole tissues of the snails using the hexadecyltrimethylammonium bromide (CTAB) method. Subsequently, the entire ITS2 region from the genomic DNA of each collected snail was amplified using the primers ITS2F (5′-CGTCCGTCTGAGGGTCGGTTGC-3′) and ETTS1 (5′-TGCTTAA GTTCAGCGGGT-3′), which are anchored in the conserved regions of the 5.8S and 28S ribosomal genes as described by Vidigal *et al* (2000).

PCR amplification was conducted following the protocol established by Vidigal *et al* (2004). Photo documentation was carried out using a gel documentation and analysis system (Clinx Science Instruments, USA). Eight representative samples of infected snails were selected for sequencing. Sequencing was performed utilizing the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. The nucleotide sequences of these eight Biomphalaria samples were subjected to the Basic Local Alignment Search Tool (BLAST) (Altschul *et al* 1990). The BLAST search revealed that the sequences exhibited a percentage similarity index ranging from 98.24% to 99.16% with samples available in the NCBI GenBank, confirming them as Biomphalaria pfeifferi. The sequences were subsequently deposited in the GenBank database under accession numbers KX644865 to KX644872 (Okeke *et al* 2020).

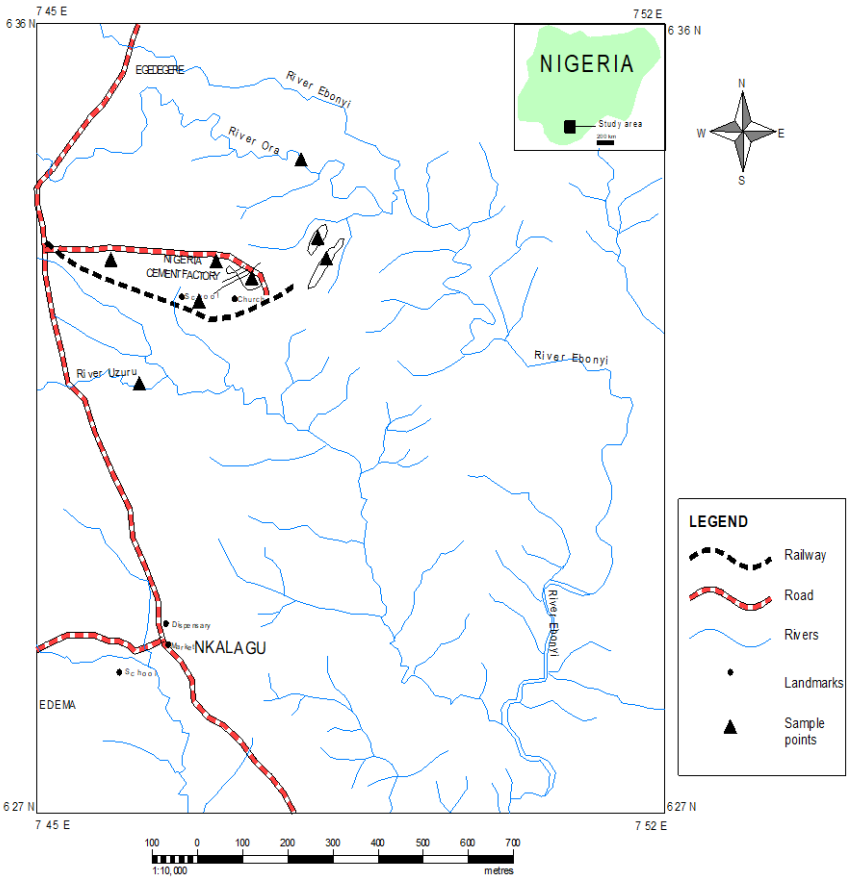

Figure 1. Map of Nigeria Cement Factory, Nkalagu, showing the flow of River Uzuru
Source: Topography Map of Nigeria (1964).

Phylogenetic analysis

To infer the evolutionary history of *B. pfeifferi* currently shedding schistosome cercariae in Nkalagu, Ebonyi State, we utilized MEGA 11 software (Tamura *et al* 2021). We gathered seven datasets of *B. pfeifferi* from various regions in Africa, all exhibiting ≤ 98% genetic identity, along with one dataset of *B. stanleyi* (98.63% identity) and one of *B. glabrata* (95% identity) from GenBank for the phylogenetic analysis. The accession numbers for these *Biomphalaria* sequences are detailed in Table 1. We calculated the evolutionary divergence between sequences as genetic distance and presented these values in a matrix. Phylogenetic trees were constructed using Bayesian Inference, with posterior probabilities assessed accordingly.

Table 1: Dataset of *Biomphalaria* ITS2 sequences retrieved from GenBank

s/n	*Biomphalaria* species	Location collected	GenBank Accession Number
1.	*B. pfeifferi*	Aso stream, Kenya	MG461588
2.	*B. pfeifferi*	Mahazoa, Madagascar	AY030364
3.	*B. pfeifferi*	Gezira, Sudan	AY030363
4.	*B. pfeifferi*	Richard Toll, Senegal	AY030361
5.	*B. pfeifferi*	Yaounde, Cameroon	AY030362
5.	*B. pfeifferi*	Cameroon	MN064848
6.	*B. pfeifferi*	Sudan	KY025444
7.	*B. stanleyi*	Lake Albert, Uganda	AY030365
8.	*B. glabrata*	Salvador, Brazil	AY030376

Results

Bayesian inference phylogenetic analysis grouped the sequence samples into four clusters (Fig. 1). The divergence of the *B. pfeifferi* samples collected from Nkalagu, Ebonyi State, Nigeria, compared to the ITS2 sequences of *B. pfeifferi* from other locations in Africa is illustrated in Table 2. One cluster indicated that sample 6 of *B. pfeifferi* from Nkalagu had very little divergence (0.004) from samples collected in Yaoundé, Cameroon; Richard Toll, Senegal; Mahazoa, Madagascar; Gezira, Sudan; Asao Stream, Kenya; Sudan; and *B. stanleyi* from Uganda. *B. pfeifferi* samples 1 and 4 from Nkalagu were found to be related to a lineage of *B. pfeifferi* from locations in Cameroon (excluding Yaoundé), with a low divergence of 0.009. In contrast, samples 2, 3, 5, 7, and 8 showed no relatedness in lineage to the ITS2 sequences of other *B. pfeifferi* available in GenBank (Fig. 1). The divergence between samples 2, 3, 5, 7, and 8 was 0.00; however, their divergence from samples 1 and 4 was 0.622, and from sample 6 it was 0.626. The outgroup, *B. glabrata*, demonstrated a divergence of 0.283 from *B. pfeifferi* samples 1 and 4, a divergence of 0.461 from sample 6, and a divergence of 0.548 from samples 2, 3, 5, 7, and 8.

Discussion

The phylogeny of ITS 2 *B. pfeifferi* produced a tree with four main groups, with *B. glabrata* identified as an outgroup. *B. glabrata* and *B. pfeifferi* are evolutionary cousins that diverged from a common ancestor in America. While *B. glabrata* remained in America, *B. pfeifferi* established itself in Africa (Au *et al* 2023).

The low evolutionary divergence among *B. pfeifferi* samples 6, 1, and 4 from Nkalagu, when compared to *B. pfeifferi* from Cameroon, indicates a close evolutionary relationship between these Nkalagu samples and those from Cameroon. Research has shown a correlation between geographical distance and genetic distance, suggesting that sequences of *Biomphalaria* populations from nearby locations tend to cluster closely together in phylogenetic trees, as observed in the *Biomphalaria* populations from East African river systems (Magero *et al* 2025). Additionally, *B. pfeifferi* sample 6 from Nkalagu exhibited a relationship with *B. pfeifferi* samples from Senegal, Madagascar, Sudan, and Kenya, as they all clustered together in group 1. It has been noted generally that there is only a small amount of genetic differentiation among *B. pfeifferi* populations, with increased genetic differentiation occurring primarily among populations that are more distantly located (Dejong *et al* 2003; Magero *et al* 2025).

Sample 6 of *B. pfeifferi* from Nkalagu was found to have an evolutionary relationship with *B. stanleyi*. This finding contradicts the report by Magero *et al* (2025), which indicated that *B. stanleyi* clustered with Nilotic species (*B. sudanica, B. choanomphala, B. smithi*, and *B. alexandrina*) instead of with *B. pfeifferi*. However, this result aligns with the findings that demonstrated that *B. stanleyi* is genetically indistinguishable from *B. pfeifferi* and clusters within the widespread *B. pfeifferi* group (Andrus *et al* 2023).

Samples of *B. pfeifferi*, specifically samples 2, 5, 7, and 8, demonstrated high evolutionary divergence from samples 1, 4, and 6, as well as from other *B. pfeifferi* snails found in different parts of Africa. The *B. pfeifferi* snails of Nkalagu are not present in River Uzuru year-round; they appear only during the dry season and are washed away with the onset of the first rain. This constant gathering and displacement of the snails may have facilitated the introduction of genetically distinct individuals from other regions into the river, which could explain the observed high evolutionary divergence (Charbonnel *et al* 2002). Additionally, this high evolutionary divergence may be linked to the mating system of the snails, which influences gene flow. It has been reported that *B. pfeifferi* snails exhibit a high rate of preferential self-fertilization.

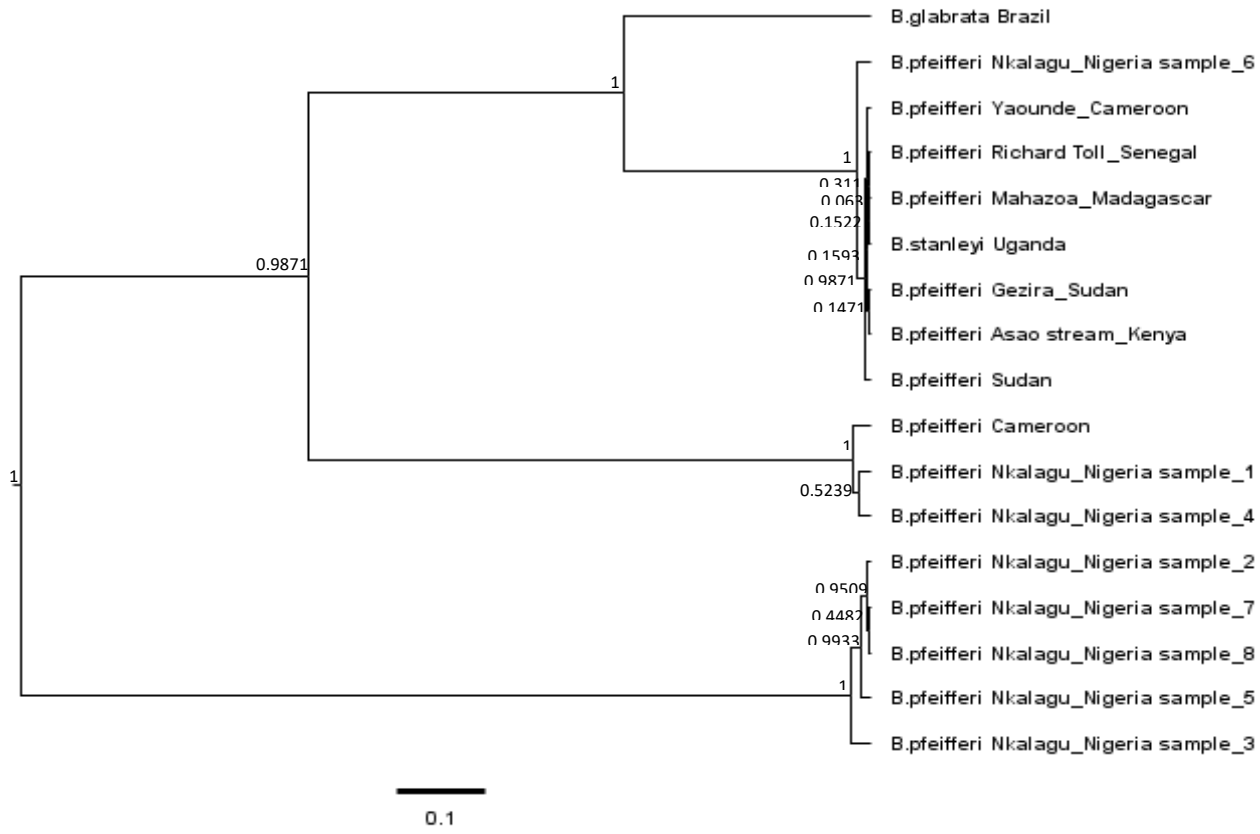

Figure 2. Bayesian Inference Phylogenetic Tree constructed using ITS2 Sequence of *B. pfeifferi* from Nkalagu and those from other locations in Africa

Table 2: Genetic distance matrix of evolutionary divergence between sequences

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 B_Nkalagu_1																	
2 B_Nkalagu_2	0.622																
3 B_Nkalagu_3	0.622	0															
4 B_Nkalagu_4	0	0.622	0.622														
5 B_Nkalagu_5	0.622	0	0	0.622													
6 B_Nkalagu_6	0.678	0.626	0.626	0.678	0.626												
7 B_Nkalagu_7	0.622	0	0	0.622	0	0.626											
8 B_Nkalagu_8	0.622	0	0	0.622	0	0.626	0										
9 B_Asao_stream_Kenya	0.678	0.622	0.622	0.678	0.622	0.004	0.622	0.622									
10 B_Mahazoa_Madagascar	0.678	0.622	0.622	0.678	0.622	0.004	0.622	0.622	0								
11 B_Gezira_Sudan	0.678	0.622	0.622	0.678	0.622	0.004	0.622	0.622	0	0							
12 B_Richard Toll_Senegal	0.678	0.622	0.622	0.678	0.622	0.004	0.622	0.622	0	0	0						
13 B_Yaounde_Cameroon	0.678	0.622	0.622	0.678	0.622	0.004	0.622	0.622	0	0	0	0					
14 B_Cameroon	0.009	0.617	0.617	0.009	0.617	0.673	0.617	0.617	0.674	0.674	0.674	0.674	0.674				
15 B_Sudan	0.678	0.622	0.622	0.678	0.622	0.004	0.622	0.622	0	0	0	0	0	0.674			
16 B_Stanleyi_Uganda	0.678	0.622	0.622	0.678	0.622	0.004	0.622	0.622	0	0	0	0	0	0.674	0		
17 B_glabrata	0.283	0.548	0.548	0.283	0.548	0.461	0.548	0.548	0.457	0.457	0.457	0.457	0.457	0.278	0.457	0.457	

While self-fertilization can lead to low genetic diversity within a local population, it can also result in high genetic differentiation among populations (Tian-Bi *et al* 2013).

In conclusion, the *B. pfeifferi* population in Nkalagu is genetically heterogeneous, comprising both well-established African lineages and highly divergent groups that may warrant further investigation to understand their origin and potential implications for schistosomiasis transmission in the region.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol*. 215(3): 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Andrus, P.S., Stothard, J.R., Kabatereine, N.B., and Wade, C.M. 2023. Comparing shell size and shape with canonical variate analysis of sympatric Biomphalaria species within Lake Albert and Lake Victoria, Uganda. *Zool. J. Linn. Soc.* 199(3): 713–722. https://doi.org/10.1093/zoolinnean/zlad052.

Au, M.F.F., Williams, G. A., and Hui, J. H. L. 2023. Status Quo and Future Perspectives of Molecular and Genomic Studies on the Genus *Biomphalaria*—The Intermediate Snail Host of Schistosoma mansoni. *Inter. J. Mol. Sci. 24*(*5*): 4895. https://doi.org/10.3390/ijms24054895.

Bandoni, S.M., Mulvey, M., and Loker, E.S. 1995. Phylogenetic analysis of eleven species of Biomphalaria Preston, 1910 (Gastropoda: Planorbidae) based on comparisons of allozymes. *Biol. J. Linn. Soc. 54*(*1*): 1–27. https://doi.org/10.1016/0024-4066(95)90034-9.

Brown, D.S. and Kristensen, T.K. 1993. Field Guides to African Freshwater Snail Identification: West Africa. Accessed from https://medbox.org/document/field-guides-to-african-freshwater-snail-identification-west-african-species 13th June 2025.

Charbonnel, N., Angers, B., Rasatavonjizay, R., Bremond, P., and Jarne, P. 2002. Evolutionary aspects of the metapopulation dynamics of Biomphalaria pfeifferi, the intermediate host of Schistosoma mansoni. *J. Evol. Biol.* 15(2): 248–261. https://doi.org/10.1046/j.1420-9101.2002.00381.x.

Dejong, R.J., Morgan, J. T., Wilson, W.D., Al-Jaser, M.H., Appleton, C.C., Coulibaly, G., D'Andrea, P. S., Doenhoff, M. J., Haas, W., Idris, M.A., Magalhães, L.A., Moné, H., Mouahid, G., Mubila, L., Pointier, J.P., Webster, J.P., Zanotti-Magalhães, E.M., Paraense, W. L., Mkoji, G.M., and Loker, E.S. 2003. Phylogeography of Biomphalaria glabrata and B. pfeifferi, important intermediate hosts of Schistosoma mansoni in the New and Old World tropics. *Mol. Ecol*. *12*(*11*): 3041–3056. https://doi.org/10.1046/j.1365-294x.2003.01977.x.

Habib, M.R., Lv, S., Guo, Y.H., Gu, W.B., Standley, C.J., Caldeira, R.L., and Zhou, X.-N. 2018. Morphological and molecular characterization of invasive Biomphalaria straminea in southern China. *Infect. Dis. Poverty*. *7(1*): 120. https://doi.org/10.1186/s40249-018-0505-5.

Hammoud, C., Mulero, S., Van Bocxlaer, B., Boissier, J., Verschuren, D., Albrecht, C., and Huyse, T. 2022. Simultaneous genotyping of snails and infecting trematode parasites using high-throughput amplicon sequencing. *Mol. Ecol. Resource 22*(*2*): 567–586. https://doi.org/10.1111/1755-0998.13492.

He, P., Hu, Y., Khan, J., Huang, Y., Yuan, Z., Sanogo, B., Gao, D., Liu, J., Wu, D., Chen, J., Wu, Z., Liang, S., Sun, X., and Lin, D. 2025. The morphology, genetic structure, and haplotype distribution of the invasive freshwater snails *Biomphalaria straminea* and *Physa acuta* in Guangdong, China. *Trop. Med. Health 53*: 98. https://doi.org/10.1186/s41182-025-00780-y.

Magero, V.O., Kisara, S., Suleman, M.A., and Wade, C.M. 2025. Population genetic structure of Biomphalaria snails found in East Africa's river systems. *Acta Tropica 267*: 107656. https://doi.org/10.1016/j.actatropica.2025.107656

Okafor, F.C. and Ngang, I. 2004. Freshwater Snails of Niger-Cem, Nkalagu, Eastern Nigeria: Observations on Some Demographic Aspects of The Schistosome-Transmitting Bulinids. *Ani. Res. Int*. *1*(*2*): 120–124. https://doi.org/10.4314/ari.v1i2.40754.

Okeke, O.C. and Ubachukwu, P.O. 2017. Trematode infections of the freshwater snail *Biomphalaria pfeifferi* from a south-east Nigerian community with emphasis on cercariae of *Schistosoma*. *J. Helmin*. *91*(*3*): 295–301. https://doi.org/10.1017/S0022149X16000353.

Okeke, O.C., Akinwale, O.P., Ubachukwu, P.O., Gyang, P.V., Henry, E.U., Nwafor, T.E., Daniel, B.M., Ebi, S.E., Anorue, C.O., Chukwuka, C.O., and Dido, M.S. 2020. Report of high prevalence of schistosome infection in *Biomphalaria* snails from a geographic area with no previous prevalence of human schistosomiasis in Nigeria. *Acta Trop*. *210*: 105326. https://doi.org/10.1016/j.actatropica.2019.105326

Osman, A., Andrus, P.S., Fang, Y., Elhassan, I., Zhou, X., Nour, B.Y.M., and Zhao, L. 2025. Genetic Diversity and Infection Prevalence of Biomphalaria pfeifferi (Krauss, 1848), the Intermediate Snail Host of Schistosoma mansoni in Gezira State, Sudan. *Int. J. Mol. Sci. 26*(*19*): 9567. https://doi.org/10.3390/ijms26199567.

Philippe, H., Germot, A. and Moreira, D. 2000. The new phylogeny of eukaryotes. *Curr. Opin. Genet Dev*. *10*(*6*): 596–601. https://doi.org/10.1016/S0959-437X(00)00137-4.

Standley, C.J., Wade, C.M. and Stothard, J.R. 2011. A Fresh Insight into Transmission of Schistosomiasis: A Misleading Tale of *Biomphalaria* in Lake Victoria. *Plos One*. *6*(*10*): e26563. https://doi.org/10.1371/journal.pone.0026563.

Tchami Mbagnia, M.C., Melachio Tanekou, T.T., Kengne Fokam, A.C., Nguiffo Nguete, D., Wondji, C.S. and Njiokou, F. 2020. PCR-based molecular identification of two intermediate snail hosts of *Schistosoma mansoni* in Cameroon. *Parasit. Vectors*. *13*: 158. https://doi.org/10.1186/s13071-020-04033-1.

Tian-Bi, Y.N., Jarne, P., Konan, J.N., Utzinger, J., and N'Goran, E.K. 2013. Contrasting the distribution of phenotypic and molecular variation in the freshwater snail *Biomphalaria pfeifferi*, the

intermediate host of *Schistosoma mansoni*. *Heredity* *110*(*5*): 466–474. https://doi.org/10.1038/hdy.2012.115.

Tuan, R., and Santos, P. dos. 2007. ITS2 variability of Biomphalaria (Mollusca, Planorbidae) species from the Paranapanema Valley (São Paulo State, Brazil): Diversity patterns, population structure, and phylogenetic relationships. *Genet. Mol. Biol*. *30*: 139–144. https://doi.org/10.1590/S1415-47572007000100024.

Vidigal, T.H.D.A., Spatz, L., Kissinger, J.C., Redondo, R.A.F., Pires, E.C.R., Simpson, A.J.G. and Carvalho, O.S. 2004. Analysis of the first and second internal transcribed spacer sequences of the ribosomal DNA in Biomphalaria tenagophila complex (Mollusca: Planorbidae). *Mem. Inst. Oswaldo Cruz*. 99(2): 153–158. https://doi.org/10.1590/s0074-02762004000200007.

Vidigal, T.H., Kissinger, J.C., Caldeira, R.L., Pires, E.C., Monteiro, E., Simpson, A.J. and Carvalho, O.S. 2000. Phylogenetic relationships among Brazilian Biomphalaria species (Mollusca: Planorbidae) based upon analysis of ribosomal ITS2 sequences. *Parasitology*. *121*: 611–620. https://doi.org/10.1017/s0031182000006831.

WHO. 2023. Schistosomiasis. https://www.who.int/news-room/fact-sheets/detail/schistosomiasis Date accessed: 23rd December 2025.

ORCID
Ogochukwu Chiamah: https://orcid.org/0000-0002-2517-6585