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SCHISTOSOMA MANSONI (TREMATODA: SCHISTOSOMATIDAE) OCCURRENCE IN *BIOMPHALARIA PFEIFFERI* (GASTROPODA: PLANORBIDAE) IN WATER BODIES OF KINDIA PREFECTURE (REPUBLIC OF GUINEA)

© 2025 **E. Dmitrieva[1], S. Diakité[2], P. Koïvogui[2], N. Pronkina[1], V. Uppe[1], L. Konate[2], M. Sow[2], A. Balde[2], M. Camara[3], D. Polevoy[1], R. Machkevsky[1], E. Vodiasova[1], I. Keita[2], and A. Diallo[2]**

[1]A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
[2]Guinea Centre for Marine and Coastal Research, Conakry, Republic of Guinea
[3]Institute of Applied Biology, Kindia, Republic of Guinea
E-mail: *evdmitrieva@ibss-ras.ru*

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Molluscs of the genera *Biomphalaria* and *Bulinus*, intermediate hosts of human-pathogenic *Schistosoma* trematodes, were found in 8 freshwater bodies in the western region of Kindia Prefecture of Guinea. While *Bulinus* snails were free from schistosome infection, *Biomphalaria* specimens were parasitized, with infection foci revealed in 4 water bodies. Morphological analysis of shells of infected snails confirmed that they belong to the species *Biomphalaria pfeifferi*. The movement behavior, body shape, internal anatomy, and morphometric characteristics of the cercariae shed from the snails matched those of *Schistosoma mansoni*. The obtained ITS1 rDNA gene sequences showed 100% identity with homologous loci of *S. mansoni* parasitizing on humans and rats in Africa and Brazil. In contrast, they differed by 6 nucleotide substitutions from those of the closest relative, *S. rodhaini*, and by 2–6 substitutions from those of *S. rodhaini* × *S. mansoni* hybrids. Phylogenetic analysis strongly supported (100% bootstrap) the clustering of the sampled sequences with that of *S. mansoni*, distinct from those of *S. rodhaini* and hybrid lineages, confirming species identification. This study reports the first documented evidence of *S. mansoni* infection in *B. pfeifferi* and its molecular characterization in the Republic of Guinea. Infected snails (n = 32) were significantly larger on average than uninfected ones (n = 1,110) in samples where trematodes were found. Analysis of environmental factors revealed no effect of pH, dissolved oxygen, or water temperature within their observed ranges during the study period (October and November) on occurrence of *B. pfeifferi* and prevalence of infection with *S. mansoni*. Notably, *B. pfeifferi* exhibited tolerance to slightly hypoxic conditions, likely explaining their persistence in excrement-contaminated waters and facilitating schistosome larvae transmission. The presence of schistosomes was associated with specific biocenosis features, including slack or slow-flowing water and abundant submerged vegetation. All infected water bodies were located within urban areas. Obtained data are significant for developing schistosomiasis control strategies in the region.

Keywords: *Schistosoma*, schistosomiasis, natural focus of infection, intermediate hosts, *Biomphalaria*, ITS1 rDNA, Republic of Guinea

Human schistosomiasis is a tropical disease caused by six trematode species of the genus *Schistosoma* Weinland, 1858. Out of them, two species, *S. haematobium* (Bilharz, 1852) and *S. mansoni* Sambon, 1907, are responsible for 99% of schistosomiasis infections worldwide. The World Health Organization considers schistosomiasis to be one of the most serious tropical diseases, with approximately 90% of cases occurring in Africa [Onasanya et al., 2021]. Furthermore, the prevalence of schistosomiasis has increased among the African population over the past two decades [Salari et al., 2020]. Because of human migration from Africa to the Eurasian continent, climate warming, and the associated risk of introducing the trematode intermediate hosts, molluscs, to new areas, studying various aspects of schistosome ecology is relevant not only in African countries, but also in other ones, including Russia. Thus, publications have reported the recent spread of schistosomiasis in Corsica and Portugal, even among residents who have not visited endemic areas [Gabrielli, Garba Djirmay, 2023].

The spread of schistosomiasis is associated with two key factors: water contamination by human excrement containing parasite eggs and the presence of infected molluscs in natural water bodies. Current control programs focus on analyzing the infection rate in the population. However, human infection originates from free-swimming larvae: cercariae produced by schistosome sporocysts that parasitize snails. Even if parasite eggs are prevented from entering water, infected snails will continue to produce new cercariae for some time. Moreover, "human" *Schistosoma* species can parasitize other mammals [Aula et al., 2021] meaning that animal reservoirs allow the infection to circulate independently of humans. Therefore, preventing and treating infection in humans alone is insufficient to control the spread of schistosomiasis. It is also necessary to monitor the presence of the parasite in molluscs in water bodies.

A large number of various kinds of research, from morphology, biology, and ecology to genomic surveys, transcriptomic works, and development of PCR diagnostics, is focused on trematodes that cause human schistosomiasis, and also on other representatives of the family Schistosomatidae Stiles & Hassall, 1898 which they belong to (*e. g.*, [Alzaylaee et al., 2020; Gandasegui et al., 2016; Sato et al., 2018]). However, given the diversity of climatic conditions in regions where these helminths are revealed, the spread and variability of their mollusc hosts, the possible inclusion of "human" schistosomes in parasite systems of various reservoir and random hosts (both definitive and intermediate ones) [Aula et al., 2021], and the occurrence of interspecific hybrids [De Elías-Escribano et al., 2025; Savassi et al., 2020; Webster et al., 2013], data on their morphological, ecological, and molecular genetic characteristics in different habitats are still insufficient. For example, in literature, there is no information on the occurrence of *Schistosoma* species in molluscs in the Republic of Guinea. Notably, in the monograph [Brown, 2005], the presence of *S. mansoni* and *S. haematobium* in two molluscs, *Biomphalaria pfeifferi* (Krauss, 1848) and *Bulinus globosus* (Morelet, 1866), within Guinea is stated by registration of trematodes in humans and the occurrence in this region of both mollusc species, which are the main hosts of these parasites in Africa. In the genetic database GenBank NCBI (National Center for Biotechnological Information), among 556,222 records for various schistosome DNA fragments, there are no deposited nucleotide sequences of representatives of this genus from the Republic of Guinea [Nucleotide database, 2024].

Currently, two *Schistosoma* species pathogenic to humans, *S. mansoni* and *S. haematobium*, are known to be common among the population of Guinea. Both species are native for the territory of the republic [Aula et al., 2021]. According to the analysis of human surveys conducted in 1989–2019, the prevalence of the parasites varies 1 to 15% in different areas [Guilavogui et al., 2023]. However, some publications provide significantly higher values. Thus, in Forested Guinea, the occurrence of *S. mansoni*

in schoolchildren reached 86.1%, and that of *S. haematobium*, 75% [Hodges et al., 2011]. Besides, in the region neighboring Guinea (Guinea-Bissau, Cameroon, Equatorial Guinea, Gabon, and Nigeria), another species parasitizing humans occurs: *S. guineensis* [Kane et al., 2003; Webster et al., 2006]; interspecific hybrids were revealed as well [Savassi et al., 2020]. In the Republic of Guinea, it has not been registered so far. Given that species identification based on egg morphology (the most common method applied for schistosomiasis pathogens) may be inaccurate, especially for hybrids [De Elías-Escribano et al., 2025], it is necessary to confirm the data using molecular genetic markers. At the same time, accurate species identification is crucial for diagnosing and treating, assessing epidemiological consequences, and developing control strategies for natural foci of infection.

In connection with the above, the aims of the paper are to characterize the occurrence of human pathogenic schistosomes in molluscs in Kindia Prefecture (the Republic of Guinea), to identify *Schistosoma* species based on the morphology of cercariae and DNA sequences, and to analyze the relationship between the indicators of mollusc infection with schistosomes and environmental parameters.

MATERIAL AND METHODS

Sampling. In October and November 2024, on the territory of the Koya and Kindia prefectures (Kindia Region, the Republic of Guinea), 27 sites in the coastal zone of 21 water bodies were surveyed (see the map: https://www.google.com/maps/d/edit?mid=1JeRUjZ5VpumfQxLM7R-GmriHB73BXss&usp=sharing).

The following parameters were recorded at the sampling sites: water temperature and pH, dissolved O_2, and characteristics of the biocenosis – the presence of vegetation, the bottom type (rocky, sandy, or silty), and the relative flow velocity (slack water, weak flow, and strong flow). Physical and chemical parameters were measured with a multifunctional water quality tester DO-100 (China) calibrated and used in accordance with the manufacturer's protocol; the measurement error was ±1.5%. The list of the surveyed water bodies, the coordinates of the sampling sites, the hydrochemical characteristics, and the number of sampled molluscs are provided in the table (see Supplement 1: https://marine-biology.ru/mbj/article/view/498).

Mollusc species were identified based on the shell morphometric features in accordance with guides [Brown, 2005; Mandahl-Barth, 1957]. To analyze the normality of the distribution of mollusc sizes, the Shapiro–Wilk test, W, was used. To check the reliability of differences between their samples, the Mann–Whitney U test was applied. For both tests, $p \leq 0.05$.

Examination for the presence of parasites. The sampled molluscs were delivered to a laboratory and maintained in clean bottled water for 2 weeks. *Prior* to examination, those were kept in the shade for 48 h; then, the specimens were placed individually into containers with a small amount of clean water and exposed to natural light at 7:00. Emissions of cercariae were detected by examining the containers with molluscs under a Micromed MC-4-ZOOM LED stereomicroscope (China) at ×20 to ×30 magnification every 2 h until 16:00. Each sample was examined for 2–3 days. If schistosome cercariae were not found, a mollusc was dissected and examined for sporocyst infection. The detected cercariae, which by external features and movement pattern corresponded to those of the genus *Schistosoma*, and the infected molluscs were fixed in 70% and 96% ethanol, respectively, for subsequent morphological and molecular genetic studies.

The cercariae fixed in 70% ethanol were stained with alum carmine and differentiated in acidified 70% ethanol. After subsequent dehydration *via* an ethanol series (80–100%) and clearing in clove

oil, the specimens were mounted on a microscope slide in Canada balsam [Bykhovskaya-Pavlovskaya, 1985]. Measurements and description of the cercarial morphology were performed based on total mounts ($n = 17$), as well as on micrographs of live cercariae using an Olympus CX41 microscope (Japan) at ×200 to ×400 magnification, an Olympus SC50 digital camera, and CellSens Standard v. 1.18 software. Measurements of cercariae are given in mm (ranges and a mean value with the standard error in parentheses). The infection rate was characterized by the prevalence: the proportion of infected molluscs in a sample, %.

Molecular genetic investigation. Genomic DNA was isolated from a whole cercariae using a commercial PureLink Genomic DNA Mini Kit (Invitrogen, the USA) according to the manufacturer's protocol, with elution in the minimum recommended volume. The concentration and quality of the extracted DNA were determined with a Nano-500 spectrophotometer (Allsheng, China) and a Qubix fluorimeter (Syntol, Russia). Amplification of the ITS1 region of rDNA was performed with the primers BD1 (5'-GTCGTAACAAGGTTTCCGTA-3') and 4S (5'-TCTAGATGCGTTCGAARTGTCGATG-3') [Bowles, McManus, 1993]. The PCR reaction solution contained 5X ScreenMix (Evrogen, Russia), 5 pmol of each primer, and 5 ng·μL^{-1} of total genomic DNA. The amplification protocol was as follows: initial denaturation at +95 °C for 3 min; 35 cycles (+95 °C, 30 s; +55 °C, 30 s; +72 °C, 30 s); and a final extension at +72 °C for 7 min. The amplification products were detected by electrophoresis in a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light. The PCR products were sequenced in both directions with a BrilliantDye Terminator v3.1 kit (NimaGen, the Netherlands) on a Nanophor-5 genetic analyzer (Institute for Analytical Instrumentation of RAS, Russia).

The ITS1 sequence obtained was aligned with similar sequences available in GenBank NCBI for *S. mansoni* (PP658717, FJ750523, and JQ289742), *S. bovis* (PP312969, PP313016, and PP312959), *S. haematobium* (LC726151, PP963804, and PP963802), *S. rodhaini* (AF531312), and a *S. mansoni* × *S. rodhaini* hybrid (EU599364–EU599378) using MUSCLE algorithm implemented in MEGA 11 [Tamura et al., 2021]. *Schistosoma turkestanicum* sequence (MF145062) was used as the outgroup. A phylogenetic tree was constructed by the maximum likelihood and the GTR + G model (generalized time reversible with gamma distribution) [Rossi, 2018]. Bootstrap analysis with 1,000 repetitions was used to assess the reliability of the clusters.

RESULTS

Occurrence of *Schistosoma* trematodes. These trematodes were found in molluscs of the genus *Biomphalaria* Preston, 1910 in 4 out of the 27 water bodies surveyed in Kindia Prefecture (Table 1). Out of the 1,376 *Biomphalaria* snails examined, cercariae and sporocysts of schistosomes were registered in 32 ind. The percentage of infected snails in samples ranged within 1.2–5.6.

Notably, in addition to schistosomes, other trematodes were revealed in *Biomphalaria* molluscs: sporocysts and cercariae of the families Plagiorchidae and Echinostomatidae, as well as apharyngeal brevifurcocercariae and metacercariae, which could not be identified. Two *Biomphalaria* specimens infected with schistosomes were also co-infected with plagiorchiid sporocysts.

Identification of a mollusc species. Our samples covered specimens of three morphotypes corresponding to the genera *Biomphalaria*, *Radix* Montfort, 1810, and *Bulinus* O. F. Müller, 1781. No schistosome infection was revealed in representatives of the latter two genera.

Table 1. Characterization of *Biomphalaria* sp. samples and prevalence of *Schistosoma* sp.

Date	Sampling site	*Biomphalaria* sp.				PI, %
		Number, ind.	Shell length, mm	Number of infected molluscs, ind.	Shell length of infected molluscs, mm	
27.10.2024	Molokhoure 1	56	4.0–7.0	0	–	0
28.10.2024	Molokhoure 1	90	5.5–8.0	5	6.0–8.0	5.56
	Molokhoure 2	82	5.0–8.5	2	7.0	2.44
29.10.2024	Lac Bamba	106	5.0–8.0	0	–	0
	Foulayah 1	14	4.5–6.5	0	–	0
	Foulayah 2	24	5.0–6.5	0	–	0
31.10.2024	Yabara 1	34	4.5–6.0	0	–	0
	Yabara 2	327	5.0–10.0	6	6.0–7.5	1.83
	Sinanya fontaine	163	5.5–9.5	2	7.0; 8.0	1.23
01.11.2024	Molokhoure 1	172	6.0–9.5	7	6.5–9.0	4.07
	Molokhoure 2	154	6.5–9.0	7	7.0–9.0	4.55
	Sinanya fontaine	73	5.5–7.0	1	6.0	1.37
08.11.2024	Molokhoure 1	20	4.5–7.0	1	7.0	5.0
	Sinanya fontaine	61	5.0–8.0	1	7.0	1.64
In total, 8 sites		1,376	4.0–10.0	32	5.5–9.0	2.33

Note: PI is prevalence of infection. For coordinates of sampling sites, see Supplement 1: https://marine-biology.ru/mbj/article/view/498.

Biomphalaria representatives (Fig. 1) had an oval and flattened shell coiled in a single plane, making 3–4 whorls, with a smooth upper surface and a slightly obtuse-angled underside, featuring a deep umbilicus. The shell length (SL) was 4.0–10.0 mm (1,110 ind.), and the shell width (SW) was 3.5–7.0 mm (50 ind.). The aperture length (AL), its width (AW), and the umbilicus length (UL) were 3.0–5.0 mm (50 ind.), 2.0–3.0 mm (50 ind.), and 1.5–3.0 mm (50 ind.), respectively. Thus, the umbilicus occupied about ⅓ of the shell length. The height of the last whorl (SH) was 2.0–3.5 mm (50 ind.); it was approximately equal to the aperture width. Based on morphometric and morphological characteristics of shell, these molluscs were identified as *B. pfeifferi* [Brown, 2005; Mandahl-Barth, 1957].

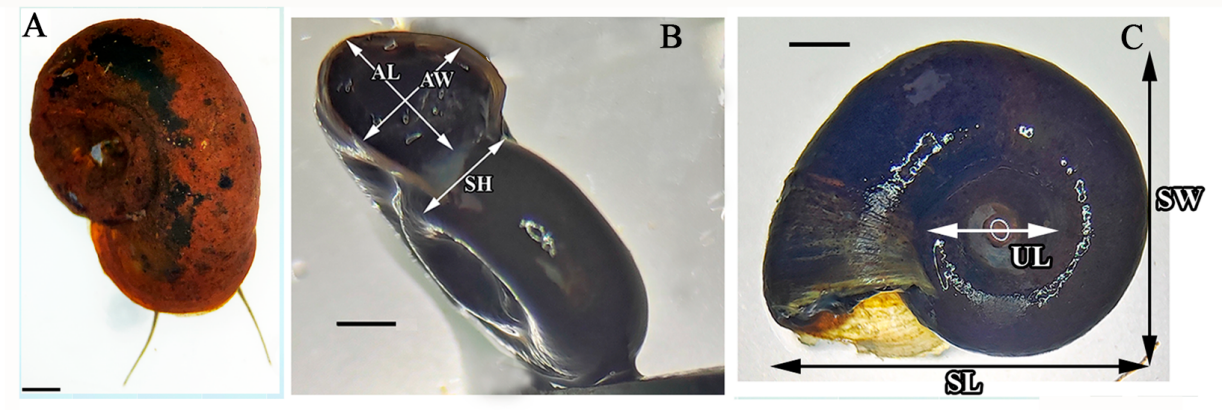

Fig. 1. *Biomphalaria pfeifferi* from water bodies in Kindia Prefecture (Guinea) (A) and scheme of the mollusc shell measurements (B, C). AL, aperture length; AW, aperture width; SH, the height of the last whorl (shell height); SL, shell length; SW, shell width; UL, umbilicus length. Scale bars are 1 mm

Identification of *Schistosoma* species based on morphological data. Daughter sporocysts and cercariae were found in *B. pfeifferi*; those were identified as trematodes of the genus *Schistosoma* based on their morphological characters and movement patterns (see Supplements 2 and 3: https://marine-biology.ru/mbj/article/view/498). Two daughter sporocysts, 0.365 and 0.488 mm long and 0.122 mm wide, were elongated sacs filled with cercariae of varying degree of maturity (Fig. 2A) which is consistent with the description of sporocysts of this genus [Ataev et al., 2016; Meuleman et al., 1980].

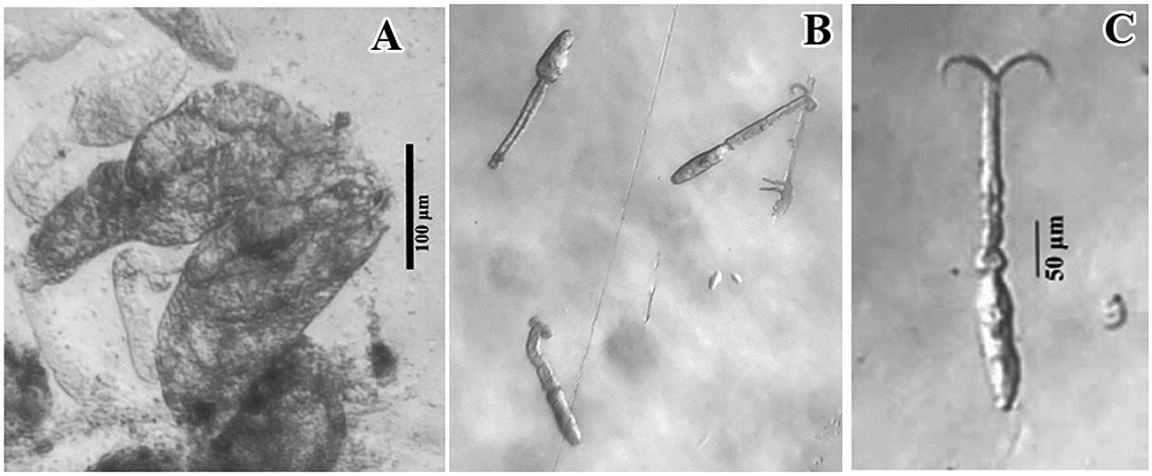

Fig. 2. *Schistosoma mansoni* sporocysts (A) and cercariae (B, C) from a mollusc *Biomphalaria pfeifferi* sampled in Kindia Prefecture (Guinea)

Cercariae are apharyngeal brevifurcocercariae without eye spots, with a typical forked tail (Fig. 2B, C, Fig. 3). Body oval, 0.122–0.139 mm long [(0.123 ± 0.003) mm], 0.044–0.058 mm maximum wide [(0.048 ± 0.009) mm], and 0.042–0.058 mm wide at the level of the ventral sucker [(0.046 ± 0.002) mm]. Tail stem cylindrical, slightly tapering distally; 0.144–0.180 mm long [(0.162 ± 0.008) mm] and 0.042–0.058 mm wide at the anterior end [(0.046 ± 0.002) mm]. Furcae 0.055–0.067 mm long [(0.062 ± 0.002) mm] and 0.006–0.012 mm wide [(0.009 ± 0.0006) mm]. Body and tail covered with minute spines. Oral sucker muscular, oval, 0.042–0.058 mm long [(0.046 ± 0.002) mm] and 0.027–0.033 mm wide [(0.03 ± 0.0005) mm], occupying about ⅓ of the body. Large pre- and postacetabular penetration gland cells in five pairs, fill nearly ⅔ of the body. Two pairs of preacetabular glands located anterior to the ventral sucker, with one pair, elongated and cylindrical, dorsally to the second pair, spheroidal. Three pairs of postacetabular glands, smaller and spheroidal ones, positioned posterior to the ventral sucker, slightly overlapping. Penetration gland ducts extend anteriorly and open at the anterior end of the body. Ventral sucker well-developed, 0.013–0.016 mm long [(0.015 ± 0.0005) mm] and 0.016–0.02 mm wide [(0.017 ± 0.0006) mm], placed in the posterior third of the body, 0.088–0.101 mm from the anterior end [(0.095 ± 0.002) mm]. Digestive system represented by esophagus: thin-walled tube with a spherical terminal dilation reaching the middle of the body. Mouth opening ventral. Nervous system consists of nerve cells forming clusters connected by thin fibers (neuropil) at the esophagus level. In the examined specimens, ciliated flame cells (cyrtocytes) and fine excretory capillaries arising from them not observed. Two larger excretory canals run anteriorly on either side of the body; two short canals located on either side of the anterior part of the tail stem; and a single excretory canal placed along the middle of the tail stem, opening at the tips of the furcae. Reproductive system undifferentiated and represented by some spherical cells posteriorly to the ventral sucker.

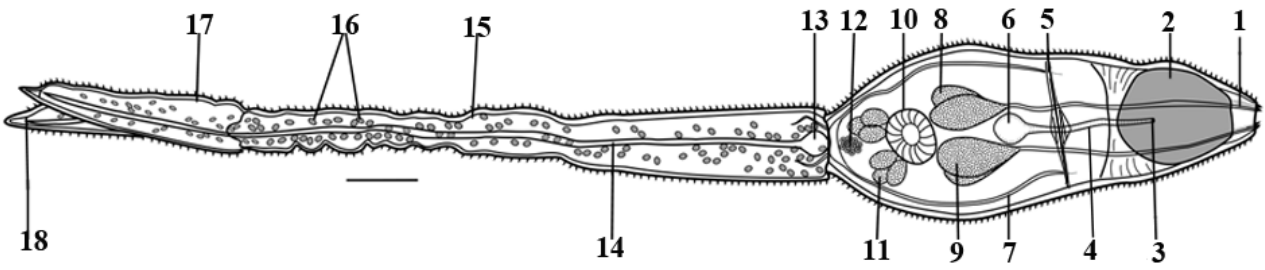

Fig. 3. Body shape and scheme of the internal morphology of *Schistosoma mansoni* cercariae from *Biomphalaria pfeifferi* molluscs sampled in Kindia Prefecture (Guinea): 1, penetration gland ducts; 2, oral sucker; 3, mouth; 4, esophagus; 5, neuropile; 6, caecum; 7, excretory ducts; 8, dorsal preacetabular gland; 9, ventral preacetabular gland; 10, ventral sucker (acetabulum); 11, postacetabular glands; 12, primordia of the reproductive system; 13, excretory bladder; 14, excretory canal; 15, tail; 16, sensilla on the tail surface; 17, tail furcae; 18, excretory pore. Scale bar is 0.02 mm

In general, the described cercariae are morphologically and morphometrically consistent with those of *S. mansoni* and *S. rodhaini* [Dorsey et al., 2002; Faust, 1919; Fripp, 1967; Skryabin, 1951; Stirewalt, 1974]. These species have nearly identical cercariae and both use *Biomphalaria* molluscs as intermediate hosts, but the second species has not been recorded in West Africa [Steinauer et al., 2008]. Taking this into account, the cercariae found were identified as *S. mansoni*.

Identification of *Schistosoma* species based on molecular genetic data. The ITS1 region (518 base pairs) was sequenced based on DNA extracted from *Schistosoma* cercariae from a mollusc *B. pfeifferi* sampled in Kindia Prefecture (the Republic of Guinea). Its sequence is available in GenBank NCBI under the accession number PV771189.

The obtained ITS1 fragment showed 100% identity with three similar DNA sequences of *S. mansoni* from Africa and Brazil (PP658717, FJ750523, and JQ289742). Comparison with a sequence of the closely related species, *S. rodhaini* (AF531312), revealed 6 nucleotide substitutions (98.96% identity). Comparison with sequences of *S. haematobium* (LC726151, PP963804, and PP963802) showed 95–96% identity (22–28 substitutions), and with *S. bovis*, 95% identity (28 substitutions). At the same time, this DNA locus differed by 2–6 substitutions (98.96–99.66% identity) from those of *S. mansoni* × *S. rodhaini* hybrid (EU599364–EU599378). However, phylogenetic analysis revealed that the sequenced sample and *S. mansoni* sequences formed a strongly supported clade, distinct from *S. rodhaini* and its hybrid with *S. mansoni* (Fig. 4). This result confirms that the found parasite belongs to *S. mansoni*.

Effect of mollusc size on *Schistosoma mansoni* occurrence. Sizes of the studied *B. pfeifferi* specimens were relatively small: the length of the molluscs did not exceed 1 cm (Table 1), while it can reach 1.5 cm. The material was sampled at the onset of the cold dry season (October and November), when *Biomphalaria* abundance was just starting to increase due to juveniles. Obviously, this affected the size composition: the predominance of small individuals caused a deviation from the normal distribution among uninfected snails (Table 2). At the same time, infected individuals, which were encountered much less frequently, showed a normal size distribution in most samples.

The mean length of *B. pfeifferi* infected with schistosomes was greater than that of uninfected individuals, but the differences between the samples were significant only for one sample (that with the number of infected snails exceeding 10) and when comparing all infected and uninfected molluscs (Table 2).

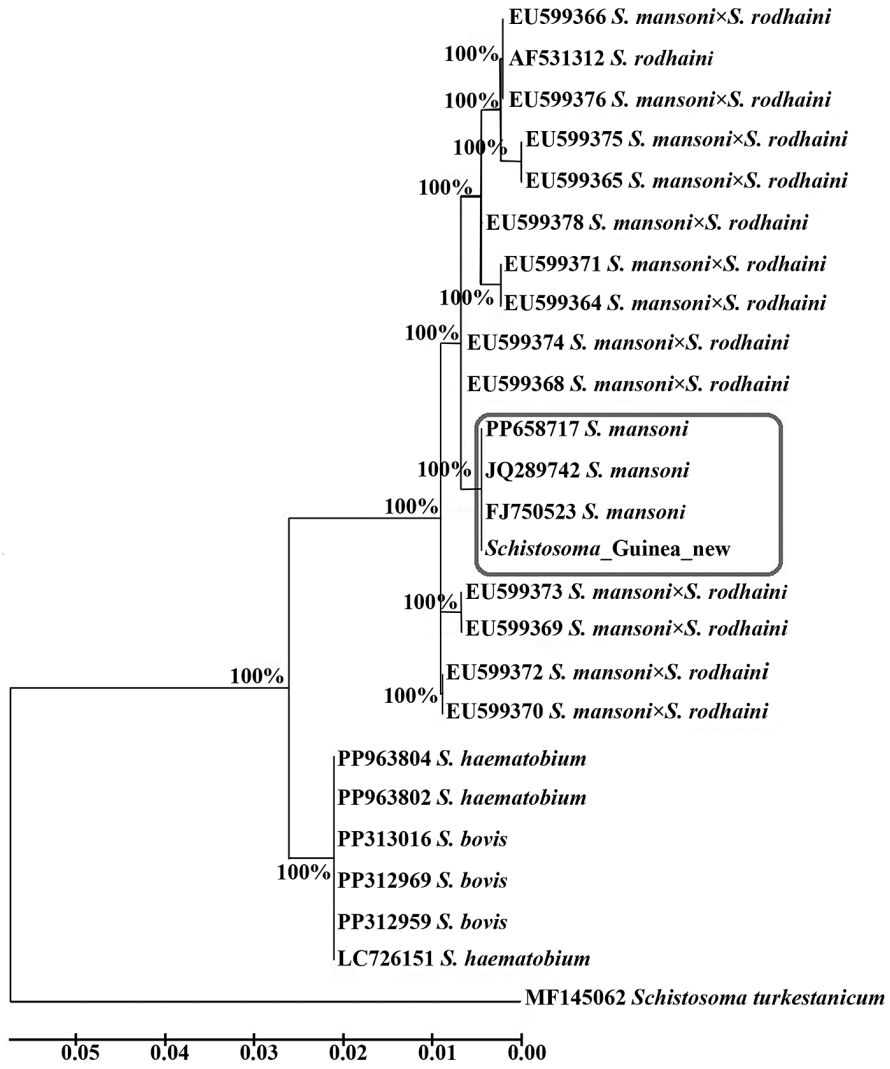

Fig. 4. Maximum likelihood phylogenetic tree based on ITS1 sequence showing the relationships between the *Schistosoma mansoni* sample from Guinea and related schistosome species available in GenBank NCBI. Nodal numbers are bootstrap support. Alignment length is 518 base pairs

Table 2. Characteristics of the distribution normality (Shapiro–Wilk test, *W*) of mollusc shell length and the significance of differences (Mann–Whitney *U* test) between samples of infected and uninfected specimens

Sampling site	Uninfected molluscs				Infected molluscs				Uninfected *vs.* infected molluscs	
	Number, ind.	Median length, mm	*W*	*p*	Number, ind.	Median length, mm	*W*	*p*	*U*	*p*
Molokhoure 1	269	7.0	0.94	< 0.01	13	7.5	0.94	**0.48**	1,177	**0.046**
Molokhoure 2	227	7.0	0.96	< 0.01	9	7.5	0.78	0.01	633	0.054
Yabara 2	321	7.0	0.94	< 0.01	6	6.8	0.96	**0.80**	914	0.833
Sinanya fontaine	293	6.5	0.92	< 0.01	4	7.0	–	–	–	–
All samples	1,110	7.0	0.95	< 0.01	32	7.3	0.94	**0.06**	12,192	**< 0.01**

Note: *p*-values indicating normal distribution and significance of differences between medians are highlighted in bold.

Effect of environmental parameters on *Schistosoma mansoni* occurrence. All water bodies where *Biomphalaria* molluscs, potential hosts of schistosomes, were recorded, were located within the city of Kindia (see Supplement 1: https://marine-biology.ru/mbj/article/view/498). Four sampling sites where schistosome invasion was revealed covered water bodies near private houses, schools, and public toilets with no wastewater treatment system (Figs 1, 2 in Supplement 1: https://marine-biology.ru/mbj/article/view/498); those are used by the local population for household needs and agricultural work.

Moreover, all water bodies in which molluscs of the genus *Biomphalaria* were registered were shallow, with slack or slow-flowing water, and abundantly overgrown with sedge and/or rice. However, among water bodies where *Biomphalaria* snails were not detected, half have the same biocenosis characteristics, and the ranges of physical and chemical parameters of water (pH, temperature, and dissolved oxygen) overlap (Table 3). A comparison of water bodies with molluscs as hosts but without cases of schistosome invasion and water bodies with reported invasion also revealed an overlap of the values of the analyzed abiotic factors.

The reduced dissolved O_2 content is obviously not a limiting factor for either *Biomphalaria* representatives or schistosomes. Thus, several water bodies where these molluscs and trematodes were revealed had the values < 5 mg·L^{-1} (see Table 3). In the biocenoses, the environmental acidity ranged from slightly acidic to slightly alkaline. No relationship between these factors and the occurrence of molluscs in water bodies was determined. Notably, the highest density of *Biomphalaria* populations was recorded at the Yabara 2 station where the environment was slightly acidic (see Supplement 1: https://marine-biology.ru/mbj/article/view/498).

Table 3. Comparative characteristics of surveyed water bodies with *Biomphalaria pfeifferi* and *Schistosoma mansoni* and without these species

Occurrence of *Biomphalaria* and *Schistosoma* in samples	Number of sampling sites	Physical and chemical parameters			Biocenosis characteristics			
		pH	T, °C	O_2, mg·L^{-1}	Current	Bottom type	Depth, m	Vegetation
No molluscs	19	4.5–6.8	+27…+34	3.3–10.3	S, 50%, W, 50%	R, 13%, Sn, 37%, Sl, 50%	0.5–1.5	In, 50%, Ab, 50%
Uninfected molluscs	4	6.3–6.8	+28…+34	4.3–5.0	W, 100%	Sl, 100%	0.02–0.5	Ab, 100%
Infected molluscs	4	5.8–7.8	+29…+32	4.5–6.1	W, 100%	Sl, 100%	0.02–0.4	Ab, 100%

Note: in the biocenosis characteristics, the proportions of sites with the corresponding parameters from the total number of sampling sites are indicated. Current (flow velocity): S, strong flow; W, weak flow or slack water. Bottom type: R, rocky; Sn, sandy; Sl, silty. Vegetation: In, an insignificant amount of semi-submerged and submerged vegetation; Ab, abundant semi-submerged and submerged vegetation.

DISCUSSION

Despite the fact that *S. mansoni* natural range covers the Republic of Guinea [Aula et al., 2021], with an infection rate up to 86% in the population in some regions [Hodges et al., 2011], data on the prevalence of infection in an intermediate host, *B. pfeifferi*, were obtained there for the first time.

Morphology-based identification of schistosome cercariae is challenging due to a small set of characters used and the existence of similar species and hybrids [Steinauer et al., 2008]. Thus, schistosome cercariae from *Biomphalaria* snails sampled in freshwater bodies of Kindia Prefecture (the Republic of Guinea) are morphologically similar to both *S. mansoni* and *S. rodhaini* which parasitizes the same mollusc. Moreover, in nature, these species can form hybrids [Morgan et al., 2003]. So far, *S. rodhaini* has been registered only in East Africa [Steinauer et al., 2008], although we cannot exclude that this species may be recorded in other regions where its hosts occur. To distinguish *S. mansoni* and *S. rodhaini*, it was proposed to use the ITS1 region of the ribosomal DNA cluster which showed stable differences between these species in three base pairs [Steinauer et al., 2008]. The ITS1 sequence obtained in this study differed from that of *S. rodhaini* in these base pairs and three more ones. This allows us to confidently identify the cercariae we found as *S. mansoni*. Thus, *B. pfeifferi* infection with these trematodes in Guinean water bodies has been proven for the first time.

The prevalence of infection (see Table 1) was generally consistent with the infection level of *Biomphalaria* snails with *S. mansoni* trematodes in Africa: 5.6% on average [Hailegebriel et al., 2020]. However, a recent study in Côte d'Ivoire, a neighboring country to Guinea, reported significantly higher *S. mansoni* occurrence: up to 100% of infected molluscs in a sample (56% on average) [Sokouri et al., 2024]. Noticeable (differing by orders of magnitude) seasonal variations in the infection with schistosomes in *Biomphalaria* molluscs have previously been established, as well as regional variations in the revealed dynamics. Thus, in Senegal and Uganda, *S. mansoni* occurrence in *B. pfeifferi* varied 0.08 to 3.7% and 8.7 to 13.3%, respectively, and was higher during the rainy seasons [Andrus et al., 2023; Bakhoum et al., 2021], while in Ethiopia, the values ranged 1.5 to 10.6% and were higher during the dry season [Hailegebriel et al., 2022]. So, to assess levels of *S. mansoni* infection for *Biomphalaria* molluscs in Guinean water bodies and to reveal seasonal peaks, similar studies should be carried out during the wet season.

Furthermore, molluscs infected with schistosomes were found only in water bodies within the city of Kindia. According to surveys of 2010–2016, the infection level of the city population with this trematode ranged 4.5 to 34% [Guilavogui et al., 2023]. Importantly, in 2010, for the age group of 1–17 years, the value was 5%, and in 2016, it was 34%. It is obvious that the infection levels of molluscs and final hosts, humans, are interconnected. It is necessary to continue studying *S. mansoni* occurrence in water bodies of the region in order to determine the reasons for the increase in the infection level among people.

It has been previously shown as follows: abiotic and biotic environmental conditions can noticeably affect both the occurrence of molluscs, potential hosts of *Schistosoma* trematodes, and the spread of cercariae [Brown, 2005]. First of all, the growth rate and population size of *Biomphalaria* snails depend on abiotic factors: depth, flow velocity, bottom type, and physical and chemical parameters of water [McCreesh, Booth, 2014]. Thus, the highest population density of molluscs was recorded for a depth of < 30 cm, silty bottom, and weak flow [Brown, 2005; Magero et al., 2025; McCreesh, Booth, 2014]. This is consistent with the results of our studies: *Biomphalaria* snails were found only in shallow bodies (< 60 cm), with a weak flow or slack water and with a silty bottom (Table 3).

However, we did not reveal a dependence of *Biomphalaria* occurrence and its infection with schistosomes on temperature, pH, and dissolved O_2 content in water; this is likely to result from the small range of values of the parameters analyzed (Table 2) within one month of the study. As shown

for neighboring Senegal, the abundance of molluscs and *S. mansoni* occurrence in them are positively correlated with the values of temperature and oxygen and negatively with pH (parameters which vary across the seasons) [Bakhoum et al., 2019].

Notably, most surveyed water bodies *Biomphalaria* snails were found in were characterized by relatively low levels of dissolved O_2: 4.3–5.0 mg·L^{-1}. Moderate eutrophication accompanied by a drop in the oxygen content in water has previously been shown to facilitate the development of *Biomphalaria* representatives [Hoover et al., 2020]. Such a tolerance of these snails to O_2 deficiency in water contributes to their occurrence in excrement-contaminated water and forms conditions for mollusc infection with schistosome larvae.

Temperature is an important factor that not only limits the geographic distribution of *B. pfeifferi* to tropical and subtropical regions but also regulates its fecundity and mortality [Bakhoum et al., 2021]. Experiments have shown that *Biomphalaria* molluscs are active at +18…+32 °C, while the optimal range for their reproduction and survival is +20…+26 °C [Sturrock, 1965]. In Senegal, for example, the highest density of *Biomphalaria* snails was recorded at +20…+32.5 °C [Bakhoum et al., 2021], and in East African water bodies, those were not found at sites where the temperature exceeded +30 °C [Magero et al., 2025]. Within this study, *Biomphalaria* representatives were sampled from water bodies with the water temperature reaching +34 °C. The temperature conditions the molluscs are adapted to seem to vary by region; in the Republic of Guinea, a range of +30…+34 °C does not limit *B. pfeifferi* occurrence.

Water temperature affects not only mollusc population, but also the development of sporocysts and the production of schistosome cercariae. According to the results of experiments, an increase in temperature from +23 to +33 °C reduces the development period of *S. mansoni* sporocysts in molluscs [Stirewalt, 1974]. The emission of cercariae is limited to a range of +16…+35 °C, decreasing by approximately three times at temperatures below +18 °C or above +33 °C [Pflüger, 1980]. After analyzing a large set of empirical data on the effect of temperature on both molluscs and schistosomes, I. H. Aslan *et al.* [2024] established a theoretical temperature optimum for the spread of *S. mansoni* in sub-Saharan African waters: +23…+27 °C. These values are lower than the temperatures at which schistosome-infected molluscs were found in Guinean waters (Table 3); this may evidence for the fact that infection level in the region may be higher (under different temperature conditions) than the level established in the present study.

Another characteristic of the biocenosis, which is considered a key factor affecting the spread of schistosomes, is the presence of semi-submerged and submerged vegetation. The high abundance of aquatic plants is positively related with the abundance of mollusc hosts of *S. mansoni* [Brown, 2005]; also, it may be positively correlated with the number of schistosome cercariae produced by infected snails [Haggerty et al., 2020]. In the present study, *B. pfeifferi*, both infected with *S. mansoni* and uninfected, were found only in water bodies abundantly covered with semi-submerged plants: sedge and rice (Table 3).

It was also shown that the level of schistosome infection depends on the mollusc size. Thus, as established experimentally, the size of *Biomphalaria* snails was negatively correlated with susceptibility to *S. mansoni* infection, and this seems to be due to their increased immunity with age. The smallest molluscs (1.5–2.9 mm) had the highest infection level and produced the largest number of cercariae [Spaan et al., 2023]. However, the relationship between the size and age of molluscs and their infection with schistosomes under natural conditions is more complex, is mediated by many factors (the number of snail generations *per* year, their population density, and the survival of infected individuals), and can vary

depending on the season [Woolhouse, 1989]. In our samples, infected *B. pfeifferi* were on average larger (see Table 2), but the largest and smallest snails were uninfected. This is probably governed by the study period: October and November are the onset of the dry season marking the development of a new generation of molluscs, and they have not yet been infected with schistosomes.

The obtained results are limited to one season and region of the Republic of Guinea. Those demonstrate the need to continue research on mollusc infection with schistosomes in various regions of the country to understand the conditions of the parasite transmission to humans. It is important for developing measures to control schistosomiasis at the local level.

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ВСТРЕЧАЕМОСТЬ *SCHISTOSOMA MANSONI* (TREMATODA: SCHISTOSOMATIDAE) У *BIOMPHALARIA PFEIFFERI* (GASTROPODA: PLANORBIDAE) В ВОДОЁМАХ ПРЕФЕКТУРЫ КИНДИЯ (ГВИНЕЙСКАЯ РЕСПУБЛИКА)

Е. В. Дмитриева[1], С. Диаките[2], П. Койвоги[2], Н. В. Пронькина[1], В. А. Уппе[1], Л. Конате[2], М. Д. Соу[2], А. М. Балде[2], М. Камара[3], Д. М. Полевой[1], Р. В. Мачкевский[1], Е. А. Водясова[1], И. Кейта[2], А. И. П. Диалло[2]

[1]ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН», Севастополь, Российская Федерация
[2]Центр морских и прибрежных исследований Гвинеи, Конакри, Гвинейская Республика
[3]Институт прикладной биологии, Киндия, Гвинейская Республика
E-mail: *evdmitrieva@ibss-ras.ru*

На территории Гвинейской Республики, в западной области префектуры Киндия, в 8 пресноводных водоёмах найдены моллюски родов *Biomphalaria* и *Bulinus* — промежуточные хозяева трематод рода *Schistosoma*, патогенных для человека. Моллюски *Bulinus* были свободны от шистосом, в то время как биомфалярии были ими заражены. В 4 водоёмах выявлены очаги заражённости моллюсков этим паразитом. На основе морфологического анализа раковин заражённых моллюсков установлена их принадлежность к виду *Biomphalaria pfeifferi*. Особенности движения,

форма тела, внутренняя анатомия и морфометрические характеристики церкарий, выходящих из моллюсков, соответствовали таковым *S. mansoni*. Полученные последовательности участка ITS1 рДНК показали 100%-ную идентичность с аналогичными локусами *S. mansoni*, паразитирующих у людей и крыс из Африки и Бразилии. В то же время они отличались от таковых ближайшего вида, *S. rodhaini*, на 6 нуклеотидных замен, а от таковых его гибридов с *S. mansoni* — на 2–6 замен. Филогенетический анализ показал достоверное (100 %) включение секвенированной пробы в кластер с другими последовательностями *S. mansoni* и дистанцирование от линий *S. rodhaini* и гибридов *S. rodhaini* × *S. mansoni*. Этот результат подтверждает идентификацию вида как *S. mansoni*. Данные о встречаемости этого паразита у моллюсков, а также последовательности его ДНК получены для территории Гвинеи впервые. В целом заражённые моллюски (32 экз.) имели достоверно бóльшие средние размеры раковин, чем незаражённые (1110 экз.). Анализ влияния ряда факторов среды на встречаемость моллюсков *B. pfeifferi* и их заражённость трематодами *S. mansoni* не выявил их зависимости от pH, насыщенности кислородом и температуры воды в пределах изменчивости этих показателей в период исследования (октябрь — ноябрь). Отмечена относительная толерантность биомфалярий к дефициту кислорода в воде, что способствует, очевидно, их встречаемости в загрязнённых экскрементами водах и создаёт условия для заражения моллюсков мирацидиями шистосом. Показана связь присутствия шистосом в биоценозе с определёнными характеристиками среды, а именно со стоячей или медленно текущей водой и с наличием обильной прибрежной растительности. Кроме того, все водоёмы, в которых найден паразит, находились в черте города. Полученные результаты важны для разработки мер борьбы с шистосомозом в регионе.

Ключевые слова: *Schistosoma*, шистосомоз, природный очаг заражения, промежуточный хозяин, *Biomphalaria*, ITS1 рДНК, Гвинейская Республика