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Mapping of intermediate host snails for schistosomiasis in the Democratic Republic of Congo: a systematic review

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Abstract: Schistosomiasis is a snail-borne disease that has a considerable impact on human and animal health, particularly in sub-Saharan Africa. The intermediate hosts of the schistosome parasites are freshwater snails of the genera *Biomphalaria* Preston, 1910 and *Bulinus* Müller, 1781. In order to identify existing gaps in the spread of the disease in the Democratic Republic of Congo (DRC), this study compiled the available knowledge of the distribution, population dynamics and ecology of the intermediate hosts of schistosomiasis. A systematic literature search was conducted in PubMed, Embase and Scopus for all malacological studies on schistosoma intermediate hosts in DRC published between 1927 and October 2022. A total of 55 records were found, of which 31 met the inclusion criteria: these were published field and experimental studies conducted in the DRC and focused on snails as intermediate hosts of schistosomes. The analysis of these studies revealed that more up-to-date data on the distribution of snail intermediate hosts in the DRC are needed. Moreover, ecological factors have been less studied for *Bulinus* species than for *Biomphalaria* species. These factors play a crucial role in determining suitable snail habitats, and the lack of comprehensive information poses a challenge in snail control. This review makes it clear that there are no current malacological data in the DRC. There is a clear need for molecular and ecological research to update the exact species status and population dynamics of all potential intermediate host species. This will facilitate targeted snail control measures that complement drug treatment in the control of schistosomiasis in the country.

Keywords: *Bilharzia*, malacology, snails, *Biomphalaria*, *Bulinus*, Africa.

Schistosomiasis or bilharziosis is a parasitic disease caused by trematodes of the genus *Schistosoma* Weinland, 1858.

The main species of schistosomes that infect humans are *Schistosoma haematobium* (Bilharz, 1852); *S. japonicum* (Katsurada, 1904); *S. mansoni* Samson, 1907; *S. intercalatum* Fisher, 1934; *S. guineensis* Weinland, 1858 and *S. mekongi* Voge, Bruckner et Bruce, 1968.

Their life cycle involves freshwater snails, which act as the intermediate hosts, while humans or other mammals are the definitive hosts (Gryseels et al. 2006). The intermediate host of *S. mansoni* belongs to the genus *Biomphalaria* Preston, 1910 (Scholte et al. 2012), while intermediate hosts of *S. haematobium* and *S. intercalatum* belong to the genus *Bulinus* Müller, 1781.

Schistosomiasis poses a major public and veterinary health problem, leading to significant socioeconomic impacts in tropical regions. In humans, chronic schistosomiasis leads to anaemia, cognitive and learning disorders, stunted growth in children, and adult complications, in-

cluding liver fibrosis and hepatocarcinoma, bladder fibrosis, cancer and infertility (Aubry and Gaüzère 2021). For these reasons, schistosomiasis is ranked the second most important parasitic disease after malaria in terms of morbidity and prevalence, with more than 240 million people infected worldwide (WHO 2021).

The Democratic Republic of Congo (DRC) is the largest country in sub-Saharan Africa and one of the countries with the highest burden of schistosomiasis, as approximately 11 million school-aged children are exposed to schistosomiasis (WHO 2021). The three main species in the DRC are *S. haematobium*, *S. mansoni* and *S. intercalatum*. Research carried out within the country was reviewed in 2015, and most research effort focused on the clinical and parasitological aspects of schistosomiasis (Madinga et al. 2015). In 2015, after mapping efforts, the DRC launched a mass drug administration (MDA) campaign for school-aged children. Additionally, a recent study in the Kongo Central province found a significant reduction in schistosomiasis-based morbidity and rapid reinfection after treatment (J.Madinga, unpublished).

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Table 1. Snail collection method. *Bi.* – *Biomphalaria*; *Bu.* – *Bulinus*

Study	Study Region	Objective	Snail collection	Sampling time	Snail identification	Intermediate hosts species studied
Pilsbry and Bequaert 1927	14 sites across the country	To describe the aquatic molluscs of the Belgian Congo with reference to their geography and ecology	Not indicated	Not indicated	<i>Bulinus africans</i>	
Fisher 1934	Yakusu, Northeast of DRC	To prove that the intestinal schistosomiasis cases were due to distinct species of schistosomes.	Not indicated	Not indicated	<i>Bu. (Physopsis) africanus africanus</i>	
Fain 1951	Lake Albert, East of DRC	To assess the presence of freshwater snails including the intermediate host of schistosomiasis in Lake Albert	By hand	Not indicated	Morphological identification, but key not indicated	<i>Biomphalaria pfeifferi</i> , <i>Bi. stanleyi</i> , <i>Bi. smithi</i>
Mandal-Barth et al. 1972	Lufira, Southeast of DRC	To describe the distribution and ecology of aquatic snails and their epidemiological role in intestinal and urinary schistosomiasis	Not indicated	Not indicated	Morphological identification using Mandhal-Barth (1962) identification key	<i>Bi. pfeifferi</i> , <i>Bi. sudanica rugosa</i> , <i>Bu. (Physopsis) globosus</i> , <i>Bu. tropicus</i> , <i>Bu. forskali</i>
Bennike et al. 1976a	Kinshasa, Southwest of DRC	To support the conception that schistosomiasis has established itself as an autochthonous disease in the region of Kinshasa	Fish nets	Two to four times a year for two years, but sampling time not indicated	Morphological identification using Mandhal-Barth (1962) identification key	<i>Bi. camerunensis</i> , <i>Bu. forskali</i> , <i>Bu. globosus</i>
Bennike et al. 1976b	Kinshasa, Southwest of DRC	To support the conception that schistosomiasis has established itself as an autochthonous disease in the region of Kinshasa	Not indicated	Not indicated	<i>Bi. camerunensis</i> , <i>Bu. globosus</i>	
Colaert et al. 1977	Makelele River, Southwest of DRC	To provide for the first time evidence of local transmission of <i>Schistosoma mansoni</i> in Kinshasa	By hand, searching step by step at a rate of 200 metres per day	Not indicated	Not indicated	<i>Bi. camerunensis</i>
Malaisse and Ripert 1977	Lufira Reservoir, Southeast of DRC	To confirm that schistosomiasis was established as an autochthonous disease in the region of Kinshasa	Tweezers and use of the shovel in trails	Not indicated	Morphological identification, but key not indicated	<i>Bi. pfeifferi</i> , <i>Bi. sudanica rugosa</i> , <i>Bu. globosus</i> , <i>Bu. forskali</i>
Frandsen et al. 1978	Kisangani, Northeast of DRC	To determine the total cercarial production per 100 exposed snails for each snail population.	Not indicated	Not indicated	Not indicated	<i>Bu. globosus</i>
Frandsen 1979	Lukala, Kisangani, Mbudi, Kinshasa and Lubumbashi, east and west of DRC	To follow and discuss the dynamics of snail species found in the Lubumbashi reservoir, Southeast of DRC	Direct pick-up and bottom dredging using a rake	Not indicated	Morphological identification using Mandhal-Barth (1962) identification key	<i>Bi. pfeifferi</i> , <i>Bu. (Physopsis) globosus</i>
Malaisse et al. 1981	Konde-Kuumba village, Southwest of DRC	To assess the presence of schistosomiasis in Mayombe and propose solutions to control the disease	Not indicated	Not indicated	Not indicated	<i>Bi. pfeifferi</i> , <i>Bu. forskali</i>
De Clercq et al. 1985	Maniema, East of DRC	To explain the patchy distribution of schistosomiasis in relation to geological features and anthropogenic change	Not indicated	Not indicated	Not indicated	<i>Bi. pfeifferi</i>
Polderman et al. 1985	Kinshasa, Southwest of DRC	To show the changes in the malacological situation in Kinshasa compared to ten years ago and identify an autochthonous focus of schistosomiasis caused by <i>S. intercalatum</i>	Fish nets	Not indicated	Not indicated	<i>Bu. forskali</i> , <i>Bu. globosus</i> , <i>Bi. pfeifferi</i>
De Clercq 1987	Virunga Stream, East of DRC	To demonstrate how population densities affect the growth and snail demography in both field and laboratory setup	Quadrat sampling using a wooden frame (25×25×30 cm) and mesh sieve (0.8 mm)	Not indicated	Not indicated	<i>Bi. pfeifferi</i>
Loreau and Baluku 1987a,b	Iwiro and Bilala, East of DRC	To compare the population dynamics of <i>Biomphalaria pfeifferi</i> in two streams (the artificial drain of Bilala and another natural stream)	Wooden frame (25×25×30 cm), 15 samples each month	Not indicated	Not indicated	<i>Bi. pfeifferi</i>
Baluku and Loreau 1989	Virunga Stream, East of DRC	To assess the ecology of snails, their diet, populations dynamics and their predators to inform on biological control	Quadrat method	Not indicated	Morphological identification using Mandhal-Barth (1962), Brown <i>Bi. pfeifferi</i> , <i>Bu. truncatus</i> , <i>Bu. forskali</i> (1994) identification keys	

Table I. continued									
Chartier et al. 1992	Ituri, Northeast of DRC	To establish an inventory of freshwater molluscs in the Ituri region and to precise the frequency of the various species responsible for transmitting animal and human trematodiasis method	Collection of snails by the “man-time”	30 minutes per sites by three to four people	Morphological identification using Mandhal-Barth (1962) identification key	<i>Bi. pfeifferi, Bi. africanus, Bi. forskalii, Bi. sudanica.</i>			
Chartier et al. 1993	Ituri, Northeast of DRC	To relate environmental factors and particularly the type of pasture where cattle graze to the presence of the intermediate hosts.	Not indicated	Not indicated	Morphological identification using Mandhal-Barth (1962) identification key	<i>Bi. pfeifferi, Bi. sudanica, Bi. africanus, Bi. forskalii</i>			
Bagalwa and Baluku 1997	Katana, East of DRC	To describe the distribution of snails in the Katana region and to determine the main foci of <i>Schistosoma haematobium</i> and <i>S. intercalatum</i>	A small mesh net, 30×20 cm with a rectangular opening and S. rectangular opening in the “man-time”	Ten minutes per person per site and Brown (1994) identi-globusus	Morphological identification using Mandhal-Barth (1962) identification key	<i>Bi. pfeifferi, Bu. truncatus, Bi. forskalii, Bi. sudanica.</i>			
Tchuen Tchuenté et al. 1997	Kinshasa, Southwest of DRC	To reassess the prevalence of <i>S. intercalatum</i> in Kinshasa	Not indicated	Not indicated	Morphological identification using Mandhal-Barth (1962) identification key	<i>Bi. pfeifferi, Bu. forskalii, Bi. pfeifferi</i>			
Bagalwa and Baluku 1998	Lwiro	To specify the periods of high risk of human infection and the favourable time for applying snail control methods in this recent focus of schistosomiasis	Mesh net of less than 2 mm and with a rectangular opening of 30×20 cm	Not indicated	Morphological identification using Brown (1994) <i>Bi. pfeifferi</i> identification keys	<i>Bu. globosus, Bu. forskalii, Bi. pfeifferi</i>			
Pointier et al. 2005	Mangungu River, South west of DRC	To investigate the introduction of a new intermediate host snail of <i>S. mansoni</i> in Kinshasa and to show that this species is indistinguishable from the Brazilian <i>Bi. tenagophila</i> but quite distinct from <i>Bi. camerunensis</i> from Cameroon	By hand	One sample per site and per month. Three genetic markers were used: ITS1, ITS2, 18S rRNA	Molecular identification. Molecular identification using Mandhal-Barth (1962) and Brown (1994) identification keys	<i>Bi. pfeifferi, Bi. smithi, Bi. stanleyi, Bi. forskalii, Bi. camerunensis, Bi. tenagophila</i>			
Schlütheiß et al. 2011	Orientale province and North-Kivu, East of DRC	To provide malacological information with relevance to conservation efforts	Sieves and a dredge	Not indicated	Morphological identification using Mandhal-Barth (1962) and Brown (1994) identification keys	<i>Bi. pfeifferi, Bu. forskalii, Bu. truncatus</i>			
Batumike et al. 2014	Katana region, East of DRC	To make an inventory of freshwater snails in the Katana region and to specify the frequency of the various species that may be involved in transmitting animal and human trematodes.	Stirrup net, with a small mesh (> 2 mm) and rectangular opening (30×20 cm)	Ten minutes per site and Brown (1994) identification keys	Morphological identification using Mandhal-Barth (1962) and Brown (1994) identification keys	<i>Bi. pfeifferi, Bu. forskalii, Bu. globosus</i>			
Ndakala et al. 2015	Kalengu river, East of DRC	To carry out a general inventory of all the aquatic macrofauna of the Kalengu River	Beach seine 45 cm in circumference and 2 mm mesh, traps 10 cm in circumference and 0.5 mm mesh made from local material	Not indicated	Morphological identification using Brown (1994) <i>Bi. pfeifferi</i> and identification keys	<i>Bi. pfeifferi, Bu. forskalii</i>			
Shabani et al. 2016	Mambasa, Northeast of DRC	To make an inventory of aquatic invertebrates of the Mambasa area	Hand scoop net of 0.5 mm of mesh, sieve or Not indicated plastic container	Not indicated	Morphological identification using Brown (1994) <i>Bi. pfeifferi</i> identification key	<i>Bi. pfeifferi, Bu. forskalii</i>			
Ruffin et al. 2018	Ruzizi plain, East of DRC	To determine the diversity and abundance of benthic invertebrates collected in natural ponds of Ruzizi Valley and their degree of similarity.	Not indicated	Not indicated	Morphological identification using Brown (1994) <i>Bi. pfeifferi, Bu. forskalii, Bu. globosus</i>	<i>Bi. pfeifferi, Bu. forskalii, Bu. truncatus</i>			
Atila et al. 2021	Kimpese, Southwest of DRC	To identify intermediate hosts of schistosomiasis in the Kimpese region	Snail collection over a distance of less than 10 m. A metal clamp, a 1 mm mesh net and hands with gloves.	Not indicated	Morphological identification using Mandhal-Barth (1962) identification key	<i>Bi. pfeifferi, Bu. forskalii, Bu. globosus</i>			
Habib et al. 2021	Kinshasa, Southwest of DRC	To review the invasion patterns of <i>Biomphalaria</i> spp., the intermediate host of <i>S. mansoni</i>	Not indicated	Not indicated	Morphological identification using Brown (1994) <i>Bi. pfeifferi, Bu. forskalii, Bu. truncatus</i>	<i>Bi. tenagophila</i>			

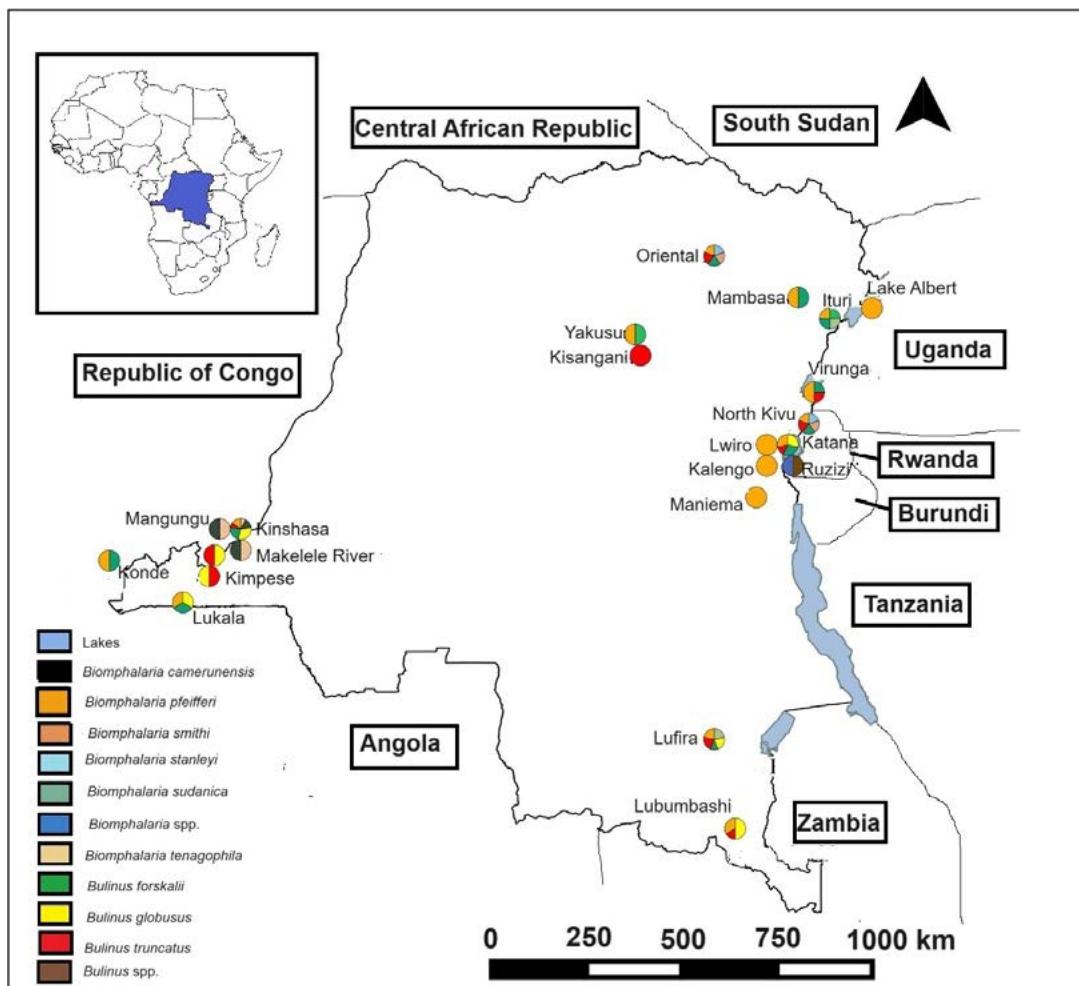


Fig. 1. Snail distribution in Democratic Republic of Congo.

Furthermore, other studies have also shown that MDA alone is insufficient in stopping disease transmission or avoiding re-emergence in endemic areas (Sokolow et al. 2016, Tchuem Tchuenté et al. 2017). Therefore, snail vector control is crucial to complement MDA and to reach elimination in areas where the prevalence is low.

For effective and sustainable snail control, detailed information on the species involved in the disease transmission, their distribution and population dynamics, and the spatial distribution of their breeding sites are fundamental. Our systematic review aims to compile a comprehensive synthesis of the literature on the intermediate host snails of schistosomiasis and to understand the species diversity, population dynamics and ecology, and the transmission sites in the DRC.

MATERIALS AND METHODS

Search strategy

This literature review was conducted per the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Liberati et al. 2009). The search was carried out for all the articles published from 1927 to October 2022 on malacology surveys for the intermediate host of schistosomiasis in DRC until 29 November 2022. PubMed (Medline), Embase and Scopus were utilised for the search. The key terms used were “*Schistosom**”, *Bilharz**,

Biomphalaria, *Bulin**, *Macology**, *Snail**, *Mollus**, *Planorb**, *Congo* and *Zaïre*”. The Boolean operators “AND” and “OR” were used to combine the search terms (full string in Supplementary Material S1). We used Patient intervention, comparison and outcome (PICO) to formulate the research objective.

Eligibility criteria

The criteria for the article selection were determined in advance of the search exercise. The search was limited to the field and experimental surveys in English and French and published in peer-reviewed journals. The selected studies had to have reported on the intermediate host snails of schistosomiasis. Grey literature was also included.

The title, abstract, key terms, authors' names, year and journal name of the identified articles were exported to an Excel spreadsheet. Exclusion of the duplicate reports was conducted by importing the references into the reference manager “*Mendeley*” and afterwards uploaded to the Rayyan web application (Rayyan QCRI) for the title and abstract screening (Ouzzani et al. 2016). Two researchers screened individual titles and abstracts independently (Germain Kapur and Cecilia Wangari Wambui). In instances where the title and abstract eligibility were uncertain, the full text was assessed instead to ensure valuable articles were not eliminated.

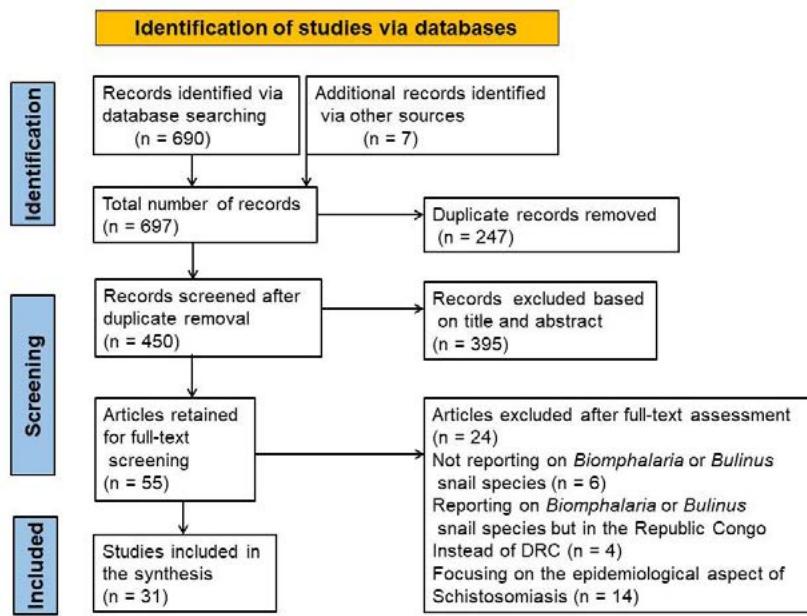


Fig. 2. Selection of papers.

Data collection

For data management, a table outlining eligible papers for this review was created with the following information: the author and year of publication, study objective; collection material and methods; intermediate host snails collected and geographical location of the sampling site in DRC (Table 1). Relevant outcomes of the studies for this review are reported in the results section. We used AMSTAR as the quality assessment tool for this study (Shea et al. 2017).

RESULTS AND DISCUSSION

Characteristics of the retrieved records

A total of 690 articles were extracted from three databases, of which 170 were found by PubMed, 154 by Embase and 366 by Scopus. Seven additional articles were identified through snowball sampling; so that articles cited by initially found papers met our selection criteria and were included in the study. After removing the duplicates, 450 articles were appropriate for the title and abstract screening. A total of 55 studies were retained for full-text screening, where 24 were later excluded because the snail species surveyed were not the intermediate hosts of schistosomiasis (*Biomphalaria* or *Bulinus*) or the study was in the Republic of Congo instead of the DRC or the study was centred on the epidemiological aspect of schistosomiasis. Finally, a total of 31 studies were incorporated into the review (Fig. 1); an outline of the studies is compiled in Table 1.

The data collected were organised in the following sections: (1) snail collection and identification methods; (2) the geographical distribution of the schistosomiasis intermediate host snails; (3) the population dynamics of the snails; (4) the ecological and water conditions of the sites where the snails were collected; and (5) method used to check snail infection rate and infection susceptibility by the schistosome parasites.

Snail collection and identification methods

Different collection methods were applied for the retrieved studies, ranging from the use of simple scooping nets to direct snail picking by tweezers (Table 1). The selection of the method used for snail collection often relied on the characteristics of the snail habitat, including factors such as vegetation presence, turbidity, bottom type (muddy or rocky), water current and water level. This choice aims to optimise the number of specimens collected. Only five studies reported the snail collection effort as shown in Table 1. Snail identification in the studies was primarily conducted through morphological means, utilising identification keys by Mandahl-Barth (1962) and Brown (1994). However, an exception was made by Pointier et al. (2005), who employed internal transcribed spacer 1 and 2 (ITS1 and ITS2) and 16S ribosomal DNA (16S rDNA) molecular markers for identification purposes.

Geographical distribution

A total of six *Biomphalaria* (*Bi.*) and four *Bulinus* (*Bu.*) species that have been documented in DRC include *Bi. camerunensis* Boettger, *Bi. pfeifferi* (Krauss), *Bi. sudanica* (Martens), *Bi. tenagophila* (d'Orbigny), *Bi. smithi* Preston, *Bi. stanleyi* Smith, *Bu. africanus* Krauss, *Bu. forskalii* Ehrenberg, *Bu. globosus* Morelet and *Bu. truncatus* (Audouin). These species are distributed in a heterogeneous way throughout the country (Fig. 2). The most widespread species are *Bi. pfeifferi*, *Bu. forskalii* and *Bu. truncatus*, respectively, in that order. *Biomphalaria pfeifferi* was found in almost all studies conducted except in a few sites, such as Kisangani, Lukala (Frandsen et al. 1978), Mangungu (Pointier et al. 2005) and Ruzizi (Ruffin et al. 2018).

Some species have only been found at one site, notably *Bi. tenagophila* in the Mangungu River in Kinshasa, the capital city of the DRC (Pointier et al. 2005, Habib et al. 2021). The 20 large specimens collected in 1994,

were initially considered to be *Bi. camerunensis*. Later in 2005, Pointier et al. (2005) confirmed the species to be *Bi. tenagophila* through molecular analysis and suspected to have been introduced to Kinshasa at the end of the 1960s through the aquarium trade. However, the species have not been found since in all rivers in Kinshasa and was replaced by *Bi. pfeifferi* and *Bu. forskalii* with low abundance (Pointier et al. 2005). The Lufira reservoir, which is situated in the southeast of DRC, has more sympatric species, namely *Bi. pfeifferi*, *Bi. sudanica*, *Bu. forskalii* and *Bu. globosus* (Mandahl-Barth et al. 1972, Malaisse and Ripert 1977).

Population dynamics of *Biomphalaria* species

In 1968, Mandahl-Barth surveyed the distribution and the ecology of the aquatic snails in the Lufira reservoir, located in the Katanga region of southeastern DRC. During the study, the species that were collected include *Bi. pfeifferi*, *Biomphalaria sudanica rugosa* Martens, and *Bu. globosus*. It should be noted that while *Bi. sudanica rugosa* was infected with *Schistosoma mansoni* in the laboratory, it is not known to be responsible for the transmission of *S. mansoni* in the region, probably due to unfavourable environmental conditions. The snail species involved in the transmission of *S. mansoni* in the region is *Bi. pfeifferi*.

In another survey conducted between 1982 and 1984 (once per month for 20 months) in Virunga stream at Lwiro (eastern DRC), the population dynamics of *Bi. pfeifferi* was discontinuous, influenced by seasonality (Loreau and Baluku 1987a). The maximum density was recorded at the beginning of the dry season (June) and the minimum in the middle of the rainy season (January), with a peak frequency just after breeding activities between June and November.

This confers with another study in the Kô and Deh (west of Ivory Coast), where they observed high densities of *Bi. pfeifferi* and *Bu. globosus* at the end of the dry season and beginning of the rainy season (Yapi et al. 2014). Similarly, observed *Bu. truncatus* in an artificial dam in Burkina Faso characterised by a slight increase in the densities at the start of the rainy season, maximum densities during periods of low temperatures, followed by a decrease in numbers when the water level falls and the temperature rises (Poda et al. 1994).

The growth rate and demography of *Bi. pfeifferi* from Virunga stream were also examined under crowding and uncrowding conditions in the laboratory and compared with the field conditions (Loreau and Baluku 1987a,b). Snail sizes in the field were influenced by the sampling techniques and their growth predictions matched those of the uncrowded laboratory populations. The small size, the lack of pigmentation and the sheltering behavior of young snails during unfavourable conditions favour their escape from sampling (Loreau and Baluku 1987a,b). The age structure, generation time and life expectancy revealed one primary generation yearly in the field, despite continuous reproduction (Loreau and Baluku 1987a,b). The observation differed from the laboratory study by Loreau and

Baluku (1987a,b), who reported a generation time of 5.3 months with a maximum egg production of 5 months.

To track biological parameters of snails in the field, a quadrat sampling using a frame was used during the 24 months of the study. The contents of the frame were cleaned on a sieve. To determine the egg production interval, the eggs removed from the frame were counted and measured. For the monitoring of lifespan, both young and adult snails collected from the frame were marked and monitored for the duration of the study.

The snails' life expectancy in the field was higher than that of the laboratory setup, with duration of up to 19 months (Loreau and Baluku 1987a,b). The survival rate was 10 to 30 times lower (2.7% in 1982 and 8.5% in 1984) than that of the snails in the laboratory setup (81.5%). Life expectancy at laying was lower in the field (2.0 weeks in 1982 and 3.5 weeks in 1984) than in the laboratory (23.4 weeks in 1982 and 24.8 weeks in 1984) (Loreau and Baluku 1987a,b).

Population dynamics of *Bulinus* species

None of the articles retrieved in the search had studied the population dynamics of *Bulinus* spp. in depth in the DRC over the last five years. Nevertheless, as briefly described Pilsbry and Bequaert, Fisher also reported *Bu. (P.) africanus africanus* (Krauss) in 1934 (Pilsbry and Bequaert 1927, Fisher 1934). At that time, its distribution was seasonal with high densities during the high rainy season (December to April). High snail abundance corresponded to the time when rivers, streams and estuaries were full and characterised by a wash grass along the banks. Snails were found in the bays, inlets and on the submerged grass along the banks of the Congo River. In addition, during this season, thousands of snails occurred on the foreshore of villages like Yakusu and Yatumbo (northeast of DRC) (Fisher 1934).

Ecological conditions of the collection sites of *Biomphalaria* species

Biomphalaria pfeifferi typically occurs in a biotope consisting of rivers, streams, reservoirs with slow currents, muddy bottoms and abundant aquatic vegetation (De Clercq et al. 1985). Baluku et al. (1989) defined the environmental niche of *Biomphalaria* as slow current, high light intensity, abundant aquatic vegetation rich in macrophytes and unicellular algae (phytobenthos and periphyton). Furthermore, *Bi. pfeifferi* is rarely found in polluted sites (Bagalwa and Baluku 1997) and is very often associated with *Lymnaea natalensis* Krauss (Batumike et al. 2014).

In the 1960s, researchers observed abundant *Bi. pfeifferi* populations in artificial lakes created for cassiterite mining in Maniema, Kivu province (eastern DRC). Consequently, villages surrounding the mining sites became highly endemic to intestinal schistosomiasis (Polderman et al. 1985). Physico-chemical analysis showed that water bodies populated by snails were characterised by higher pH, lower calcium concentration and lower conductivity than those without snails. Gillet and Wolfs (1954) described the

intermediate host snails' habitat as high pH environments between 6.8 and 9.2 and slow-moving water. In the Lufira region (southeastern DRC), Mandahl-Barth et al. (1972) described *Bi. pfeifferi* being absent in the lakes and widely distributed in other freshwater habitats (streams, rivers and marshy areas). *Biomphalaria sudanica* inhabited reedbeds at the lake's edge and especially in marshland with *Cyperus* plants.

Biomphalaria snails mainly prefer ponds with stagnant water or low-velocity streams with an optimum velocity lower than 0.3 m/s (Moyroud et al. 1983). In Virunga stream, water flow was negatively correlated with *Bi. pfeifferi* densities and positively correlated by the fact that the water current carried away juveniles. This could be attributed to the inability of juveniles to resist the speed of the water (Loreau and Baluku 1987a,b). The observed low fecundity of *Bi. pfeifferi* in the stream could be explained by adult snails sparing energy to resist the current at the expense of reproductive effort (Loreau and Baluku 1987a,b).

Combined presence of *Biomphalaria* and *Bulinus* species

In the Ituri region (northeastern DRC), Chartier et al. (1993) reported an association between the environmental factors and the type of pastures the cattle fed on with the presence of intermediate snail hosts for schistosomiasis, namely *Bu. africanus*. The field was characterised by an average altitude ranging between 950 and 1,300 metres, the presence of wood, the absence of agricultural activities and schist soil. In addition, the prevalence of *Bi. sudanica* had a negative correlation with rainfall, while the prevalence of *Bi. sudanica* and *Bu. forskalii* was significantly correlated with the altitude. However, the abundance of different snail species was not significantly associated. In the same province (Ituri), another study assessed the abundance of freshwater invertebrates in 2016 along the rivers, ponds and streams of Mambasa, northeastern DRC. Out of a total of 1,270 invertebrates collected, 17.32% were *Bi. pfeifferi* and 0.39% *Bu. forskalii* (Shabani et al. 2016). The abundance was higher in sites characterised by aquatic plants, such as *Phragmites*, *Panicum*, *Typha* and *Hyparhenia*.

Ruffin et al. (2018) conducted a field survey to investigate the benthic invertebrate community species diversity and abundance in the Ruzizi Valley natural ponds of Uvira territory (eastern DRC). The collection site was characterised by mud, detritus and aquatic vegetation, and scooping ranged between 0.5 to 0.75 metres. Out of the total invertebrate specimens (6,360) collected, 3.9% were identified as *Biomphalaria* spp. and 9.8% as *Bulinus* spp. *Bi. pfeifferi* is well-known as the main intermediate host of *S. mansoni* in the Ruzizi Valley region (Shabani et al. 2016, Ruffin et al. 2018).

Ecological conditions of the collection sites of *Bulinus* species

Bulinus truncatus is the local snail host for the Zairian strain of *Schistosoma haematobium* and was previously described as *Bu. natalensis* (Küster), but later identified

as *Bu. truncatus* (Mandahl-Barth et al. 1972). However, in some southern parts of DRC, *S. haematobium* is transmitted by *Bu. globosus* where *Bu. truncatus* is not present (Frandsen et al. 1978). *Bu. truncatus* was mainly collected in freshwater with dead leaves devoid of macrovegetation (Bagalwa and Baluku 1997). Mandahl-Barth et al. (1972) and Batumike et al. (2014) reported *Bu. (physopsis) globosus* to live in a wide variety of freshwater biotopes. These included permanent springs, streams, marshy vegetation and reedbeds along rivers or lakes. *Bulinus forskalii* was mainly found in temporary freshwater habitats, like bays of marshy streams (Bagalwa and Baluku 1997). In 1997, Cecchi and colleagues conducted field sampling of intermediate host snails of schistosomiasis at the dams, pools and rivers near the border of Burkina Faso and Mali (Cecchi et al. 2016). They found *Bu. truncatus* always present in temporary pools and dams but rarely in the rivers, as it prefers stagnant and sunny environments. While *Bu. globosus* was present in the rivers but not in the dams, *Bu. forskalii* was always present in the temporary pools and *Bi. pfeifferi* never in the temporary pools (Cecchi et al. 2016). In most cases, *Bulinus* spp. are more resistant to desiccation, where they bury themselves in the mud than the *Biomphalaria* spp., hence able to survive in temporary freshwater bodies (Maes et al. 2021).

Infection and susceptibility of snails by the schistosome parasites

Snail infection was determined through three different kind of tests: cercariae shedding test, dissection and molecular analysis (Bennike et al. 1976a, Colaert et al. 1977, Frandsen et al. 1978, Malaisse et al. 1981, De Clercq 1987, Atila et al. 2021). Atila (Atila et al. 2021) placed the snails in an oven and set the temperature between 25 and 30°C to stimulate cercaria shedding. They dissected the snail that did not shed and observed them under a microscope. In contrast, did a molecular analysis to determine the snail infection by the parasites (Tchuem Tchuenté et al. 1997).

Of the studies that checked the snail's infection by the schistosome parasites, only four mentioned the infection rate: (1) Atila et al. (2021) reported a 17% infection rate of *Bi. pfeifferi*, 17% for *Bu. globosus* and 2% for *Bu. forskalii*; (2) Bennike et al. (1976a) reported a 70% infection rate of *Bi. camerunensis* by *S. mansoni*; (3) Colaert (Colaert et al. 1977) collected 87 *Bi. camerunensis* in the Lubudi River (Kinshasa, south-west of DRC) and 138 in the Makelele River (Kinshasa, south-west of DRC) and exposed them to the light for shedding; none of the snails from the Lubudi River were infected, while those from the Makelele River had a 66% infection rate; (4). De Clercq et al. (1985) determined a 58% infection rate of *Bi. pfeifferi* by *S. mansoni*, through snail dissection in the laboratory.

For intermediate host snail susceptibility to infection, the miracidia of *S. mansoni* (autochthonous case from Kinshasa) and different strains of *Schistosoma intercalatum* were tested on *Bi. camerunensis* and *Bu. forskalii*, respectively (Bennike et al. 1976b). In the first trial (1969/1970), *Bi. camerunensis* infection was negative, while in the second trial (1973/1974) the infection rate

was 70%. In the first trial, *Bi. camerunensis* from Kinshasa could not be infected by the imported *S. mansoni* strain. However, this same strain was infected by other species originating from other countries such as *Bi. pfeifferi* (84%) and *B. sudanica* (8%), all originating from Tanzania as well as *Bi. alexandrina* (Ehrenberg) (2%) originating from Egypt. It should be noted that the strain of *S. mansoni* originating from Egypt succeeded in infecting *Bi. camerunensis* from Kinshasa to the tune of 60% with the Egyptian strain and 16% with the Tanzanian strain. This demonstrated that the combination of schistosome and snail originating in Kinshasa was unfavourable for the transmission of the disease.

The second trial took place later using strains of *S. mansoni* taken from patients in Kinshasa. This time, 70% of *Bi. camerunensis* from Kinshasa were infected. The same strain also infected snails from other countries, with a low percentage of 10%. The variation in infestation rates observed in the two trials can be attributed to the utilisation of two distinct sets of eggs for each trial. Consequently, this suggests the existence of two different strains of *S. mansoni* in Kinshasa, each of which utilises a separate intermediate host. The first strain is associated with *Bi. pfeifferi*, while the second strain is linked to *Bi. camerunensis*.

Additionally, it is plausible that an error occurred during the morphological identification of these species, snails became more sensitive over time, and the technique used during the final trial was refined, all of which may contribute to the observed situation (Bennike et al. 1976a). Congolese, Gabonese and Cameroonian strains of *S. intercalatum* miracidia were used to infect *Bu. forskalii*, the predominating snail in the small gutters and streams in the Kinshasa area. *Bulinus forskalii* in these water bodies was not infected by the Congolese strain of *S. intercalatum*, but with the one of Gabon and Cameroon with an infection rate of up to 97% (Bennike et al. 1976b).

The survey by Tchuem Tchuenté et al. (1997) reported the existence of *S. intercalatum* in Kinshasa, although the prevalence in humans had decreased from 30% one decade ago to 4% during the survey. Unexpectedly, no intermediate snail hosts were found in the region despite being the season of high snail density. A decade earlier, De Clercq (1987) had reported observed change in the malacological fauna in Kinshasa, where observations of *Bi. camerunensis* had been replaced by *Bi. pfeifferi*. The replacing of *Bi. camerunensis* by *Bi. pfeifferi* can be explained by competitive interactions or ecological changes that favour one species over another.

DISCUSSION

The objective of this study was to compile a synthesis of the literature on the malacological surveys of the schistosomiasis intermediate hosts in the DRC. From the retrieved studies, we collated available knowledge on the intermediate snail host distribution in DRC, factors influencing their population dynamics and ecology, and highlighted the existing knowledge gaps and recommendations for future malacology research towards snail control efforts.

Identified trends and current state of knowledge

Two-thirds (21/31) of the retrieved malacology surveys were conducted in the eastern part of DRC, which could be explained by the presence of a Natural Science Research Center in the province of South Kivu. The Research Center's malacology laboratory within the Department of Biology contributed to the realisation of these studies (Loreau and Baluku 1987a,b, Baluku et al. 1989, Bagalwa and Baluku 1997, 1998, Batumike et al. 2014) with most of them conducted before 1998. From this period, there were fewer studies in the region which could be linked to the social unrest and the presence of armed groups in the eastern part of the country that impacted the functioning of the natural science research centre (Thamba 2019).

Several ecological factors come into play to create a unique environment for the intermediate host snails of schistosomiasis, such as the flow rate of the water, the pH, the concentration of dissolved oxygen as well as mineral salts, the associated vegetation, and fauna (Moyroud et al. 1983, Cecchi et al. 2016). The biotopes differ from one genus of snails to another and vary among species of the same genus. The most critical ecological factor influencing the population dynamics is the water current. Most freshwater gastropods are poorly adapted to withstand fast currents, especially the juveniles as documented for *Biomphalaria pfeifferi* in Virunga. *Bulinus forskalii* is a ubiquitous African species and adapts to highly variable ecological conditions. It is found both in slow-flowing streams and in reservoirs cluttered with debris and aquatic plants (Cecchi et al. 2016). Similarly, *Bulinus truncatus* is known to live in an environment rich in dead leaves that accumulate due to insufficient current to carry them away (Cecchi et al. 2016).

Knowledge gaps and limitations of the study

In this section, we outline some limitations and knowledge gaps that should be considered when interpreting this study review. Firstly, snail species identification in the retrieved studies was predominantly based on morphological characteristics, which was common during the time as most studies were conducted before the widespread adoption of polymerase chain reaction (PCR) in the 1990s. Relying solely on morphology can lead to errors due to variability among the intermediate snail hosts of schistosomiasis or in the case of newly introduced species (Maes et al. 2021). An example of this occurred with *Biomphalaria tenagophila* collected along the Mangungu River in Kinshasa, initially identified as *Biomphalaria camerunensis* based on morphological identification (Pointier et al. 2005, Habib et al. 2021). Therefore, the data in this review highlights a significant gap in snail species identification, emphasising the need for future malacology research to combine morphological identification with genetic characterisation.

Secondly, it is important to note that most studies did not report on the details of their sampling efforts, such as sampling time, sample sizes and the specific methods used for snail collection. The absence of this information hinders data comparability. Employing standardised sampling techniques is crucial to enable accurate estimation

of snail abundance and distribution patterns. Additionally, standardised sampling helps reduce bias when representing snail communities in a given region, which is vital for understanding their role as intermediate hosts for various parasites. Therefore, due to the lack of standardised sampling efforts and reporting in the current study, it was impossible to generate reliable and comparable data on the abundance of intermediate host snails of schistosomiasis in DRC.

Thirdly, knowledge of the population dynamics of intermediate host snails in the DRC is inadequate and dates back more than four decades. The only detailed data available are for *Bi. pfeifferi* and from a single region (Virunga River) in the late 1980s. Furthermore, the effects of different ecological conditions on the distribution and abundance of most intermediate host snail species remain unclear. Their tolerance limits to the chemical composition of water in different types of water bodies are also not well understood or documented. This lack of data is a major problem and caution should be exercised when using the available data on *Bi. pfeifferi*. This is because the data are outdated and unrepresentative, and the variation in abundance of most snails is influenced by local geography, waterbody regime and ecological factors. Furthermore, the transmission and intensity of *Schistosoma* parasites varies between different snail species, each of which occupies different niches to some extent (Brown 1994, Hauffe et al. 2016).

Lastly, none of the studies we examined mentioned any efforts to control the intermediate host snail populations in the country. As recommended by the World health organisation (WHO), the integration of MDA with snail control to effectively reduce the burden of schistosomiasis is pertinent, as chemotherapy alone has been found to have its limitations (Sokolow et al. 2016). Japan's success story serves as a compelling example of how the complete eradication of schistosomiasis can be achieved through the implementation of both disease control strategies (Tanaka et al. 2019).

Considering these limitations, this study should primarily be utilised as a tool for future malacology studies to

address the identified knowledge gaps concerning schistosomiasis intermediate host species in the DRC. Moreover, to successfully implement effective targeted snail control measures, it is essential to regularly monitor their spatial and temporal distribution throughout the country.

In conclusion, recent data on the distribution of the intermediate host snails in the DRC are lacking, despite the widespread presence of schistosomiasis throughout the country as indicated by the Global Atlas of Helminthes Infections (GAHI 2023). The population dynamics data have only been documented for *Bi. pfeifferi* and none for *Bulinus* species, which are responsible for the transmission of *S. haematobium* and *S. intercalatum*. Moreover, comprehensive abiotic and biotic data that account for the influence of ecological factors on the population dynamics of the intermediate host snails are incomplete for most snail species. Additionally, is it important to note that there is no standardisation in snail sampling and identification methods, which hampers data comparability across different studies. Considering all these factors, conducting current malacological studies in the DRC is necessary to understand intermediate host snails and transmission by specific vector species. This will enable us to accurately assess the risk of schistosomiasis and help support control efforts in the DRC.

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