

## BULINUS SPECIES AND PREVALENCE OF SCHISTOSOMIASIS IN RIPARIAN POPULATIONS OF BENIN AQUATIC ENVIRONMENTS

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### ABSTRACT

Schistosomiasis remains a major public health concern in Benin, where *Bulinus* species serve as the intermediate hosts responsible for parasite transmission. Understanding their spatial distribution and ecological preferences is essential for improving disease control strategies. This study aimed to characterize the geographical distribution of *Bulinus* populations and to evaluate the influence of selected biotic and abiotic factors on their abundance and infection status. Between 2022 and 2023, *Bulinus* snails were collected from lake and riverine ecosystems in southern Benin. Three species were identified morphologically and molecularly: *Bulinus globosus*, *Bulinus forskalii*, and *Bulinus truncatus*. The non-parametric Kruskal-Wallis test and generalized linear models (GLMs) were applied to examine the effects of environmental indicators on both the number of collected snails and the proportion releasing cercariae.

A total of 407 *Bulinus* specimens were recorded. Statistical analyses revealed that both species and locality significantly influenced snail abundance and infection rates. Linear regression analysis indicated that several environmental parameters affected the spatial distribution of *Bulinus* populations across the study area. All three species were found to release schistosome cercariae, confirming their epidemiological importance in urinary schistosomiasis transmission.

The findings highlight a heterogeneous distribution of *Bulinus* species and their infection potential in southern Benin. Establishing an integrated ecological and molecular surveillance system is urgently needed to monitor transmission dynamics and guide effective schistosomiasis control strategies.

**Keywords:** *Bulinus* snails, Schistosomiasis, distribution, physico-chemical parameters, Benin.

### INTRODUCTION

Schistosomiasis is an acute and chronic parasitic disease that remains a major public health concern worldwide (WHO, 2022). Globally, it is the second most important water borne endemic disease after malaria in terms of morbidity and mortality (Hotez & Kamath, 2009). The World Health Organization estimates that schistosomiasis occurs in more than 78 countries, predominantly in tropical and subtropical regions, and primarily affects impoverished populations with limited access to clean water and adequate sanitation (WHO, 2018). Nearly 800 million individuals are at risk, with the African region bearing the highest disease burden (Hotez *et al.* 2014). Within sub-Saharan Africa, schistosomiasis is responsible for over 800,000 deaths annually (Lai *et al.* 2015; Aubry, 2022), and in 2019, approximately

236.6 million people required preventive chemotherapy (WHO, 2022). In Benin, schistosomiasis remains endemic, with an average national prevalence of 20%, corresponding to one infected person in five, and ranging from 0.4% to 91% across agro-ecological zones (PNLMTN, 2016). Transmission occurs when humans come into contact with freshwater contaminated with schistosome larvae released from infected snails (Wer, 2020). Six *Schistosoma* species are known to infect humans globally (Gryseels *et al.* 2006). In Benin, two species are endemic: *Schistosoma haematobium*, which causes urinary schistosomiasis and exhibits infection rates ranging from 4% to 100%, and *Schistosoma mansoni*, responsible for intestinal schistosomiasis, with infection rates reaching 74% in certain areas (Chippaux *et al.* 1990; Garba *et al.* 2000; Ibikounlé *et al.* 2009). The life cycle of schistosomes involves two hosts: humans or ruminants as definitive hosts, and freshwater snails as intermediate hosts. Among these, *Bulinus* species (gastropod mollusks) play a crucial role in transmitting *S. haematobium* and related species. *Bulinus* snails are widely distributed across Africa, parts of the Middle East, and Mediterranean regions, but significant variation exists in the compatibility between specific *Bulinus* species and schistosome parasites depending on local ecological and evolutionary conditions (Rollinson *et al.* 2000; Gryseels *et al.* 2006).

The *Bulinus* genus comprises 37 species, grouped into four main lineages: the *B. reticulatus* group, *B. africanus* group, *B. forskalii* group, and *B. truncatus/tropicus* complex (Brown, 1994). The specificity of the *Schistosoma*–*Bulinus* interaction is high, yet this relationship exhibits considerable geographic variability (Rollinson *et al.* 2001; Stothard *et al.* 2002; Njiokou *et al.* 2004). Despite their epidemiological significance, limited genetic data exist for *Bulinus* populations in West Africa. Except for isolated investigations in Nigeria (Oloyédé *et al.* 2016), Ethiopia (Mutsaky Makvaza *et al.* 2020; Olkeba *et al.* 2020), and Kenya (Opisa *et al.* 2011), the genetic structure and ecological adaptation of these gastropods remain poorly characterized. Rollinson (1985) emphasized the need to explore both ecological and genetic factors shaping the population dynamics of *Bulinus* hosts and their associated schistosome parasites, as these factors are key to understanding schistosomiasis transmission. Several ecological studies across Africa have demonstrated that both biotic and abiotic parameters—including temperature, rainfall, water velocity, salinity, and desiccation—affect snail distribution and habitat preference (Woolhouse, 1989; Rollinson, 2000; Barkia *et al.* 2015; Yigezu *et al.* 2018). However, in Benin, few studies have investigated the ecological determinants of *Bulinus* abundance, and no molecular data are available regarding their genetic variability. This research therefore aims to fill this knowledge gap by (i) assessing the spatial distribution of *Bulinus* species in southern Benin, and (ii) examining how environmental factors, particularly physicochemical water parameters, influence their abundance and infection status. The findings from this study will contribute to the development of targeted biological and ecological control strategies for schistosomiasis transmission in Benin and, by extension, in other endemic regions of Africa.

## 1. MATERIALS AND METHODS

### 1.1 Study area and sampling of biological material

This study was conducted between November 2022 and January 2023 across six sites distributed within five of the twelve administrative departments of southern Benin: Sô-Ava (Atlantic Department, located on Lake Nokoué), Djitozounmê in Aplahoué (Couffo Department), Doffé in Houéyogbé and Hédoui in Kpinou (Mono Department), Azowlissè (Ouémé Department), and Za-Kpota (Zou Department) (Figure 1). The study area lies within the sub-equatorial climatic zone of southern Benin, characterized by high relative humidity and a bimodal rainfall pattern (Adomou *et al.* 2006; Assogbadjo *et al.* 2017). The climate

alternates between two rainy seasons (April to mid-July and mid-September to October) and two dry seasons (November to March and mid-July to mid-September). Annual rainfall varies from approximately 800 mm in the southwest to 1,200 mm in the southeast, while mean annual temperatures slightly exceed 30°C. Site selection was based primarily on ecological and socio-economic criteria, particularly proximity to freshwater bodies such as rivers, lakes, and streams, which are known habitats for *Bulinus* species and important sites for schistosomiasis transmission. The selected areas are characterized by intense human-water contact resulting from agricultural activities (market gardening, annual and perennial crop cultivation), fishing (traditional and artisanal practices), aquaculture (fish ponds), and domestic uses (laundry, bathing, and other household activities). These conditions create suitable habitats for the proliferation of *Bulinus* snails and, consequently, potential hotspots for schistosome transmission.

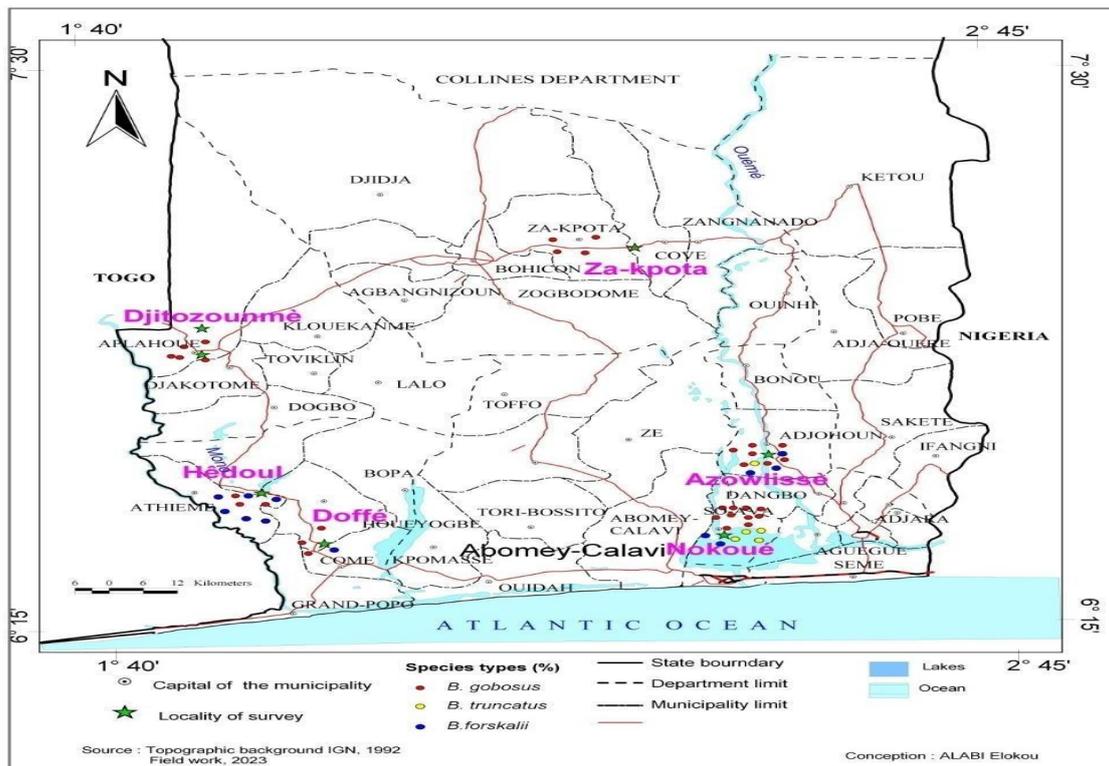


Figure 1: Geographical location of sampling sites.

*Bulinus* specimens were collected from natural freshwater bodies and retention sites. The sampling sites were selected based on direct visual inspection and preliminary sociological surveys conducted with local inhabitants, who provided information on the water bodies most frequently used for domestic, agricultural, and recreational purposes. At each identified site, *Bulinus* snails were manually collected using metal scoops and sieves. The abundance of aquatic vegetation, particularly the invasive water hyacinth (*Eichhornia crassipes*), and the physicochemical characteristics of the water (e.g., temperature, pH, dissolved oxygen, and conductivity) were measured in situ following standard procedures (Table 1). All sampling sites were geo-referenced using a Global Positioning System (GPS) device. At each location, sampling was conducted randomly for approximately two hours, covering different microhabitats within the water body to capture potential variations in snail distribution. Collected *Bulinus* specimens were placed in perforated containers to maintain aeration during transport to the laboratory. In the laboratory, the snails were examined for ecological characterization, including shell morphology and determination of schistosome cercarial

shedding to assess parasitic infestation rates for each identified species. Specimens intended for molecular analysis were preserved in 70% ethanol until DNA extraction and genetic analysis.

**Table 1:** Physico-chemical characteristics of surveyed locations

regions	Latitude and Longitude	ensity water hyacinth	emperature (°C)	pH	Dissolved oxygen (mg/l)	Conductivity (s/m)	Salinity unit (ppt)
Sô - Ava	6°28'35" N, 2°24'86" E	highest	28	7,31	6,71	45	0
Aplahoué	6°55'55" N, 1°40'48" E	weak	29,6	7,87	4,5	132,3	0,05
Houéyogbé	6°27'11" N, 1°50'80"E	weak	33,8	7,97	4,58	1321	0,6
Kpinnou	6°34'53" N, 1°45'57"E	weakest	32,2	7,02	3,68	374	0,13
Azowlissè	6°40'51" N, 2°29'11"E	high	27,9	7,01	5,06	93	0,01
Za-Kpota	7°12'20" N, 2°17'44"E	weakest	33	7,25	3,14	135	0,13

## 1.2 Morphological analysis and cercarial release test for *Bulinus* snails

Following the taxonomic criteria described by Brown (1994), *Bulinus globosus* specimens were identified by their truncated columella and the presence of microsculptured nodules forming short ridges or undulations on the shell. *B. truncatus* individuals were characterized by a straight, uniformly concave, or slightly twisted columella without truncation, whereas *B. forskalii* were recognized by their distinctly pointed apices. All identified *Bulinus* specimens were subjected to cercarial shedding tests to determine infection status. Each snail was placed individually in a glass vial containing dechlorinated freshwater and exposed to artificial light for up to four hours, following the method of Qiu (2017). Cercarial emergence was monitored at regular intervals during the exposure period. Identification of schistosome cercariae was based on morphological characteristics described by Frandsen and Christensen (1984), using a binocular microscope at 10× magnification. After examination, both infected and non-infected snails were counted and preserved in 70% ethanol for subsequent molecular analyses.

## 1.3 Statistical analysis

All statistical analyses were performed using R software (version 3.5.1). The Shapiro–Wilk test was first applied to assess the normality of the data distributions for the number of *Bulinus* snails collected and those releasing cercariae. Since both variables deviated significantly from a normal distribution ( $P < 0.05$ ), non-parametric methods were used. The Kruskal-Wallis test assessed differences among localities in the number of snails collected and in the infection rates. In addition, a generalized linear model (GLM) with a Poisson family distribution was applied to identify environmental variables significantly influencing *Bulinus* abundance and cercarial shedding.

## 2 RESULTS

### 2.1 Identification and distribution of bulins in areas

#### 2.1 Identification and Distribution of *Bulinus* Species

A total of 407 *Bulinus* specimens were collected across six sampling sites between November 2022 and January 2023, corresponding to the hydrological recession period. Based on previously established morphological criteria, three distinct species were identified: *Bulinus globosus*, *Bulinus forskalii*, and *Bulinus truncatus*. Among the collected specimens, *B. globosus* was the most abundant species ( $n = 338$ ;  $83.05 \pm 2.04\%$ ), followed by *B. forskalii* ( $n = 46$ ;  $11.30 \pm 0.64\%$ ) and *B. truncatus* ( $n = 23$ ;  $5.65 \pm 3.08\%$ ). Spatially, *Bulinus* populations were more widely distributed in Sô-Ava ( $n = 199$ ;  $26.78 \pm 0.19\%$ ) and Azowlissè ( $n = 102$ ;  $25.06 \pm 0.37\%$ ) compared to other surveyed sites (Figure 2). All three species co-occurred in Sô-Ava and Azowlissè. In contrast, *B. globosus* was the sole species recorded in Za-Kpota and Djitozounmê, whereas it occurred in sympatry with *B. forskalii* in the remaining sites. *B. truncatus* was detected only in Sô-Ava and Azowlissè, where it coexisted with the two other species. Overall, *B. globosus* showed the broadest geographical distribution, being present in all six study areas.

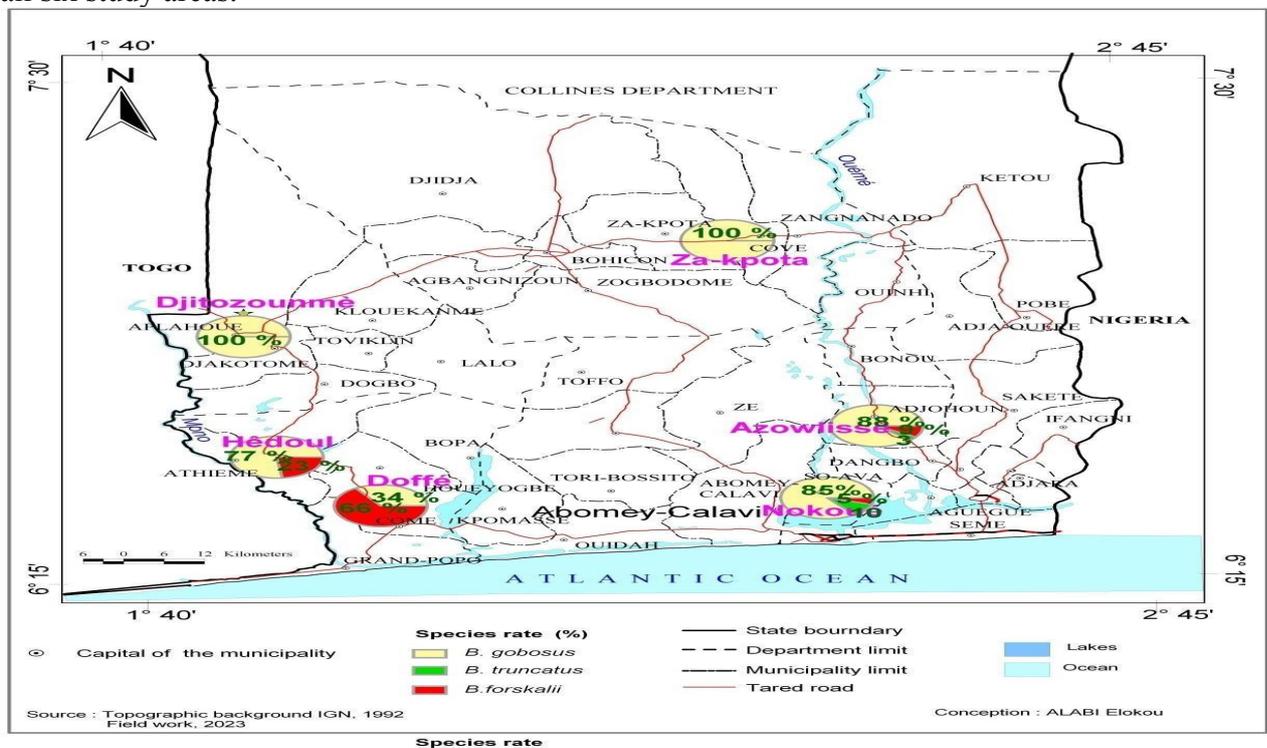


Figure 2: Geographical distribution of the different *Bulinus* species found in southern Benin

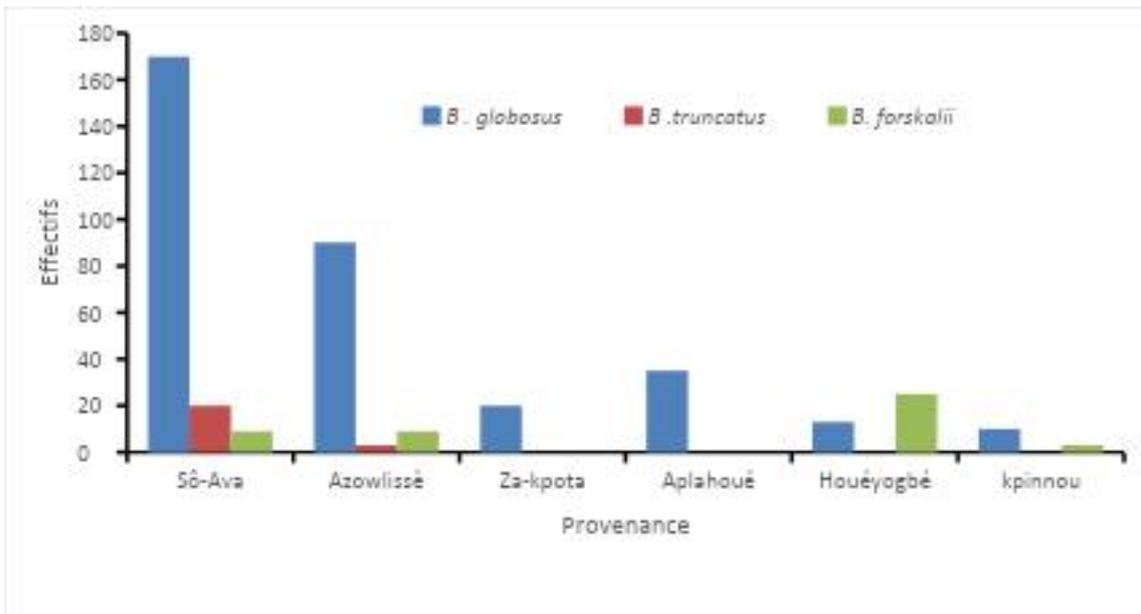


Figure 3 : Distribution of *Bulinus* species in different localities

The abundance of *Bulinus* species varied significantly across the six surveyed localities. *Bulinus globosus* exhibited the highest abundance in Sô-Ava ( $50.30 \pm 3.83\%$ ), while the lowest abundance was recorded in Kpinnou ( $2.96 \pm 5.35\%$ ). Intermediate abundance levels were observed in Azowlissè ( $26.63 \pm 4.65\%$ ), Aplahoué ( $10.35 \pm 5.14\%$ ), Za-Kpota ( $5.91 \pm 5.27\%$ ), and Houéyogbé ( $3.84 \pm 5.32\%$ ) (Figure 4). *Bulinus forskalii* was predominant in Houéyogbé, where it reached its highest relative abundance ( $54.35 \pm 9.96\%$ ), compared to other sites. In contrast, *Bulinus truncatus* displayed high local abundance in both Sô-Ava and Azowlissè, the only sites where this species was recorded (Figure 5). Results from the non-parametric Kruskal-Wallis test revealed that *Bulinus* species had a significant effect ( $p = 0.01$ ;  $\alpha = 0.01$ ) on snail abundance across the different localities. This indicates that the spatial variation observed in *Bulinus* density is strongly influenced by species identity and site-specific ecological conditions.

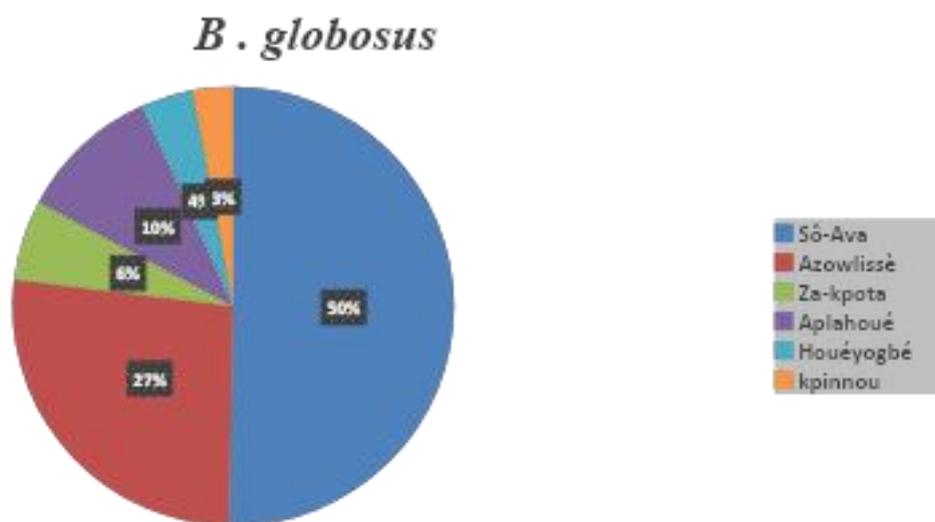


Figure 4: Abundance rates of *Bulinus globosus* in the different sampled locations.

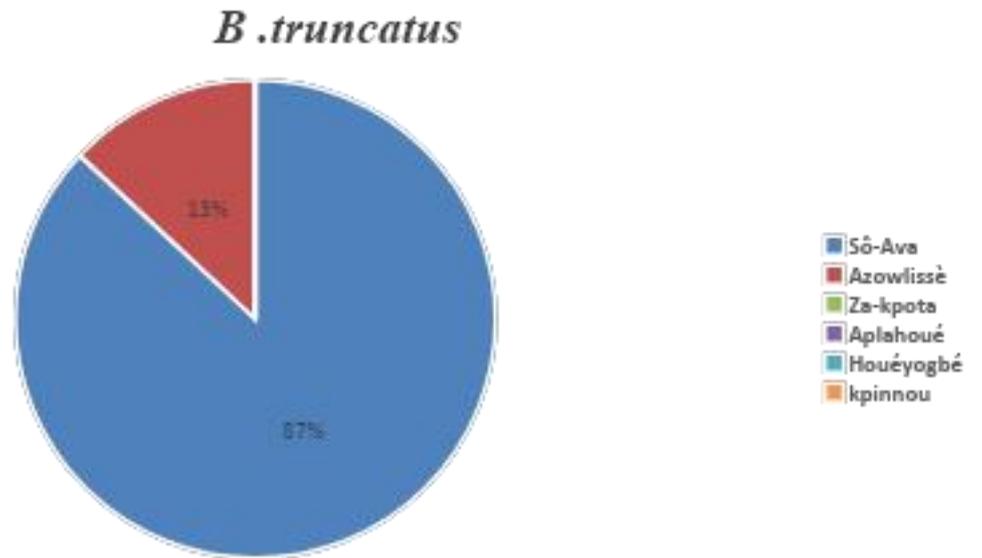


Figure 5 : Abundance rates of *Bulinus truncatus* in the different sampled locations

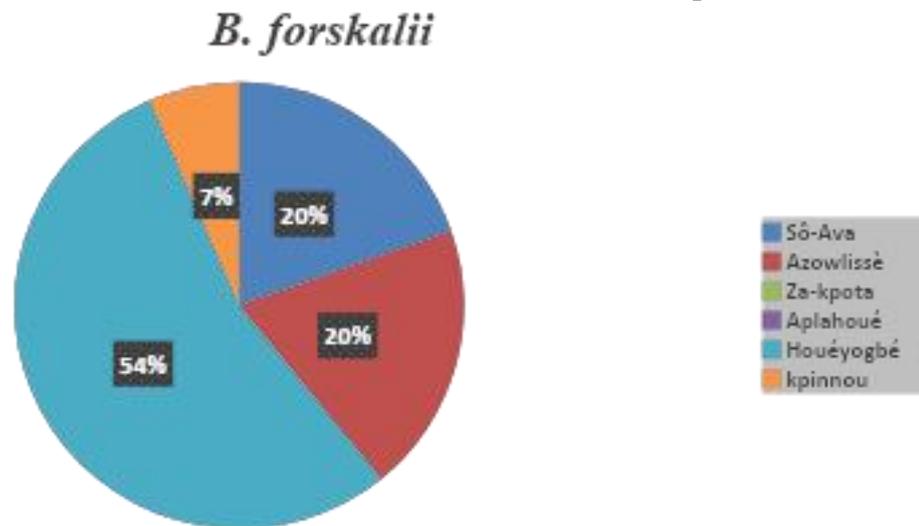


Figure 6 : Abundance rates of *Bulinus forskalii* in the different sampled locations

2 Infestation rate of *Bulinus* species

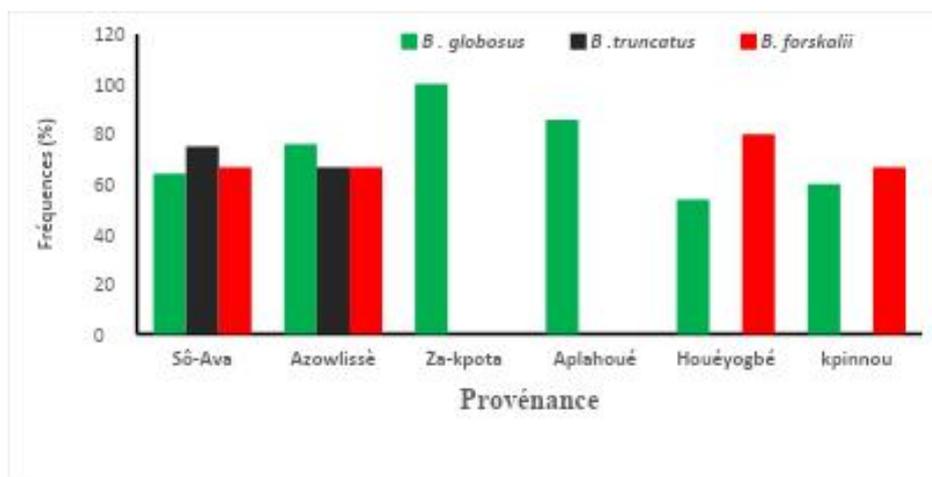


Figure 7 : Infestation rate of *Bulinus* species in different localities

The non-parametric Kruskal–Wallis test indicated a significant difference ( $p = 0.01$ ) in the number of *Bulinus* snails infested among the six sampling localities. Among the 338 *Bulinus globosus* examined for cercarial shedding, 248 individuals released cercariae, corresponding to an overall infestation rate of  $73.37 \pm 2.80\%$ . This rate, however, varied markedly between localities: Houéyogbé ( $53.84 \pm 18.84\%$ ), Kpinnou ( $60 \pm 20\%$ ), Sô-Ava ( $64.12 \pm 4.60\%$ ), Azowlissè ( $76 \pm 4.90\%$ ), Aplahoué ( $85.71 \pm 6.39\%$ ), and Za-Kpota ( $100 \pm 8.90\%$ ). Similarly, out of 46 *Bulinus forskalii* tested, 34 individuals were infested, representing an infestation rate of  $73.91 \pm 7.53\%$ . The locality-specific infestation rates were as follows: Sô-Ava ( $66.67 \pm 19.24\%$ ), Azowlissè ( $66.67 \pm 19.24\%$ ), Kpinnou ( $66.67 \pm 33.33\%$ ), and Houéyogbé ( $80 \pm 8.94\%$ ). For *Bulinus truncatus*, 17 out of the 23 snails examined released schistosome cercariae, yielding an infestation rate of  $73.91 \pm 10.65\%$ . Infestation levels varied by locality, with  $66.67 \pm 33.33\%$  recorded in Azowlissè and  $75.00 \pm 11.18\%$  in Sô-Ava. When considering all species combined, the highest overall infestation rates were observed in Azowlissè ( $69.78 \pm 5.01\%$ ) and Sô-Ava ( $68.60 \pm 4.07\%$ ), followed by Houéyogbé ( $44.61 \pm 9.56\%$ ), Kpinnou ( $42.22 \pm 17.56\%$ ), Za-Kpota ( $33.33 \pm 10.54\%$ ), and Aplahoué ( $28.57 \pm 8.24\%$ ) (Figure 6). These results clearly indicate that Azowlissè and Sô-Ava are the most epidemiologically active zones, where the coexistence of multiple *Bulinus* species and elevated infestation rates suggest favorable ecological conditions for the transmission of *Schistosoma* spp.

### 3 Factors affecting the distribution of *Bulinus* snails

#### 3.1 Effect of physico-chemical parameters on the number of *Bulinus* snails collected

The generalized linear regression model (family = *Poisson*) indicated that all evaluated environmental parameters exerted a highly significant effect ( $p < 0.001$ ) on both the total number of *Bulinus* snails collected and those releasing cercariae. The analysis revealed a negative correlation between *Bulinus* abundance and two physicochemical factors—conductivity and pH—suggesting that elevated ionic concentration and alkalinity may limit the proliferation of these snails. In contrast, the remaining variables showed a positive association with *Bulinus* density, indicating that favorable environmental conditions. Similarly, the infestation rate followed the same pattern: conductivity and pH were negatively correlated with the number of infested snails, whereas all other parameters exhibited positive correlations, implying that ecological factors supporting *Bulinus* abundance also promote schistosome transmission potential.

**Table 2:** Effects of physico-chemical parameters on the number of *Bulinus* individuals collected and those releasing cercariae

Settings	<i>Bulinus</i> collected				<i>Bulinus</i> snails releasing cercariae			
	Estimate	Standard error	Z value	Pr (> z )	Estimate	Standard error	Z value	Pr (> z )
Intercept	261,53	327,08	0,8	0,42	644,15	289,16	2,23	0,026*
Conductivity	-0,44	0,075	-5,89	3,81e-09 ***	-0,36	0,07	-5,38	7,63e-08 ***
Dissolved oxygen	166,98	12,44	13,42	< 2e-16 ***	166,27	11,02	10,55	< 2e-16 ***
pH	-373,76	52,31	-7,14	9,02e-13 ***	-308,58	47,75	-6,46	1,03e-10 ***

Salinity	1134,72	237,34	4,78	1,74e-06 ***	1020,59	213,22	4,79	1,70e-06 ***
Temperature	56,04	4,67	12,01	< 2e-16 ***	34,92	4,01	8,52	< 2e-16 ***

\*Significant at the 5% level; \*\* Significant at the 1% level; \*\*\* Significant at the 0.1% level. Alabi *et al.* 2024

### 3 DISCUSSION

Understanding the specific diversity and spatial distribution of *Bulinus* species is essential for designing efficient prevention and control strategies against schistosomiasis in endemic regions. In the present study, three *Bulinus* species: *Bulinus truncatus*, *Bulinus globosus* and *Bulinus forskalii* were identified across the six surveyed sites, each displaying distinct ecological preferences and heterogeneous distribution patterns. This uneven distribution appears to be driven by a combination of abiotic and biotic factors, particularly the density of aquatic vegetation (notably *Eichhornia crassipes*) and the physicochemical characteristics of the water (temperature, salinity, turbidity, dissolved oxygen, and conductivity), as previously observed by Rocha-Ramirez *et al.*(2007). Our results revealed that *Bulinus globosus* populations were significantly more abundant in Sô-Ava (Nokoué Lake) and Azowlissè (Ouémé River) compared to other sampling sites. This pattern could be attributed to the proliferation of *Eichhornia crassipes*, which forms dense mats on the water surface and provides a favorable ecological niche for snail colonization, feeding, and oviposition (Olkeba *et al.* 2020). The structural complexity created by aquatic vegetation is known to enhance habitat stability and resource availability for pulmonate snails. Moreover, Sô-Ava and Azowlissè are hydrologically connected, allowing constant exchanges of organisms and water-borne materials, which likely maintain high genetic and ecological connectivity between populations. The predominance of *B. globosus* and *B. truncatus* in Sô-Ava may also be related to the lake's shallow depth and weak current, conditions that favor reproduction and survival (Kalinda *et al.* 2018).

Similar findings were reported by Thomas & Tait (1984), who demonstrated that *B. globosus* thrives in shallow habitats with minimal current and abundant aquatic vegetation. Conversely, *B. forskalii* was more abundant in Houéyogbé, characterized by relatively high temperatures. This observation aligns with earlier reports highlighting the thermotolerance and ecological plasticity of *B. forskalii*, which can survive in warmer and often transient water bodies due to adaptive physiological mechanisms (Kalinda *et al.* 2017; Manyangadze *et al.* 2021). Temperature emerged as a major driver of *Bulinus* distribution, confirming its critical role in shaping snail community dynamics (Paull & Johnson 2011; McCreesh *et al.* 2014). A positive correlation was observed between temperature and *Bulinus* abundance, consistent with previous studies (Malek 1958; Hofkin *et al.* 1991). However, extreme temperatures may reduce snail density by exceeding physiological tolerance limits, as reported in other African settings (Opisa *et al.* 2011). Overall, the physicochemical parameters particularly pH, conductivity, and dissolved oxygen played a central role in determining habitat suitability for *Bulinus* species (Rumes *et al.* 2011; Marie *et al.* 2015; Alhassan *et al.* 2020). Furthermore, Sô-Ava and Azowlissè are subject to intense human activity, including fishing, agriculture, market gardening, livestock grazing, and domestic uses. Such anthropogenic pressures not only modify local hydrology and vegetation but also increase human-water contact, thereby enhancing the risk of schistosomiasis transmission (Olkeba *et al.* 2020). Hence, ecological control measures targeting *Bulinus* habitats in these areas should be prioritized to limit disease transmission.

## 4 CONCLUSION

This study identified three *Bulinus* species: *B. truncatus*, *B. globosus* and *B. forskalii* in southern Benin and demonstrated that their abundance and distribution are significantly influenced by environmental factors, notably water physicochemical properties and vegetation density. All three species were found to release schistosome cercariae, underscoring their epidemiological importance in sustaining transmission cycles in the region. The findings highlight the necessity of establishing an integrated ecological surveillance system for *Bulinus* species as part of national schistosomiasis control efforts. Such surveillance should include periodic monitoring of snail populations, mapping of transmission foci, and environmental management interventions such as aquatic vegetation control. In parallel, community-based awareness programs should be promoted to reduce high-risk behaviors associated with water contact. By integrating ecological, environmental, and public health perspectives, this study provides valuable insights into the population dynamics of *Bulinus* and offers a scientific basis for evidence driven schistosomiasis control and elimination strategies in Benin and beyond. The present study provides foundational insights into the ecological distribution and genetic diversity of *Bulinus* populations in southern Benin. Understanding the spatial heterogeneity of these snail hosts is critical for designing effective schistosomiasis surveillance programs. Our findings suggest that different *Bulinus* species occupy distinct ecological niches, which may influence the local transmission dynamics of *Schistosoma* parasite.

### Conflicts of Interest

The author declares no conflicts of interest.

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