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Population genetic structure of the freshwater snail, *Bulinus globosus*, (Gastropoda: Planorbidae) from selected habitats of KwaZulu-Natal, South Africa

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ABSTRACT

The freshwater snail *Bulinus globosus* is an important intermediate host of *Schistosoma haematobium*, the causative agent of urinary schistosomiasis. This disease is of major health concern, especially in Africa where the majority of cases have been reported. In this study the inter- and intra-genetic diversity and population genetic structure of *B. globosus* from nine locations in the KwaZulu-Natal province of South Africa was studied using four polymorphic microsatellite loci (BgZ1–BgZ4). Moderate genetic diversity was detected within populations with a mean diversity (H_E) of 0.49 ± 0.09. The majority of populations significantly deviated from Hardy-Weinberg equilibrium ($p < 0.05$), due to a deficit of heterozygotes. Such deviations may be due to founder events that were caused by bottlenecks that occurred as a result of frequent droughts and flooding that these snails' habitats are exposed to. Overall, the populations studied seem to be partially inbreeders/selfers with mean estimates of 0.24/0.38. A discernable genetic structure was elucidated among populations as evident by the mean pairwise F_{ST} of 0.58 ± 0.13. There was no significant association between genetic and geographical distance among populations, an indication of limited gene flow. This increases the chances of populations losing alleles due to genetic drift. Populations in close proximity demonstrated high genetic differentiation (58.77% total variation) due to allelic differences between them. The sample populations fell into 12 clusters, however, the populations from uMkhanyakude and uThungulu exhibited no discernable genetic structure. Genetically, the Bhobhoyi site found within the uGu district was equidistant to the two main sampling regions.

1. Introduction

Schistosomiasis caused by *Schistosma haematobium* and *S. mansoni* is a parasitic disease commonly found in tropical and sub-tropical regions and is considered as the third most important tropical disease after malaria and intestinal helminthiasis (WHO, 2014). In the year 2012 alone it was reported that an estimated 42.1 million people were treated for this disease and 249 million required preventative treatment, of which 90% lived in Africa (WHO, 2014; Kali, 2015).

In sub-Saharan Africa, *S. haematobium* and *S. mansoni* are endemic and *Bulinus globosus* and *Biomphalaria pfeifferi* are the main intermediate hosts respectively (Brown, 1980; Jarne and Delay, 1991; Nyakaana et al., 2013). *Biomphalaria* and *Bulinus* spp. are freshwater hermaphrodites that are capable of selfing or outcrossing (Jarne and Delay, 1991; Jarne et al., 1993), with different species adopting either selfing or outcrossing as the preferential reproductive mode (Mavárez et al., 2002). Their habitats range from small temporary ponds to large rivers (Brown, 1980, 1994). The common habitats of *B. globosus* are shallow waters near shores of lakes, ponds, streams and irrigation channels (Brown, 1980; Utzinger and Tanner, 2000). *Bulinus globosus* populations follow small patchily distributed habitats and their populations sizes fluctuate due to temporal instabilities (Brown, 1994; Njiokou et al., 1994). The freshwater snail *B. globosus* is an important intermediate host for *S. haematobium*, the causative agent of urinary schistosomiasis in tropical and sub-tropical countries (Brown, 1994; Nyakaana et al., 2013; Djuikwo-Tuekeng et al., 2014) as it is found in the *Bulinus africanus* species group which presents as the main intermediate host of *S. haematobium* in South Africa (De Kock and Wolmarans, 2005).

The important factors that shape the genetic structure and spatial distribution of *B. globosus* include the distribution of habitats, which is influenced by the spatial and temporal fluctuations in

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Table 1
Characteristics of the habitats where studied *Bulinus globosus* populations were collected.

Population	Site	Habitat type	Activities (use)	Geographical coordinates		*n*
				Latitude	Longitude	
uMkhanyakude district						
01	Namaneni dam (NAM)	dam	Gardening Fishing	26°59′25.00"S	32°16′12.00"E	12
02	Nsunduza dam (NSU)	dam	Gardening Cattle watering point	26°56′06.00"S	32°12′55.00"E	10
03	Skemelele (SKE)	dam	Gardening Cattle watering point Washing	27°03′00.96"S	32°15′31.18"E	10
04	Bridge (BRI)	pond	Cattle watering point	27°03′55.07"S	32°08′03.68"E	11
uThungulu district						
05	Amatikula (AMA)	trough	Cattle watering point	29°04′22.62"S	31°38′39.77"E	12
06	Obanjeni (OBA)	dam	Cattle watering point	28°55′39.90"S	31°42′32.18"E	12
07	Nyezane (NYE)	swamp	Cattle watering point	29°01′45.44"S	31°01′23.92"E	12
08	Nyoni (NYO)	dam	Cattle watering point	29°04′50.56"S	31°27′40.50"E	9
uGu district						
09	Bhobhoyi (BHO)	stream	Cattle watering point Washing	30°43′29.13"S	30°23′12.87"E	10

water availability (Brown, 1980; Djuikwo-Tuekeng et al., 2014). This results in stern population bottlenecks (Brown, 1994). The other factor is the patchily distributed habitats which results in isolation-by-distance between populations, which limits gene flow amongst them (Mukaratirwa et al., 1996a,b; Emery et al., 2003; Wilkinson et al., 2007; Djuikwo-Tuekeng et al., 2014) and encourages inbreeding and this is probably the most significant factor shaping the genetic structure of hermaphroditic freshwater snails. Habitats also vary in space with regards to environmental factors such as predation, parasitism and competition (Hoverman et al., 2011). The hermaphroditic nature of freshwater snails enables them to self-fertilize (Jarne and Delay, 1991). Although moderately high levels of inbreeding have been reported in fresh water snails in a number of studies (Wilkinson et al., 2007; Nyakaana et al., 2013; Djuikwo-Tuekeng et al., 2014), *B. globosus* normally has a mixed reproductive strategy, although adopts only one reproductive mode at any particular time, with evidence proposing outcrossing as a way to avoid inbreeding depression (Jarne and Delay, 1991). Parasites have also been found to have an effect on the genetic diversity, reproduction mode and overall structure of freshwater snail populations (Davies et al., 1999; Schulte et al., 2010; Charbonnel et al., 2002). The co-evolution of snail and parasite generally favours sexual reproduction in the former which results in increased genetic diversity (Schulte et al., 2010), such increased genetic diversity results in populations that are resistant to common parasite genotypes, and increased population subdivisions (Curtis et al., 2002).

In Southern Africa there have been a limited number of molecular techniques that have been utilized to study the genetic structure of African *B. globosus* populations. Allozyme markers depicted low levels of genetic diversity within populations (Mukaratirwa et al., 1996a) due to high frequency of self-fertilization and low gene flow. Randomly amplified DNA (RAPD) markers also demonstrated low genetic diversity within populations and high population differentiation (Davies et al., 1999). A much recent study (Djuikwo-Tuekeng et al., 2014) was conducted using microsatellite markers and depicted moderate genetic diversity accompanied by substantial genetic differentiation and migration only limited to close populations.

A deeper understanding of the geographical distribution and population structure of *B. globosus* is crucial in understanding its population genetic diversity and fitness because the species plays a major role as a vector in the transmission of schistosomiasis (Wilkinson et al., 2007; Jørgensen et al., 2013), which is a disease of great socioeconomic concern (Chitsulo et al., 2000). Against this background, four microsatellite loci previously characterized by Emery et al. (2003) were used to assess the intra- and inter-population genetic diversity of *B. globosus* populations collected from locations around the KwaZulu-Natal province in South Africa.

2. Materials and methods

2.1. Sampling area

A total of five hundred and seventeen (517) snail samples were collected from spatially distributed locations in the KwaZulu-Natal province of South Africa between 2014 and 2015. These included uMkhanyakude, uThungulu and uGu districts (Fig. 1), where *B. globosus* has previously been found and in some cases found to be implicated in the transmission of bilharzia (Appleton and Stiles, 1976). Out of the 517 samples collected approximately ninety-nine (99) were viable to be used in this study. Sampling sites were separated by geographical distances ranging from a few kilometers to several hundreds and were characterized by different water systems (Table 1). The snails were collected using metal scoops (with a 1 mm by 1 mm nylon mesh) and those that were visible were handpicked. Field identification of the samples was done through analysis of shell morphological characteristics using the field identification key by Kristensen (1987). Snails were then stored in 70% ethanol prior to DNA extraction.

2.2. DNA extraction and microsatellite amplification

DNA was extracted from the head-foot tissue of each snail using the Genomic DNA Tissue MiniPrep Kit (Zymo Research, USA), according to the manufactures protocol. Genetic variation was assessed using four microsatellite loci, BgZ1–BgZ4 (Table 2) previously used for *B. globosus* by Emery et al. (2003). BgZ5 and BgZ6 failed to amplify in all populations and were therefore, excluded from the study. The lack amplification of the previous microsatellites can be explained by the selfing nature of the *B. globosus*. This in turn results in some alleles being monomorphic and in some cases being totally lost from the populations due to inbreeding. Such reproductive tendencies in *B. globosus* can also explain the low levels of genetic diversity that are often found in the species. In that context and also basing on the previously cited literature it was conceded that the four microsatellite markers (BgZ1–BgZ4) were sufficient enough to yield informative and reliable population genetic parameters. All polymerase chain reaction (PCR) amplifications were performed in a total volume of 25 μl in a T100™ Thermal