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Shell Morphology of Three Medical Important Tropical Freshwater Pulmonate Snails from Five Sites in South-Western Nigeria

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ABSTRACT
Biomphalaria pfeifferi, *Bulinus globosus* and *Lymnaea natalensis* act as obligatory vectors of schistosomiasis and fascioliasis in Sub-Saharan Africa. Although, they are known to be widely distributed throughout Nigeria, there is the need for precise species identification to help vector control initiatives. Snail shell morphometry has previously proved useful in taxonomic studies. We thus employed seven morphological parameters of snail shells in identifying three snail vectors from five water bodies in South-Western Nigeria. Snails were sampled for 14 months and total of 718 snails were collected, which include 204 *B. pfeifferi*, 316 *B. globosus* and 198 *L. natalensis*. Eleyele Lake had 278 snails which was the highest number followed by Osun River with 64 snails. Our finding revealed *B. globosus* as the dominant snail in all the five sites, while *B. pfeifferi* and *L. natalensis* were present in only three sites. The presence of the snails in otherwise uninfected water bodies may have implications for transmission and disease control in these areas. Our findings should prove useful for improved snail identification, information, which will be invaluable for vector control in affected communities.

Key words: *Biomphalaria pfeifferi*, *Bulinus globosus*, *Lymnaea natalensis*, morphology, schistosomiasis, fascioliasis

INTRODUCTION
Freshwater snails belonging to the genus *Biomphalaria*, *Bulinus* and *Lymnaea* are common species of the subfamily Planorbidae and Lymnaeidae, respectively and are widely distributed throughout much of Sub-Sahara Africa (Mandahl-Barth, 1962; Brown, 1980; Gryseels, 1989; Jordan *et al*., 1993; WHO., 1995). Species in these genera inhabit various natural and artificial freshwater environments including shallow lakes, streams, rivers, wetlands, seasonal pools, rice paddies, irrigation canals and ponds (Brown, 1980; Gryseels, 1989; Utzinger and Tanner, 2000). Members of these families of snails are necessary intermediate hosts of blood-dwelling trematode parasites, which cause serious public health problems to man and animals in tropical and subtropical regions of the world (Brown, 1980; Gryseels, 1989; Jordan *et al*., 1993). Global losses in agriculture attributed to fascioliasis due to increase in animal mortality and reduced production is estimated in the billions. With an estimated 17 million infected and 180 million at risk of infection in endemic areas. Schistosomiasis has global prevalence of 207 million people mostly from Sub-Saharan Africa (Hotez *et al*., 2008), where the disease burden is the highest.

Some of the snails transmitting the above infections like *B. pfeifferi* and *B. globosus* transmit *Schistosoma* parasites to man causing human Schistosomiasis (Brown, 1980; Gryseels, 1989; Jordan *et al*., 1993; Opisa *et al*., 2011; Dida *et al*., 2014) while, *L. natalensis* transmits *Fasciola* parasites to animals and man causing fascioliasis (Utzinger and Tanner, 2000; Dida *et al*., 2014). *Biomphalaria pfeifferi* is mainly found in tropical regions of Africa and Southwest Arabia. It is widespread throughout Eastern, Western and Southern Africa while Northern Africa has few isolated populations in Southeast Algeria and Southern Libya (Woolhouse, 1992). *Biomphalaria pfeifferi* populations have also become established in Southern Madagascar. It is found in number of freshwater habitats including man-made water channels (Woolhouse and Chandiwana, 1989; Bandoni *et al*., 1990; Akufongwe *et al*., 1995; Sturrock, 2001). *Biomphalaria globosus* belonging to the *Bulinus africanus* group are distributed throughout Africa, with the species further extending its distribution to Madagascar (Stothard *et al*., 2001) and the Middle East (Jorgensen *et al*., 2007). *Lymnaea natalensis* is widespread in tropical Africa, but it is rare in the Northeast coastal area (Appleton *et al*., 2009).

These three snails of immense medical importance are found in Nigeria (Thomas and Tait, 1984; Betterton, 1984; Betterton *et al*., 1988) especially in the Southwest region (Ndifon and Ukoli, 1989), with habitat fragmentation that has resulted in their patchy distribution in lakes, dams, canals, ponds and rivers. Despite the fact that these snails are well distributed in Nigeria with wide geographical range, the morphological variations of these species are poorly known making precise identification problematic and information of snail distribution in local water bodies difficult to verify. This consequently impedes control efforts to limit disease transmission via vector control herculean task.

Shell morphometric is useful tool and first step in identification in mollusc taxonomy and ecological studies (Mandahl-Barth, 1962; Abdel-Malek, 1958). In malacology, shell morphology has been useful in describing, identifying, characterizing (Wullschleger and Jokela, 2002) and recognizing intraspecific morphological variations (Schniebs *et al*., 2013). It has also assisted in deducing shell structures and properties affected by environmental variations to determine (Bertin *et al*., 2012; Mahilum and Demayo, 2014) snail geographical distribution (Goodfriend, 1986). In this study, we conducted long time sampling of *Biomphalaria pfeifferi*, *Bulinus globosus* and *Lymnaea natalensis* from five water bodies, which include rivers and lakes in South-Western, Nigeria. Morphometric analyses were carried out on the collected snails, to better determine the range of variation in bid to improving species delineation.

MATERIALS AND METHODS

Study sites: Five water bodies which include three rivers and two lakes were selected for this study. The different water bodies are (1) Orori River, Ogun state (Latitude: 7°14’00” N, longitude: 3°02’00” E),(2) Bareke River, Ogun State (Latitude: 7°14’02” N, longitude: 3°02’ 01” E), (3), Eleyele Lake, Oyo State (Latitude: 7°25'36" N, longitude: 3°51'58" E)(4) Eko-Ende Lake, Osun State (Latitude: 7°55'60" N, longitude: 4°34'60" E) and (5) Osun River, Osun State (Latitude: 7°46 N, longitude: 4°34 E). Thereis recent report of urinary schistosomiasis in communities around; Orori and Bareke rivers, Ogun State (Morenikeji and Idowu, 2011; Salawu and Odaibo, 2013, 2014) and Eko-Ende Lake and Osun River, Osun State (Hassan *et al*., 2012; Babatunde *et al*., 2013). There are also reports of animal fascioliasis around some of these water bodies (Adedipe *et al*., 2014).

Survey design: The study was conducted from October, 2012 to December, 2013. The snail collection was done early in the morning between 8.00 am and 12.00 pm using flat dip-net scooping as described by Ritchie *et al*. (1962) and Demian and Kamel (1972), however hand picking

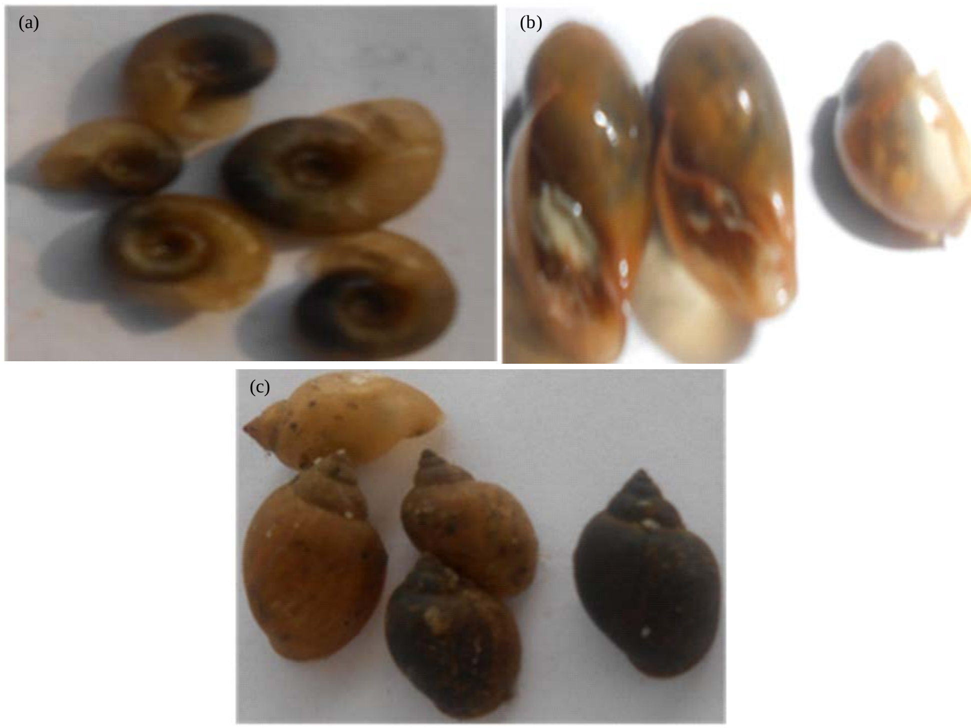

Fig. 1(a-c): Shells of (a) *Biomphalaria pfeifferi*, (b) *Biomphalaria globosus* and (c) *Lymnaea natalensis*

was employed in areas that were woody or rocky in nature. The collected snails were gathered in sterile plastic container and *in situ* identification was done using an identification key was employed in areas that were woody or rocky in nature. The collected snails were gathered in sterile plastic container and *in situ* identification was done using an identification key (Mandahl-Barth, 1962). A total of 718 snails were collected across the five sampling sites at the end of the sampling period, 204 *B. pfeifferi*, 316 *B. globosus* and 198 *L. natalensis*. Morphological parameters were measured using vernier caliper. The selected measurable parameters were Shell Height (SH), Shell Width (SW), Aperture Height (AH), Aperture Width (AW), Spiral Length (SL) and Aperture Circumference (AC) (Fig. 1). The measurable and non-measurable morphological parameters were selected after properly reviewing the available taxonomic shell descriptions from literature that were practical for use in snail identification (Pace, 1973; Brandt, 1974). A total of 6 continuous shell parameters were measured along imaginary straight lines as shown in Fig. 2. The raw data was entered into Excel and saved for analysis.

Data analysis: Data analysis was done on Excel 2013, using the scatter plot for shell height against shell width and aperture height against aperture width. The linear regression equation and R squared were analyzed for each graph.

RESULTS

A total of 718 Pulmonate snails were collected from the five water bodies across south-western Nigeria (Table 1-3). *Biomphalaria pfeifferi* snails were seen in only three sites out of the five sites sampled, where 204 snails were collected. The highest number of *B. pfeifferi* (100 snails) were collected from Eleyele Lake in Oyo state, while 28 snails were collected from Osun River (Table 1)

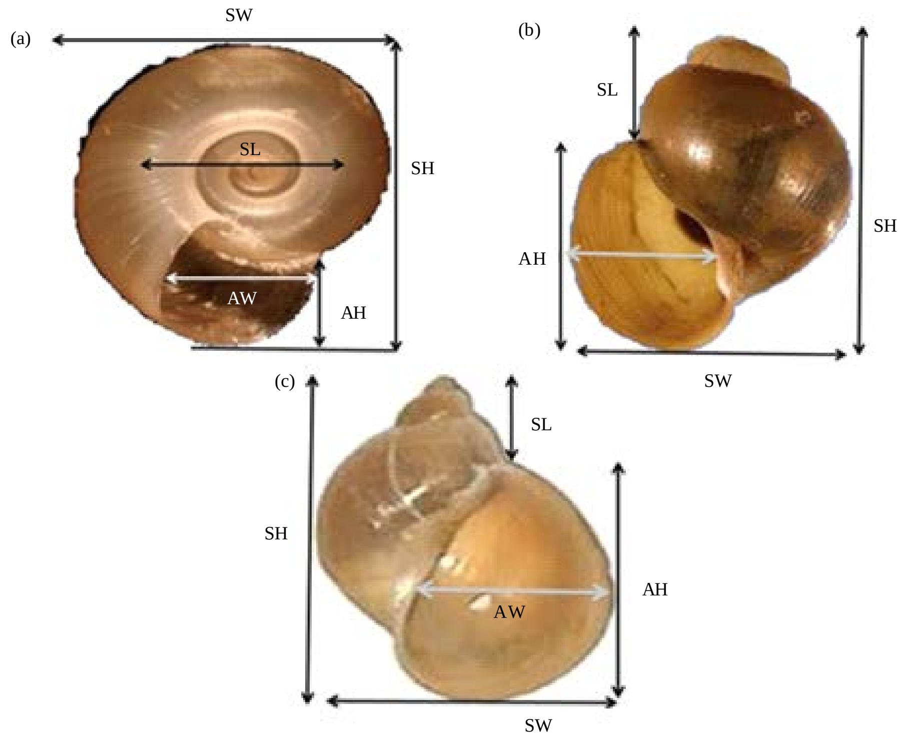

Fig. 2(a-c): Measurements made on the shells of (a) *Biomphalaria pfeifferi*, (b) *Bulinus globosus* and (c) *Lymnaea natalensis*

Table 1: Location, number of snails and measurement of 6 characters of *Biomphalaria pfeifferi* (n = 204)

Locations	Eko-Endo lake	Osun river	Eleyele lake
States	Osun state	Osun state	Osun state
No. of snails	64	26	114
Characters (X±SE)			
SH	2.47±0.13 (1.30-3.90)	2.06±0.16 (1.30-3.95)	2.82±0.13 (1.80-3.95)
SW	7.98±0.26 (6.00-10.20)	7.56±0.25 (6.00-11.00)	8.65±0.24 (5.00-12.00)
AH	2.28±0.12 (1.00-3.65)	1.83±0.15 (1.00-3.90)	2.95±0.12 (1.50-3.90)
AW	2.76±0.09 (1.90-3.70)	2.67±0.09 (1.85-3.50)	3.12±0.08 (2.00-4.00)
SL	2.06±0.14 (0.90-3.70)	1.51±0.18 (0.80-3.70)	2.46±0.11 (1.50-3.90)
AC	8.89±0.27 (7.00-11.70)	8.08±0.35 (6.00-12.20)	9.85±0.29 (7.00-14.00)

SH: Shell height, SW: Shell width, AH: Aperture height, AW: Aperture width, SL: Spiral length, AC: Aperture circumference

Table 2: Location, number of snails and measurement of 6 characters of *Bulinus globosus* (n = 316)

Locations	Orori river	Bareke river	Eko-Endo lake	Osun river	Eleyele lake
States	Ogun state	Ogun state	Osun state	Osun state	Oyo state
No. of snails	60	50	68	24	114
Characters (X±SE)					
SH	9.48±0.45 (5.10-13.00)	8.38±0.62 (5.00-14.00)	10.56±1.19 (4.00-12.80)	6.88±0.41 (5.20-9.20)	8.87±0.36 (5.00-14.00)
SW	5.86±0.39 (1.90-8.50)	4.47±0.47 (1.50-9.30)	6.53±0.98 (1.50-8.50)	4.33±0.38 (2.50-6.10)	5.19±0.32 (1.50-9.30)
AH	6.70±0.48 (2.00-10.00)	5.47±0.53 (2.00-9.50)	7.71±1.01 (2.00-10.00)	4.76±0.42 (3.00-7.10)	6.10±0.36 (2.00-10.00)
AW	3.59±0.24 (1.20-5.30)	3.13±0.26 (0.90-5.50)	4.17±0.47 (1.70-5.30)	2.77±0.30 (1.80-4.10)	3.34±0.18 (0.90-5.50)
SL	2.56±0.17 (1.00-4.00)	2.44±0.27 (0.60-6.00)	2.34±0.35 (0.50-3.00)	1.16±0.12 (0.70-1.80)	2.38±0.16 (0.60-6.00)
AC	17.85±1.30 (4.50-24.90)	16.92±1.51 (4.00-26.00)	21.74±1.66 (12.00-24.10)	18.89±0.89 (15.00-4.00)	18.12±0.92 (4.00-26.00)

SH: Shell height, SW: Shell width, AH: Aperture height, AW: Aperture width, SL: Spiral length, AC: Aperture circumference

Biomphalaria globosus species were seen in the entire sites sampled with total number of 316 snails collected with the highest number (114 snails) collected from Eleyele lake

Table 3: Location, number of snails and measurement of 6 characters of *Lymnaea natalensis* (n = 198)

Location	Eko-Endo lake	Osun river	Eleyele lake
State	Osun State	Osun State	Oyo State
No. of snails	35	14	50
Characters (X±SE)			
SH	13.30±0.41 (9.00-19.00)	11.39±0.29 (8.80-14.00)	13.04±0.32 (9.00-19.00)
SW	7.89±0.23 (5.50-10.00)	7.96±0.21 (5.50-10.00)	7.84±0.21 (5.00-11.20)
AH	9.12±0.27 (5.80-12.00)	6.72±0.17 (4.70-8.40)	8.97±0.21 (5.80-12.00)
AW	5.22±0.13 (4.00-6.50)	4.60±0.10 (4.00-6.00)	5.11±0.11 (4.00-7.00)
SL	4.30±0.11 (3.00-6.00)	3.61±0.14 (1.80-5.00)	4.23±0.15 (2.00-7.00)
AC	22.89±0.49 (18.00-29.00)	20.83±0.34 (18.50-5.50)	22.66±0.44 (18.00-30.00)

SH: Shell height, SW: Shell width, AH: Aperture height, AW: Aperture width, SL: Spiral length, AC: Aperture circumference

and the lowest (24 snails) from Osun River (Table 2). *Lymnaea natalensis* were seen in only three sites, with 198 snails collected (Table 3).

The *B. pfeifferi* morphological parameters measured are presented in Table 1. The snails from Eleyele Lake had the highest shell height (2.82±0.13), shell width (8.65±0.24), aperture height (2.95±0.12), aperture width (3.12±0.08), spiral length (2.46±0.11) and aperture circumference (8.08±0.35), while those from Osun River had the least shell height (2.06±0.16), shell width (7.56±0.25), aperture height (1.83±0.15), aperture width (2.67±0.09), spiral length (1.51±0.18) and aperture circumference (9.85±0.29). *Biomphalaria globosus* morphological parameters measured are presented in Table 2.The snails from Eko-Endo Lake had the highest shell height (10.56±1.19), shell width (6.53±0.98), aperture height (7.71±1.01), aperture width (4.17±0.47) and aperture circumference (21.74±1.66) while, the once from Orori River had the highest spiral length (2.56±0.17). The least shell height (6.88±0.41), shell width (4.33±0.38), aperture width (2.77±0.30) were observed from snails collected from Osun River, while the least spiral length (2.38±0.16) and aperture circumference (18.12±0.92) were observed from snails collected from Eleyele Lake. The least aperture height (6.70±0.48) was observed from snails collected from Orori River. Morphological parameters measured on *L. natalensis* are presented in Table 3. The snails from Eko-Endo Lake had the highest shell height (13.30±0.41), aperture height (9.12±0.27), aperture width (5.22±0.13), spiral length (4.30±0.11) and aperture circumference (22.89±0.49), while the once from Osun River had the highest shell width (7.96±0.21). The snails from Osun River had the least shell height (11.39±0.29), aperture height (6.72±0.17), aperture width (4.60±0.10), spiral length (3.61±0.14) and aperture circumference (20.83±0.34). The least shell width was observed from snails from Eleyele Lake.

The relationship of snail species' shell length to shell width, aperture length to aperture width show linearity (Fig. 3-4). The highest and lowest coefficient of determinant (R-square) for aperture length against aperture width and shell length against shell width was observed for *B. globosus* and *B. pfeifferi*, respectively.

DISCUSSION

Mollusca are large part of the global invertebrate fauna of freshwater and marine habitats (Strong *et al*., 2008). Thus identifying these snails is of value to Zoologists, Malacologists and Conchologists because of the role they perform as intermediate hosts of the larvae of variety of trematode parasites that cause diseases of humans and animals (Appleton, 1996; Correa *et al*., 2010). Besides, the taxonomic information obtained from snail shells can be used in deducing and interpreting evolutionary history and relationship between species (Chiu *et al*., 2002). Moreover, malacological studies that confirm the presence of snail species that have being incriminated as

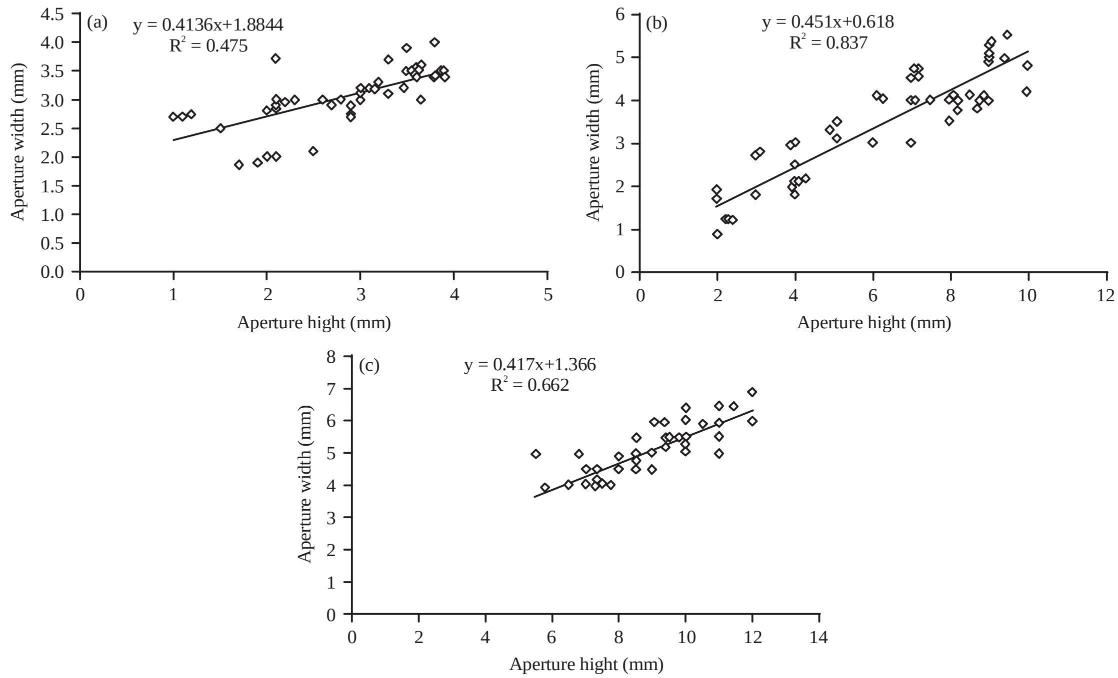

Fig. 3(a-c): Relationship of aperture length to aperture width of (a) *Biomphalaria pfeifferi,* (b) *Bulinus globosus* and (c) *Lymnaea natalensis*

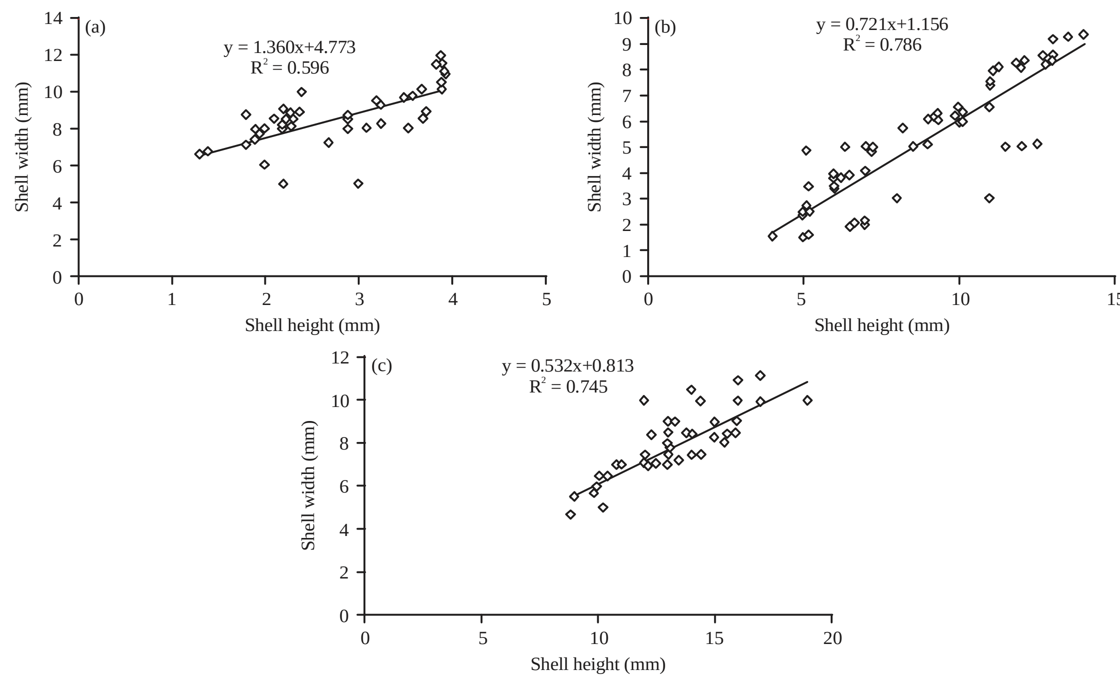

Fig. 4(a-c): Relationship of shell length to shell width of (a) *Biomphalaria pfeifferi*, (b) *Bulinus globosus* and (c) *Lymnaea natalensis*

medically or veterinary important species could explain the presence, potential risk and/or the spread of diseases such as schistosomiasis and fascioliasis to areas that were not formally endemic for these diseases (De Kock *et al*., 2004). The spread of schistosomiasis and fascioliasis in most African countries particularly to areas where the disease was not endemic has been attributed to the presence of the intermediate snail vector populating man-made habitats, such as irrigation canals and small dams, which have been known to serve as sites for disease transmission (Adenowo *et al*., 2015). Various studies have shown that the presence of snail intermediate host may determine the prevalence and explain the increased transmission of trematode infections in these areas. Thus, the determination of the distribution of the snail intermediate hosts of molluscan-borne diseases through e.g., shell morphology represent an important advance for epidemiological studies that are central to making public health-control decisions for control of snail vectors of trematode infections (Ibikounle *et al*., 2008). There is need for better documentation of these snail species in an endemic country like Nigeria where there is paucity of information from endemic sites. This work therefore, sought to employ seven morphological parameters of snail shell in identification of three snail vectors from five water bodies in South-Western Nigeria.

Although, no report exists of schistosomiasis around Eleyele Lake, the presence of both *B. pfeifferi* and *B. globosus* in the lake may pose the risk of the disease being introduced to the surrounding communities in the future. This is because fishing and other agricultural activities are being carry out daily, therefore allowing human water contact. Studies of populations living in areas endemic for schistosomiasis have shown that unavoidable contact with water for farming, fishing and recreation have led to increased transmission of schistosomiasis (Olubode *et al*., 2011). Despite the fact that there are no reports of human schistosomiasis in communities around Eleyele lake, PCR analysis of water samples collected from this Lake indicated the presence of *S. haematobium* cercariae (Akande *et al*., 2012).

Some water bodies like Eko-Endo Lake, Osun River and Eleyele Lake are rich in molluscan fauna. These water bodies habour the three pulmonate snails studied in this work. *Biomphalaria pfeifferi* and *B. globosus* are found in waters across Sub-Sahara Africa where their role as intermediate hosts in the transmission of human schistosomiasis are well documented (Mandahl-Barth, 1962; Brown, 1980; Gryseels, 1989; Jordan *et al*., 1993; WHO., 1995; Dejong *et al*., 2003). *Lymnaea natalensis* is also found throughout Africa with a wider distribution, occurring from north to south of the continent (Appleton *et al*., 2009). The three pulmonate snails studied are well distributed and are abundant in Nigeria, particularly in the South (Ndifon and Ukoli, 1989) which could account for the high endemicity of schistosomiasis, fascioliasis and other molluscan-borne diseases in this region of country (Brown, 1980; Ndifon and Ukoli, 1989). Our study observations indicate *B. globosus* to have the widest distribution based on the water bodies sampled and was the most abundant species. This may explain why *S. haematobium* has wider distribution than *S. mansoni* around the studied area since its intermediate vector is *B. globosus* (WHO., 2010).

We observed that Eleyele and Eko-Endo Lakes are rich in terms of molluscan fauna. In addition to the highest numbers of *B. pfeifferi*, *B. globosus* and *L. natalensis* collected from these two sites. We also collected other snail species such as *Melanoides tuberculate* (Prosobranch). However, these were of lesser interest to this work and were not investigated further. Besides, the snail shells collected from these lakes were bigger when compared to those collected from the rivers in this study, though it was not our aim to compare snails collected from the lakes to those collected from rivers. linear relationship was observed when shell length to shell width of each snail species was compared. similar relationship was also observed for aperture length when compared to aperture width. This pattern of association has been observed in many studies, both among freshwater (Chiu *et al*., 2002; Elkarmi and Ismail, 2007) and marine snail species (Ismail *et al*., 2000).

This study presents the morphological features of the shell of *B. pfeifferi*, *B. globosus* and *L. natalensis* from five sites from South-Western Nigeria. The findings in this study should prove useful for snail identification by taxonomist and epidemiological field researchers working around potential transmission sites. Although, shell morphology of snails is rich in taxonomic information (Dung *et al*., 2013; Pesic and Gloer, 2013), studies employing alternative tools such as molecular identification, using housekeeping genes (ITS, COI and 16S), can be used *pari passu* with findings from morphological identification. To this end we are currently characterizing these snails using molecular identification tools, which will further help in delineating these species.

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