academicJournals

Vol. 17(9), pp. 269-278, 28 February, 2018
DOI: 10.5897/AJB2017.16003
Article Number: 14B951456134
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Shell morphology and the radula structures of two closely related bulinid snails intermediate host of *Schistosoma haematobium* in Nigeria

Opeyemi Gbenga Oso* and Alexander Bababunmi Odaibo

Parasitology Research Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria.

Received 25 March 2017; Accepted 1 November, 2017

Bulinid snails act as intermediate host of schistosomes and the presence of the snail gives schistosomiasis an expansive characteristics. Schistosomiasis is of medical and veterinary importance in the tropical and subtropical regions. The shell shape and structure of radula teeth of bulinids are often specific to a species or genus, and are widely used for gastropod species identification. Bulinid species collected from schistosome endemic areas of Ogun State, South-western Nigeria were used for this study. Shell morphometrics were recorded using vernier caliper while the buccal mass of each snail was removed and permanent slides of the radulae were made according to standard procedure. There was a significant difference in the shell height, width, aperture height and aperture width between *Bulinus globosus* and *Bulinus jousseaumei* ($p<0.05$). The average shell height measurement for *B. globosus* was 7.6±1.9 mm, while *B. jousseaumei* measured 5.1±1.6 mm. Each transverse row of *B. globosus* radula had a ratio of 26:8:1:8:26 while *B. jousseaumei* had a ratio of 25:8:1:8:25. The marginal teeth of *B. globosus* possessed five cusps while *B. jousseaumei* possessed six cusps. The differences observed in shell, radula ratio and cusps in both species could be used to differentiate both species.

Key words: Radula, shell, *Bulinus* species, identification, Nigeria.

INTRODUCTION

Digenetic trematodes of the genus *Schistosoma* are causative agents of schistosomiasis in human and have an indirect lifecycle with freshwater snails serving as the intermediate host. Urinary schistosomiasis is often transmitted by different species of the genus *Bulinus* (Brown, 1994).

The use of shell and internal anatomy of snails have been very useful in the past for identifying and separating *Bulinus* species, although, these characteristics are also thought to be problematic in their use in phylogenetic studies (Inaba, 1969; Brown, 1994; Stothard and Rollinson, 1996). The variability in internal anatomy tends to occur as a result of selective processes in snail species (Bargues et al., 2001; Remigio, 2002). However, some of these characteristics such as distal genitalia, prostate, shell and radula teeth have been useful in the identification of snail species (Walter, 1968; 1969, Mimpfoundi and Ndassa, 2005). Shell characteristics

*Corresponding author. E-mail: opeyemi.immaculate@gmail.com; alexodaibo@yahoo.com.

have been said to be of limited value for the identification of bulinid species (Stothard et al., 1997). However, early species descriptions were grouped into genera based on shell characteristics, especially while in the field before using other methods of identification.

The radula has been described as a chitinous ribbon-like series of nearly colourless transverse tooth rows resting on top of the radula membrane (Radwin and Wells, 1968). The way a radula function is often affected by size, form, and nutrient materials. The reactions between teeth and nutrient materials tend to determine the need of each species to special radula. The type, form, number, and arrangement of radular teeth in different species indicate its different functions, which include nutrition capabilities, quality of reaction between different species as well as differentiating the species.

Radula features such as number and structure of radula have been of higher importance in molluscan taxonomic relationships. It often shows general similarities or differences at the species level. These observable differences and similarities have been utilized in the classification of gastropods (Arularasan et al., 2011). Although many factors such as food, seasonal changes and sexual differences affect the structure of radula (Carlos and Helena, 2003; Matthews-Cascon et al., 2005), some radula characters are generally constant within the same species (Fretter and Graham, 1994). Moreso, radula morphology is frequently used as a taxonomic character for studies on molluscan systematics (Fretter and Graham, 1994; Padilla, 1998; deMaintenon, 2004). The shape and form of the radula teeth are typically unique to a species or genus and some features of the radula, such as tooth numbers have been used to investigate higher levels in molluscan phylogenetic studies (deMaintenon, 2004).

Apart from the above mentioned anatomical features of the snails which are used for speciation, other anatomical characters such as kidney, nervous system, pneumostome, tentacles and digestive system are equally useful in snail identification (Jackiewicz, 1990; Paraense, 1994; 1995; Ponder and Waterhouse, 1997; Jackiewicz and Buksalewicz, 1998; Samadi et al., 2000). Though anatomical characteristics have some short comings in species identification, some of the characters referred to above have proven to be more useful in discriminating snail species (Samadi et al., 2000). Therefore, the use of anatomical characters in understanding the systematics of the freshwater snails is equally important.

Although molecular biomarkers have played important role in species identification (Stothard, 1996; Hebert et al., 2003; Akinwale et al., 2015), morphological characteristics such as shell and radula structure continue to be the primary means of identification of freshwater snails (Schander and Willassen, 2005). *Bulinus globosus* and *Bulinus jousseaumei* are two closely related snails, both of which are intermediate hosts of the parasitic flatworm known to cause schistosomiasis (Salawu and Odaibo, 2012; Stothard et al., 2013; Akinwale et al., 2015; Mkize et al., 2016; Hassan et al., 2016; Chibwana and Nkwengulila, 2017). The study therefore describes the morphological differences in the shell and radula of two snail intermediate hosts of schistosome in South-western Nigeria.

MATERIALS AND METHODS

Snail species (expand methods)

A total of 917 *B. globosus* and 543 *B. jousseaumei* specimens were collected from water contact sites in Yewa North Local Government Area of Ogun State (latitude 7°15' N and longitude 3° 3' E). The snails were preserved in 70% ethanol for dissection.

Shell morphometrics

The following linear measurements were recorded using a vernier caliper: shell height (H), shell width (W), shell aperture height (AH), and shell aperture width (AW) according to Chiu et al. (2002).

From the values obtained for each linear measurement, the following ratios were calculated: shell height/shell width (H/W); shell height/aperture height (H/AH); shell height/aperture width (H/AW); shell width/aperture height (H/AW) and aperture height/aperture width (AH/AW).

Dissection and preparation of radula

The head region was opened by an incision from the mantle edge between the tentacles. The body walls were relaxed and fixed with pins, exposing the buccal mass and penial complexes. The buccal mass was macerated in 7.5% sodium hydroxide (NaOH) for 2 h at 60°C. The freed radula was washed in water and membranes were removed under a dissecting microscope. Radula was transferred to a drop of 10% glacial acetic acid on a slide and orientated with its teeth uppermost and straightened out. The acid was left to evaporate.

A drop of Mallory stain was placed on the radula for 2 to 3 mins after which the radula was rinsed in running water. The radula was then rinsed in 2% oxalic acid, 96% ethanol and xylene (Mandahl-Barth, 1962). A drop of Canada balsam was used to mount the radula at room temperature. These radulae were imaged using a light microscope.

Determination of radula teeth formula

Each row of radula teeth consists of one central tooth (C) which is always found in the middle, on each side of the central teeth are the lateral teeth (L) and then beyond the lateral teeth are the marginal teeth (M). Different species of snails have different numbers of lateral and marginal teeth.

Statistical analysis

The mean, standard deviation, minimum, maximum values, independent t-test and the general linear regression were performed on shell character, using statistical package SPSS version 22.0.

Table 1. Mean and range values of *Bulinus globosus* in Yewa North LGA.

Shell morphometrics	Minimum Value (mm)	Maximum Value (mm)	Mean ± SD (mm)
Height (H)	3.0	13.5	7.6 ± 1.9
Width (W)	2.0	9.5.0	5.5 ± 1.2
Aperture height (AH)	0.5	10.0	5.7 ± 1.4
Aperture width (AW)	0.5	6.5	3.5 ± 0.9
Height Width ratio (H/W)	0.2	2.1	1.4 ± 0.2
Height and Aperture Height ratio (H/AH)	0.8	17.0	1.4 ± 0.5
Height and Aperture Width ratio (H/AW)	1.3	7.0	2.2 ± 0.4
Width and Aperture Height ratio (W/AH)	0.7	12.0	1.0 ± 0.5
Aperture Height and Aperture Width ratio (AH/AW)	0.1	3.0	1.7 ± 0.3

Table 2. Mean and range values of *Bulinus jousseaumei* in Yewa North LGA.

Shell morphometrics	Minimum Value (mm)	Maximum Value (mm)	Mean ± SD (mm)
Height (H)	2.0	11.0	5.1 ± 1.6
Width (W)	1.2	8.0	3.9 ± 1.1
Aperture height (AH)	1.5	8.0	4.3 ± 1.3
Aperture width (AW)	1.0	6.0	2.3 ± 0.8
Height Width ratio (H/W)	0.7	3.0	1.3 ± 0.2
Height and Aperture Height ratio (H/AH)	0.6	2.3	1.2 ± 0.2
Height and Aperture Width ratio (H/AW)	1.3	6.0	2.4 ± 0.6
Width and Aperture Height ratio (W/AH)	0.5	1.4	0.9 ± 0.1
Aperture Height and Aperture Width ratio (AH/AW)	1.1	5.0	2.0 ± 0.5

RESULTS

Morphometrics

On average, *B. globosus* shells measured 7.6±1.9 mm in height, 5.5±2.3 mm in width, 5.7±1.4 mm in aperture height and 3.5±0.9 mm in aperture width (Table 1). The longest *B. jousseaumei* shell height recorded was 11.0 mm, although this species' mean shell height was 5.1±1.6 (Table 2).

Figures 1 and 2 show the adult *B. globosus* and *B. jousseaumei*. The relationship of bulinids shell morphometrics showed linearity (Figures 3, 4, 5 and 6). Analysis of shell height, width, aperture height and aperture width shows that *B. globosus* was significantly larger than *B. jousseaumei* ($p<0.05$).

Radula morphology

Bulinus jousseaumei

The general radula ratio is M:L:C:L:M. Generally, bulinid species have the same radula shape (Figures 7 and 9) however there is variation in different parts of the radula amongst different species. The radular teeth ratio of *B. jousseaumei* was 25:8:1:8:25 and it consist of a single row of central teeth found at the middle of the radula, eight pairs of lateral teeth (Figure 8a) and 25 pairs of marginal teeth (Figure 8b). Variation in the mesocone showed that the cusps of the central radula were intermediate while the lateral cusps were angular.

The cusps of the central teeth were small and reduced in size. The lateral teeth were broad and asymmetrically tricuspid. The endocone was short and fused with the mesocone while the mesocone was broader than the ectocone. The marginal teeth which is the last morphological tooth type, comprised the outermost group of the teeth on each side of a transverse row. *B. jousseaumei* teeth possessed six cusps. Variation in the mesocone of the lateral radula teeth was angular while the central teeth were intermediate.

Bulinus globosus

The radula teeth ratio of *B. globosus* was 26:8:1:8:26, which consist of a single row of central teeth found at the

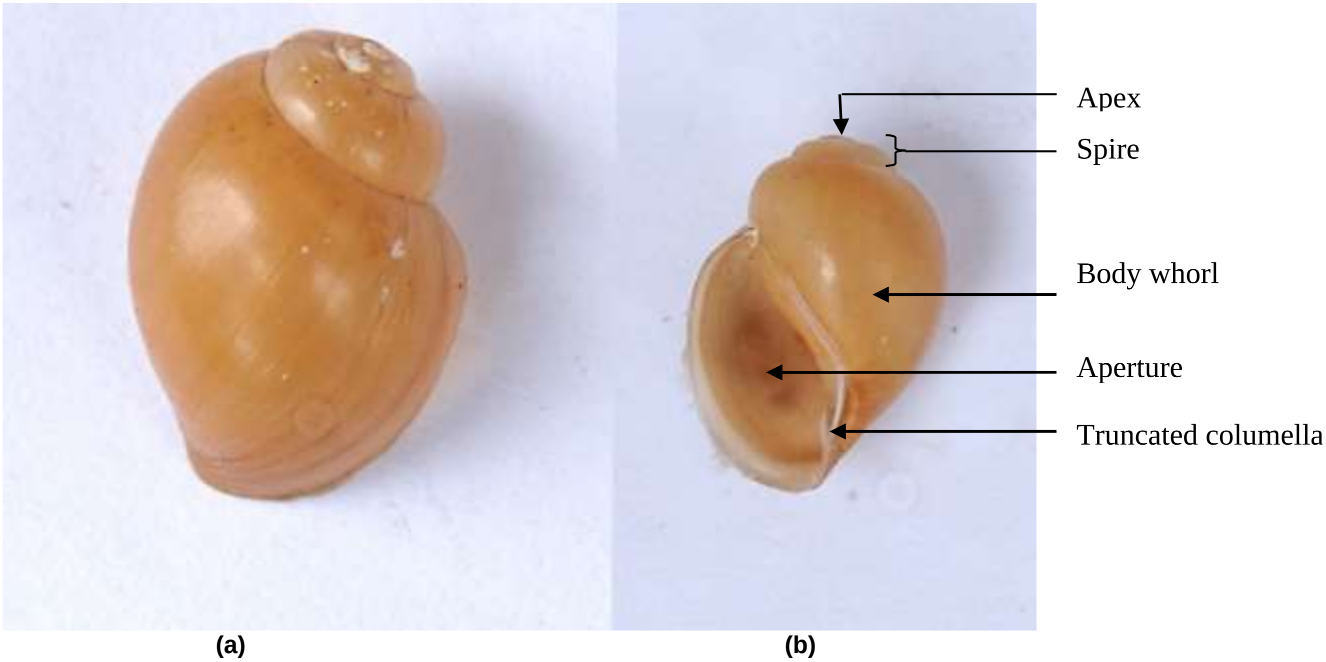


Figure 1. Abapertural (a) and Apertural (b) view of *Bulinus globosus* (x3). The shell is sinistral and the columella truncated.

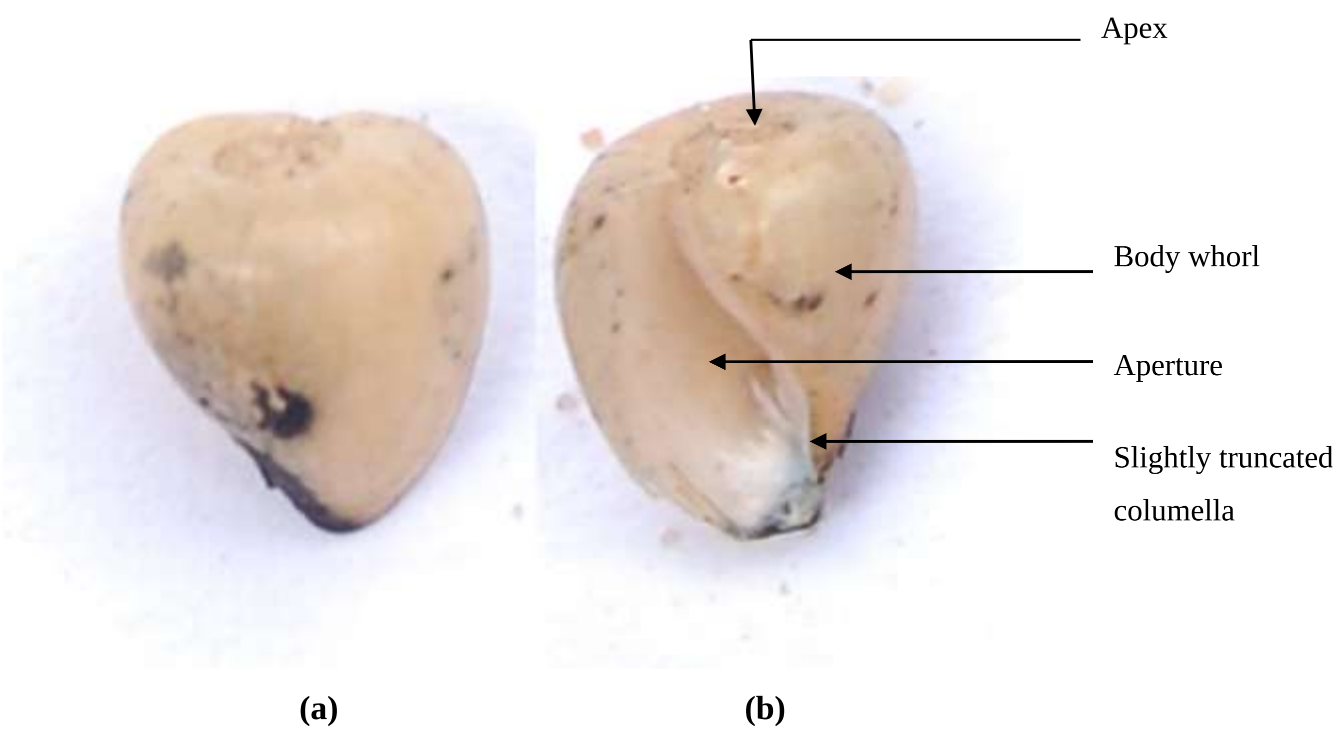


Figure 2. Abapertural (a) and apertural (b) view of *Bulinus jousseaumei* (x3). The shell has a depressed spire and it is sinistral.

middle of the radula, eight pairs of lateral teeth (Figure 10a) and twenty-six pairs of marginal teeth (Figure 10b). The cusps of the central teeth were small and reduced in size. The lateral teeth were broad and asymmetrically tricuspid.

The endocone was short and fused with the mesocone

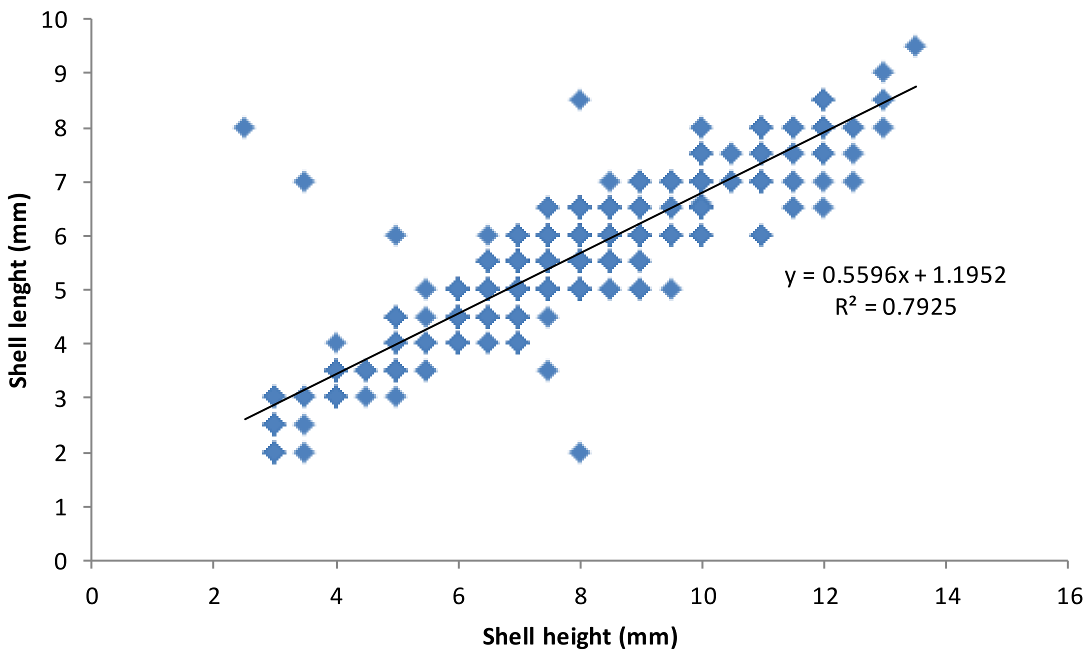


Figure 3. Relationship between Shell Height and Shell Width of *Bulinus globosus.*

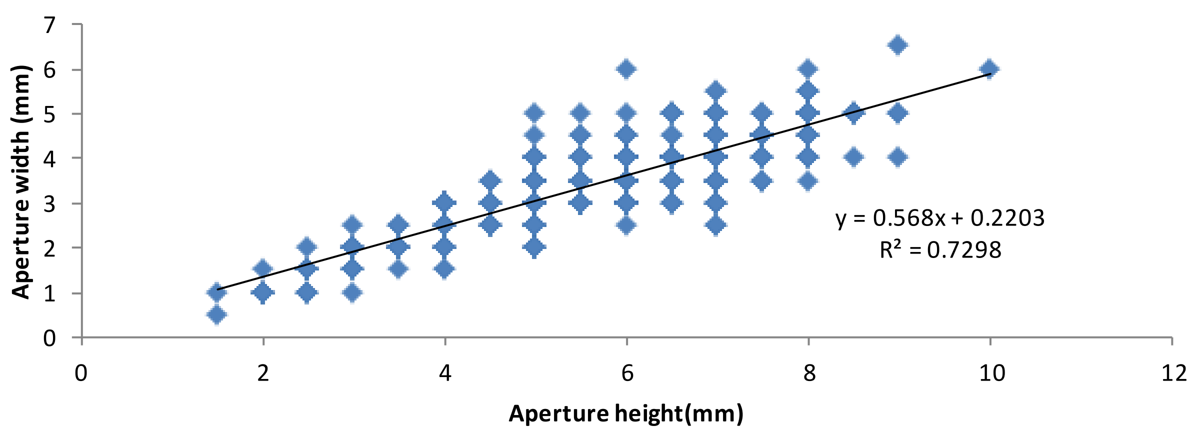


Figure 4. Relationship between aperture height and aperture width of *Bulinus globosus.*

while the mesocone was broader than the ectocone. The marginal teeth of *B. globosus* possessed five cusps.

DISCUSSION

Generally, shells of freshwater snails provide important taxonomic information that can be used to show differences in species as well as reveal evolutionary relationships in different taxa. Shell characters such as the spire height, and width of *B. globosus* have been widely reported from different schistosome endemic areas of Nigeria, however *B. jousseaumei* was only recently reported in Nigeria (Salawu and Odaibo, 2012).

The significant difference observed in all the shell characters (shell height, width, aperture length and aperture width) between *B. globosus* and *B. jousseaumei* showed that all these shell characteristics can be used to differentiate these species. The significant difference in the morphometrics of *B. globosus* and *B. jousseaumei* recorded in this study had been observed in other similar pulmonates (Monzon et al., 1993). The long-spired and short-spired form in *B. globosus* and *B. jousseaumei* respectively has been reported in previous observation.

The line of best-fit plot shows the positive trend in the measured characters. In most cases, shell height forms

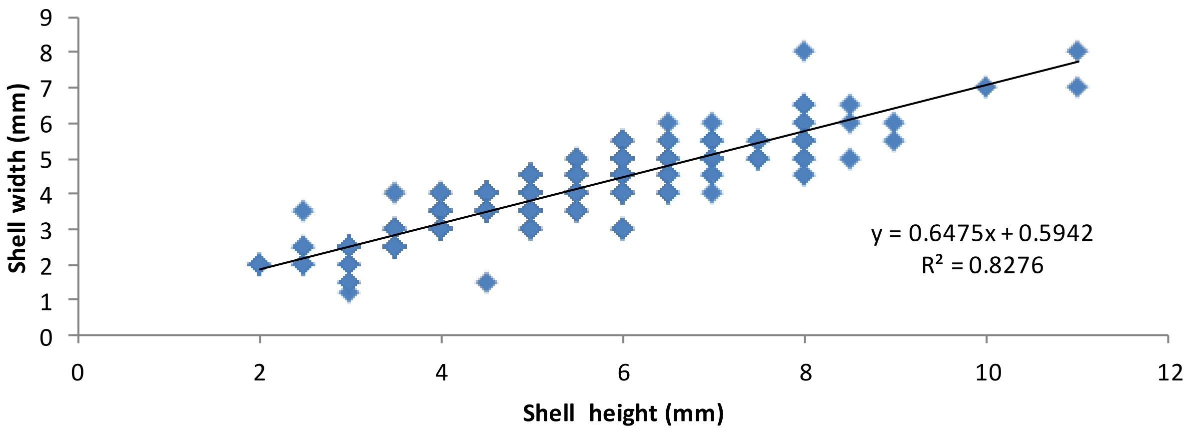


Figure 5. Relationship between shell height and shell width of *Bulinus jousseaumei.*

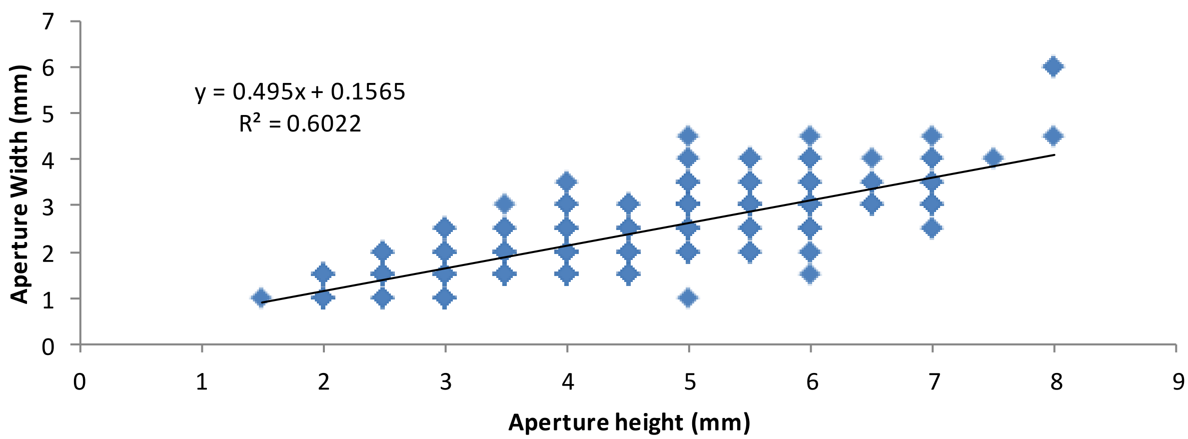


Figure 6. Relationship between Aperture Height and Aperture Width of *Bulinus jousseaumei.*

part of the taxonomic characters in differentiating snail species (Chiu et al., 2002). Environmental changes without genetic change can result in distinct non-genetic changes in shell morphology. Calcium contributes to shell formation in gastropods (Jokinen, 1982), although calcium level in the environment acts as a limiting factor and selective pressure on shell morphology (Rundel et al., 2004). The higher the calcium in the environment, the thicker the shell formed and there is a narrow aperture in the snail (Rundle et al., 2004).

Most time calcium correlated positively with water pH (Hunter, 1990) and in the presence of low calcium and low pH in the environment, snail shells are eroded easily (Glass and Darby, 2009). In this study, the short spire found in *B. jousseaumei* could be a result of underutilization of calcium from the environment. Water flow also affects shell morphology. This factor often affects snail shell in large lakes compared to shallow river bodies (Trussell, 1997), in our study area, snails were collected from shallow river bodies. Snails with thick shell are often associated with high water flow. The thick shell of the snail minimizes the shell damage, when the snails are dislodged by high water current (Trussell, 1997; Minton et al., 2008). Also, under the shallow areas, shells with very short spire are common and look alike (Shileyko, 1967).

In this study, each transverse row of radula of *B. globosus* had a radula formula of 26:8:1:8:26 while *B. jousseaumei* had formula of 25:8:1:8:25. The difference in the morphology in these two species focuses attention on the use of radula morphology for the species differentiation. Soft parts of snails have been proved useful for species identification; however, some studies argued that such anatomical characteristics are too variable and should be avoided for phylogenetic studies because these anatomical characteristics are prone to selective processes which could hamper the normal formation of these characters (Bargues et al., 2001; Remigio, 2002).

However, distal genitalia, prostate and radula teeth

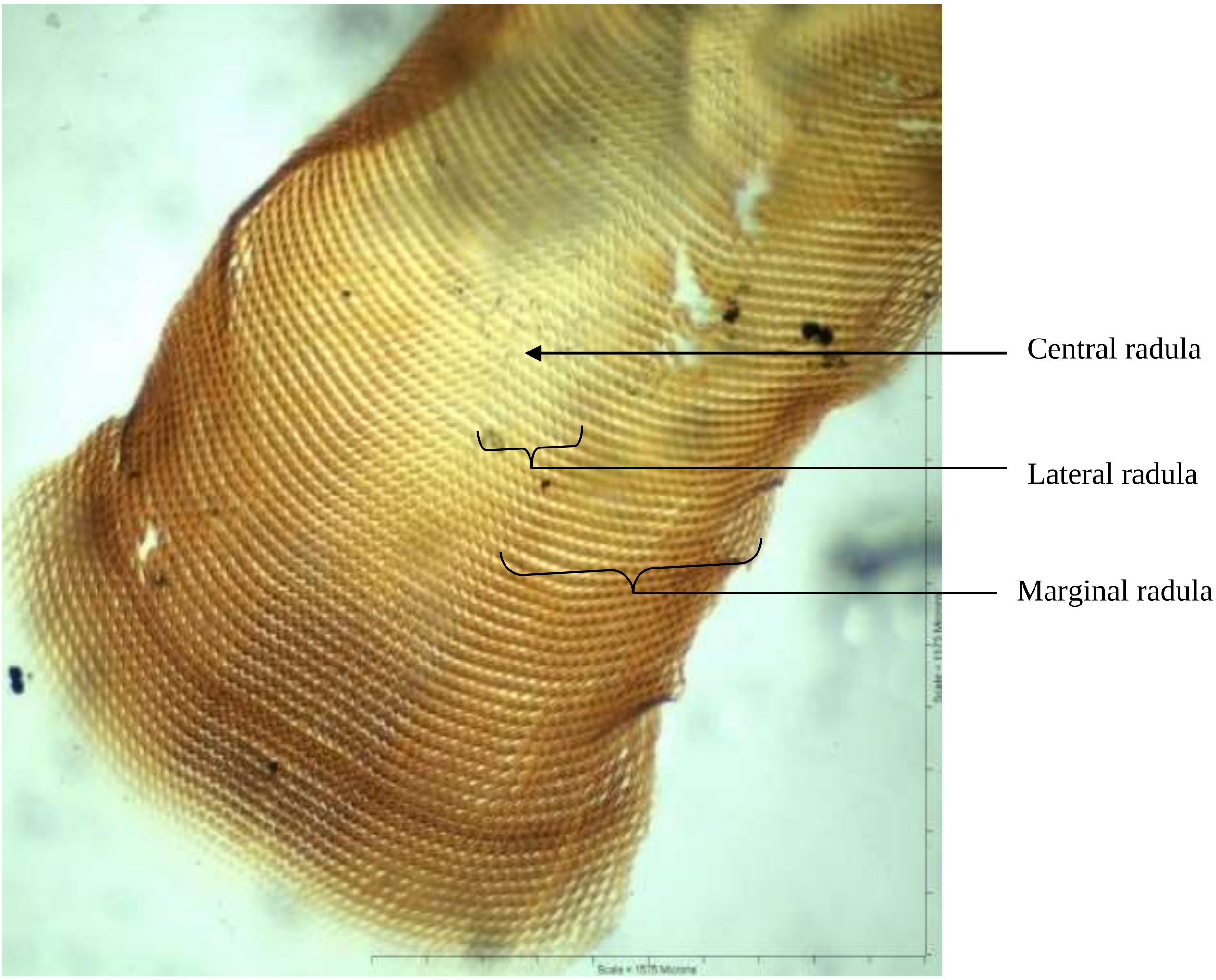


Figure 7. Whole radula of *B. jousseaumei*. Scale bar: 575 microns.

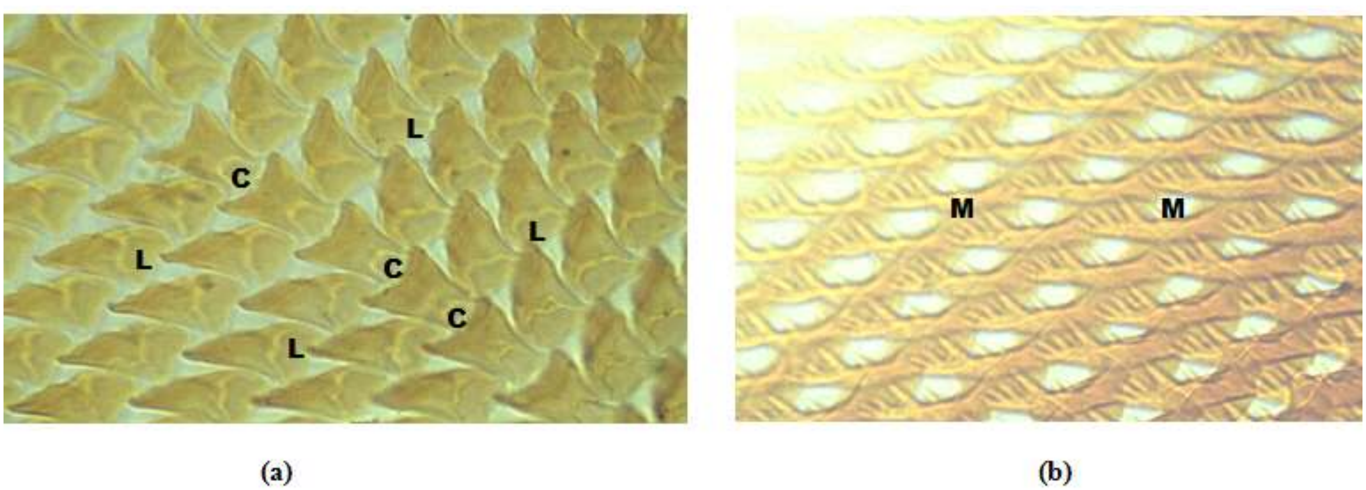


Figure 8. (a) *Bulinus jousseaumei* (Lateral (L) and central (C) teeth). Scale bar: 1575 microns. The mesocone cusp of the central radular tooth is intermediate while the lateral radular cusp is angular. The endocone of the lateral radula is fused with the mesocone. (b) *Bulinus jousseaumei* (marginal (M) teeth). Scale bar: 1575 microns. The marginal radula of *B. jousseaumei* possessed six cusps each.

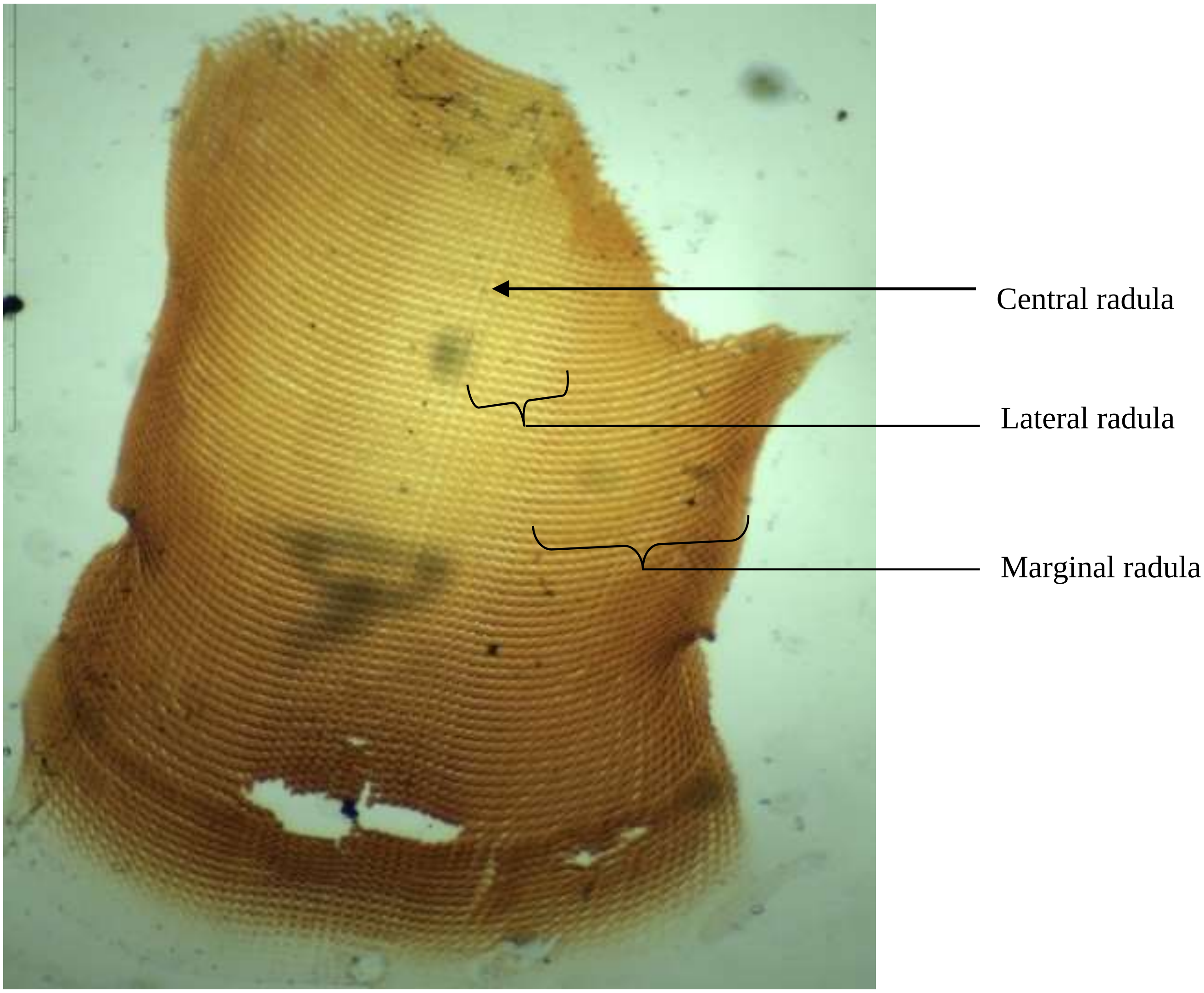
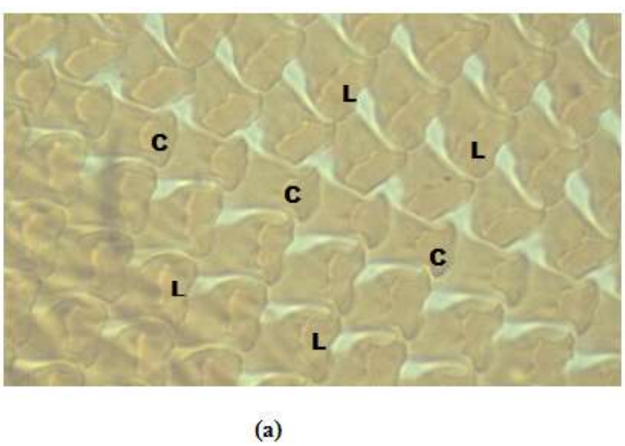


Figure 9. Whole radula of *B. globosus.* Scale bar: 575 microns.



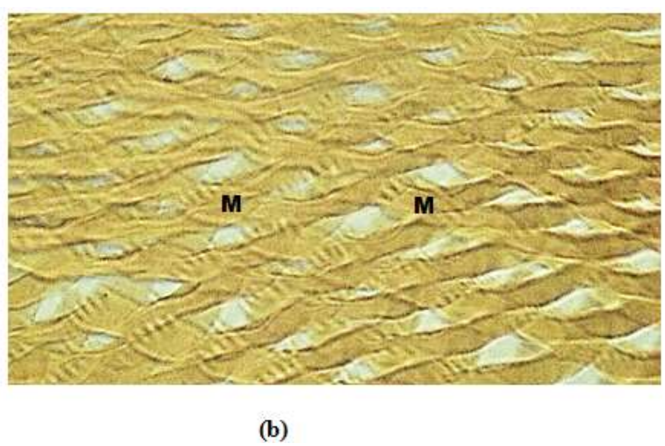


Figure 10. (a) *Bulinus globosus* (Lateral (L) and central (C) teeth). Scale bar: 1575 microns. The mesocone of central radula is intermediate while the lateral radula cusp is angular. The endocone of the lateral radula is fused with the mesocone. **(b)** *Bulinus globosus* (marginal (M) teeth). Scale bar: 1575 microns. The marginal radula of *B. globosus* possessed five cusps each.

were useful characters that can be used for successful species identification (Hubendick, 1951; Walker, 1968). Besides, the formula of radula (Ponder and Lindberg, 1996), shape, size and structure of the cusps (Kilburn, 1988; Monzon et al., 1993) of radula, has provided the needed information for the taxonomy of different snail species. The unicuspid characteristic of the central tooth of bulinids in this study was similar to observations in Mozambique (de Azevedo et al., 1961).

Conclusion

Shell and radula morphology of bulinid species could be adopted as part of the characteristics used for the classification of these species, as clear differences occurred between the shell and radulae of *B. globosus* and *B. jousseaumei*. These observable differences in these bulinid species can ensure correct species identification with the aim of controlling schistosome in the area. Moreso, the similarity between these two intermediate hosts of schistosomes need quick and cost effective means of differentiation.

Thus, this study observed differences in conchological measurements and radula morphology of these species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge the Institute of Infectious Disease of Poverty (IIDP) for the scholarship award to OGO. We are also grateful to the Head of Communities in Yewa North Local Government Area, Ogun State, for allowing us to carry out this study in their communities. This study forms part of OGO's Doctoral Thesis.

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