International Journal of Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Zoological Research

ISSN 1811-9778
DOI: 10.3923/ijzr.2022.1.7

Research Article
Comparative Differences in Radula Characteristics of *Schistosoma* Snail Intermediate Host in the *Forskalii* Group

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Abstract

Background and Objective: Snails in the *forskalii* group act as intermediate host of schistosomes and the availability of these snails in freshwater bodies increase the risk of schistosome infection. In tropical and sub-tropical countries, schistosome infection is still common among people living in rural and urban communities. Radular teeth of freshwater snails are often specific to a species or genus and are widely used for species identification. Hence, this study compared the radula structures of *Bulinus* species in the *forskalii* group with the aim of providing observable differences within the species. **Materials and Methods:** The snail species were collected from schistosome endemic rural areas and preserved in ethanol for this study. Shell morphometrics was measured using a Vernier caliper while permanent slides of the radulae were dissected and stained using Mallory. Descriptive statistics, One-way analysis of variance (ANOVA) and LSD post hoc tests were performed on shell characters. **Results:** The means shell length (mm) of *B. camerunensis*, *B. senegalensis* and *B. forskalii* were 5.8±0.9, 7.4±0.2 and 7.1±1.2, respectively. The radula of each species had a ratio of 18:17:1:17:18. The marginal teeth of *B. forskalii* possessed seven cusps, *B. camerunensis* possessed six cusps while *B. senegalensis* possessed twelve cusps. **Conclusion:** The differences observed in shell characteristics and radulae morphology in the three species could be used to differentiate these species in middle and low-income areas.

Key words: Radula, planorbids, *Bulinus* species, morphology, cusp, mallory stain, schistosome

Citation: Oso, O.G. and A.B. Odaibo, 2022. Comparative Differences in radula characteristics of *Schistosoma* snail intermediate host in the *Forskalii* group. Int. J. Zool. Res., 18: 1-7.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Studies involving *planorbidae*, a group of freshwater snail intermediate hosts of schistosomes, both in humans and animals have evolved many years ago[1]. In the tropical and sub-tropical countries, colonization of new habitats by different species of snail intermediate host is not reducing, hence, the probability of increased schistosome transmission is inevitable[2]. According to a WHO report, among different strategies adopted for controlling the spread of schistosome parasite is the quick and correct identification of *planorbidae* in different areas where transmission is currently on-going[3]. In Nigeria, snails in the *forskalii* group have been incriminated as the intermediate host of schistosomes[4]. Different method has been used in the identification of *Bulinus* species found in this group, among which are shell morphology, use of soft part like the radula, digestive system, reproductive system as well as molecular methods[1,5-7]. Although the molecular method is considered to be one of the best methods in characterizing snail species, the high cost of this method and expertise limits its use in many tropical countries, yet, there is need for quick and correct identification of *planorbidae* in order to target the correct snail intermediate host through the application of control strategies. The use of soft parts, among which is radula morphology have been successfully used in identification of snail species in different studies [8]. This is due to variations that occur in different parts of the radular teeth. Although radula teeth in general are useful for macerating food materials by snail species, variations in the central, lateral and marginal teeth have enhance their use in snail systematics.

Bulinus forskalii (Ehrenberg, 1831), *Bulinus camerunensis* (Mandahl-Barth, 1957) and *B. senegalensis* (Muller, 1781) were all categorized under *Bulinus forskalii* group[1], using shell morphology. Though shells have been used to differentiate some snail species, due to close similarity in shell morphology, misidentification is often possible. However, the use of other identification forms such as the radula morphology often aids shell morphology in species systematics. Although *Bulinus forskalii* and *Bulinus camerunensis* have been reported in Nigeria as snail intermediate host of schistosoma parasite, *Bulinus senegalensis* was recently reported in Nigeria and has been incriminated as an intermediate host of schistosoma parasite[4]. This study aims to differentiate freshwater snails in *forskalii* group that was reported in Nigeria using morphological differences in their radula structures.

MATERIALS AND METHODS

Study area: The study was carried out at Parasitology Research Unit Laboratory, Department of Zoology, University of Ibadan, Ibadan, Nigeria, from February, 2017-October, 2018.

Snail species: *Bulinus senegalensis* (Fig. 1a,b), *Bulinus camerunensis* (Fig. 2a,b) and *Bulinus forskalii* (Fig. 3a,b) specimens were collected from water contact sites in Ogun State (latitudes 6°52′ 08″ N to 7°25′ 28″N and longitudes 2°43′ 09″ E to 3°07′ 13″ E). The snails were preserved in 70% ethanol for dissection. The front part of

Fig. 1(a-b): (a) Abapertural and (b) Apertural view of *Bulinus senegalensis* (x3)
Whorls are evenly curved and not carinate. The whorls are more rounded with no shoulder angle. The Shell is sinistral. The aperture is relatively higher and the sculpture is much finer

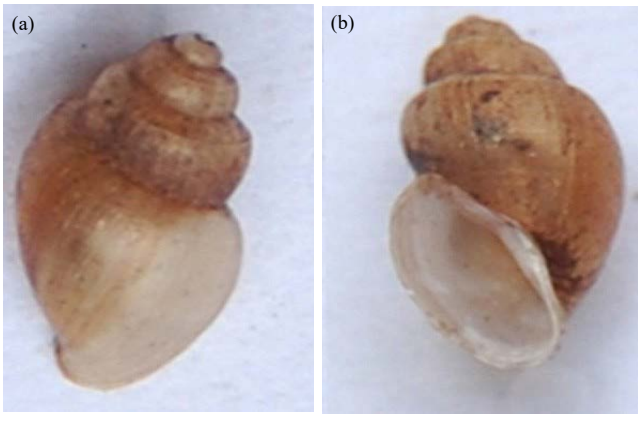

Fig. 2(a-b): (a) Abapertural and (b) Apertural view of *Bulinus camerunensis* (x10)
Shell is sinistral and carinate

Fig. 3(a-b): (a) Abapertural and (b) Apertural view of *Bulinus forskalii* (x4)
Spire is stepped and whorls carinate while the shell is sinistral

each snail species was incised, the body walls were then fixed with surgical pins exposing the interior part. Buccal mass was macerated in 7.5% sodium hydroxide for 2 hrs. Running water was used in removing particles from the freed radula. The radula was transferred to a drop of 10% glacial acetic acid on a slide, orientated with its teeth facing upward and straightened out. The radula was stained with Mallory stain for 2-3 min after which the radula was rinsed in running water. The radula was cleared in 2 % oxalic acid, 96% ethanol and xylene[9]. The radula was mounted with a drop of Canada balsam at room temperature, these radulae were imaged using a binocular microscope.

Vernier caliper (CP72678-00, VW|R, USA) was used to measure the shell Height (H), shell Width (W), shell Aperture Height (AH) and shell Aperture Width (AW)[6]. Ratios were also 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). Central teeth (C) which are in the middle, lateral teeth (L) and marginal teeth (M) were recorded to determine the difference among them.

Statistical analysis: One-way analysis of variance (ANOVA) and LSD post hoc tests were performed on shell characters using the required statistical package (SPSS).

RESULTS

The radula teeth formula of *B. senegalensis* was 18:7:1:7:18. The radula formula of *B. forskalii* and *B. camerunensis* was the same as *B. senegalensis*. Variation in the mesocone of the lateral and central radula teeth was angular. The variation in the mesocone of the lateral and central teeth of *B. forskalii* was intermediate (Fig. 4a). The lateral and central teeth of *B. camerunensis* followed the same pattern as *B. senegalensis* (Fig. 4b). The radula of *B. senegalensis* consists of a single row of central teeth found in the middle of the radula, seven pairs of lateral teeth (Fig. 4c). The marginal teeth of *B. forskalii* were multi cuspid having seven cusps (Fig. 5a). The marginal teeth of *B. camerunensis* were multi cuspid, with six cusps on each half of the transverse row (Fig. 5b). The radula of *B. senegalensis* consists of eighteen pairs of marginal teeth (Fig. 5c). The central teeth cusps of *B. senegalensis* were small and distinct while the lateral teeth were broad and asymmetrically tricuspid. The endocone of *B. senegalensis* was short compared to mesocone while the mesocone was broader than both endocone and ectocone. The marginal teeth of *B. senegalensis* were multi cuspid with twelve cusps on each transverse row.

Table 1 summarized the measurements recorded on all shell length, width, aperture length, aperture width from the three field collected snail species in *forskalii* group (*B. forskalii*, *B. camerunensis* and *B. senegalensis*) and their ratios were calculated. The mean shell length of *B. camerunensis* was 5.8±0.9 mm with the lowest value of 3.4 mm, the mean shell length of *B. senegalensis* was 7.4±0.2 mm with the lowest value of 4 mm while the mean shell length of *B. forskalii* was 7.1±1.2 mm.

The differences among the means of the measured morphometrics on all the three species in *forskalii* group collected from the field were determined by one-way analysis of variance (ANOVA). LSD post-hoc test was carried out to compare pair wise differences on the measured characters. Analysis of variance showed a significant difference in the length of all the three species, however, post-hoc test indicated no significant difference between the lengths of *B. forskalii* and *B. senegalensis* ($p = 0.377$) while significant differences occurred between *B. camerunensis* and *B. forskalii* ($p = 0.001$), *B. camerunensis* and *B. senegalensis* ($p = 0.001$). On the other hand, no significant difference ($p = 0.192$) occurred in the mean shell width of all three species. For aperture length, ANOVA showed significant difference among the three species, however, post hoc test showed no significant difference between *B. forskalii* and *B. senegalensis* ($p = 0.490$) while the mean aperture length of *B. forskalii* and *B. camerunensis* ($p = 0.019$), *B. camerunensis* and *B. senegalensis* ($p = 0.025$) differed significantly. Though aperture width of the three snail species differed significantly with the aid of ANOVA, however, post hoc test showed no significant differences between *B. forskalii* and *B. senegalensis* ($p = 0.149$), *B. forskalii* and *B. camerunensis* ($p = 0.739$) but *B. camerunensis* and *B. senegalensis* differed

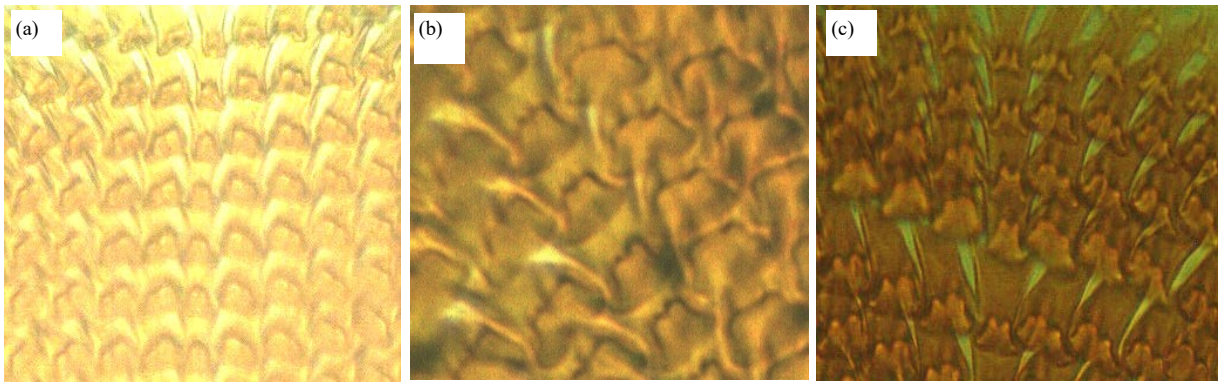

Fig. 4(a-c): Microscopic view of (a) *Bulinus forskalii* (Lateral and central teeth), (b) *Bulinus camerunensis* (Lateral and central teeth) and (c) *Bulinus senegalensis* (Lateral and central teeth) ×1575 microns

[a,b,c]Mesocone cusp of the central radula is intermediate while the lateral Radula cusp is angular. The endocone of the lateral radula is fused with the ectocone

Table 1: Mean and range values of collected field specimens of *B. forskalii*, *B. camerunensis* and *B. senegalensis*

	Snail species		
Shell morphometrics	*B. forskalii*	*B. camerunensis*	*B. senegalensis*
Length (L)			
L±SD	7.1±1.2	5.8±0.9	7.4±0.2
range	5.5-11.0	3.4-8.0	4.0-10.0
Width (W)			
W±SD	3.0±0.6	2.8±0.6	3.0±0.6
range	2.5-5.0	2.0-6.5	2.0-4.5
Aperture Length (AL)			
AL±SD	2.9±0.7	2.6±0.7	2.8±0.8
range	2.0-5.0	1.5-6.0	1.5-5.0
Aperture width (AW)			
AW±SD	1.6±0.4	1.6±0.4	1.8±0.4
range	1.5-3.0	0.5-4.0	1.0-2.5
Length Width ratio (L/W)			
L/W±SD	2.4±0.2	2.1±0.4	2.5±0.4
range	2.2-2.8	1.2-4.4	1.6-4.0
Length and Aperture Length ratio (L/AL)			
L/AL±SD	2.5±0.2	2.4±0.6	2.7±0.5
range	2.0-2.8	1.3-5.5	2.0-5.3
Length and Aperture Width ratio (L/AW)			
L/AW±	4.4±0.4	3.9±1.2	4.3±1.0
range	3.3-4.7	2.0-11.0	3.0-8.0
Width and Aperture Length ratio (W/AL)			
W/AL±SD	1.0±0.1	1.1±0.2	1.1±0.2
range	0.8-1.3	0.6-1.8	0.7-1.0
Aperture Length and Aperture width ratio (AL/AW)			
AL/AW±SD	1.8±0.2	1.6±0.4	1.6±0.3
range	1.3-2.0	1.0-3.0	1.3-2.5

significantly (p = 0.007). The mean length/width ratio between *B. forskalii* and *B. camerunensis* (p = 0.001), *B. senegalensis* and *B. camerunensis* (p = 0.001) differed significantly while the difference between length/width ratio between *B. forskalii* and *B. senegalensis* (p = 0.127) was not significant. The length/aperture length ratio was also determined to know the difference between the three species. Only *B. senegalensis* and *B. camerunensis* (p = 0.001) differed significantly in their length/aperture length ratio, the differences between *B. forskalii* and *B. senegalensis* (p = 0.058), *B. forskalii* and *B. camerunensis* (p = 0.360) were not significant. On the other hand, the length/aperture width ratio between

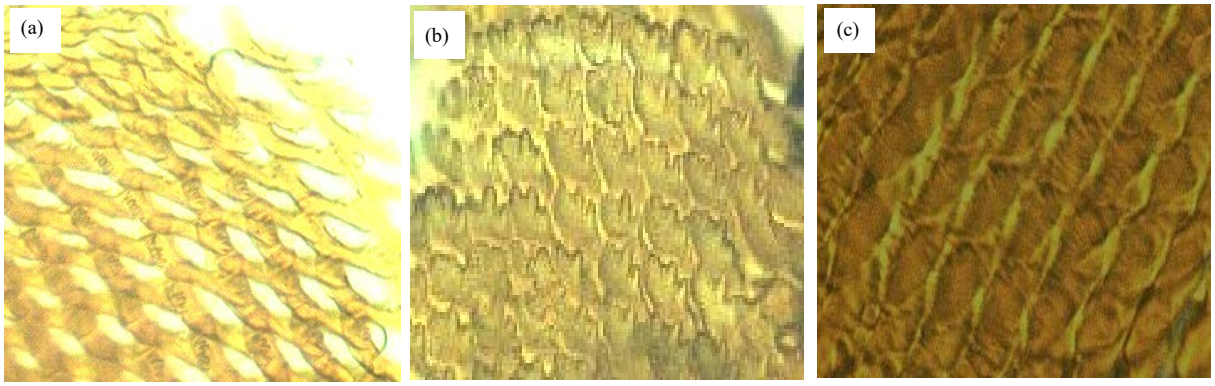

Fig. 5(a-c): Microscopic view of (a) *Bulinus forskalii* (Marginal teeth), (b) *Bulinus camerunensis* (Marginal teeth) and (c) *Bulinus senegalensis* (Marginal teeth) × 1575 microns
(a) Marginal radula of *B. forskalii* possessed seven cusps each, (b) *B. camerunensis* possessed six cusps and (c) *B. senegalensis* possessed twelve cusps

B. senegalensis and *B. forskalii* (p = 0.527) showed no significant difference while significant differences occurred between *B. forskalii* and *B. camerunensis* (p = 0.011), *B. camerunensis* and *B. senegalensis* (p = 0.009). Furthermore, the width/aperture length ratio was also determined to know the differences amongst the three snail species. Although ANOVA showed that the differences among the three species were significant (p = 0.027), however, only the difference between *B. forskalii* and *B. camerunensis* was significant (p = 0.021). The mean width/aperture length ratio did not differ between *B. forskalii* and *B. senegalensis* (p = 0.368), *B. senegalensis* and *B. camerunensis* (p = 0.061). The mean aperture length/aperture width ratio between *B. camerunensis* and *B. senegalensis* did not differ significantly (p = 0.368) while significant differences occurred between *B. forskalii* and *B. senegalensis* (p = 0.011), *B. camerunensis* and *B. forskalii* (p = 0.032).

DISCUSSION

From the result of morphometric, observable differences occurred in shell length measurement, suggesting that shell length can be used to differentiate *B. forskalii* and *B. camerunensis*, *B. camerunensis* and *B. senegalensis*, into different morphotypes. However, shell length could not be used to differentiate *B. forskalii* and *B. senegalensis* because no significant differences occurred between *B. forskalii* and *B. senegalensis*. The use of shell width cannot be used for differentiating any of these species because no significant differences occurred among the three species. The length of the aperture cannot be used to differentiate *B. forskalii* and *B. senegalensis*, however, observable differences between *B. forskalii* and *B. camerunensis*, *B. camerunensis* and *B. senegalensis*, suggest that aperture length could be used to differentiate them. In the same vein, aperture width can be used to differentiate *B. camerunensis* and *B. senegalensis* but failed to differentiate *B. forskalii* and *B. senegalensis* as well as between *B. forkalii* and *B. camerunensis*. Similar result was observed in Kenya where, observable differences were made on the shell characteristics of *Bulinus truncatus/tropicus* complex. Moreover, soft part of the species was used in addition to shell morphology[10].

The length/width ratio cannot be used to differentiate *B. forskalii* and *B. senegalensis* but the observable differences that occurred between *B. forskalii* and *B. camerunensis*, *B. senegalensis* and *B. camerunensis*, suggest that length-width ratio could be used to differentiate them. Also, length and aperture length ratio can be used to differentiate *B. senegalensis* and *B. camerunensis*, however, it cannot be used to differentiate *B. forskalii* and *B. senegalensis*. Though length and aperture width ratio cannot be used to differentiate *B. senegalensis* and *B. forskalii*, on the other hand, it could be used to differentiate *B. forskalii* and *B. camerunensis*. Width and aperture length ratio can be used to differentiate *B. forskalii* and *B. camerunensis*, on the other hand, it cannot be used to differentiate *B. senegalensis* and *B. camerunensis*, *B. forskalii* and *B. senegalensis*. Aperture length and aperture width ratio showed significant variation between *B. forskalii* and *B. senegalensis*. The presence of these bulinid species in this study is in consonance with other studies in other African countries[11,12].

Some shell measurements (shell length, aperture length, aperture width, length and aperture width ratio, width and aperture length ratio, aperture length and aperture width ratio) suggested differences between *B. forskalii* and

B. camerunensis and shell measurement (shell length, aperture length, aperture width, length or width, length and aperture length ratio, length and aperture width ratio, width and aperture length ratio, aperture length and aperture width ratio) also suggested differences between *B. forskalii* and *B. senegalensis*. On the other hand, shell measurements (shell length, aperture length, aperture width, length or width, length and aperture length ratio, length and aperture width ratio, width and aperture length ratio) showed closer affinity between *B. senegalensis* and *B. forskalii.* In the same vein, length and aperture length ratio, aperture width ratio showed closer affinity between *B. forskalii* and *B. camerunensis* from this study. Our findings is in agreement with other study where shell morphometric and radula provided a preliminary differences between *B. forskalii* and other snail species[13,14]. Identification of *B. forskalii* and subsequent infection in the species was able to provide a guide towards the control of schistosomiasis in the affected areas.

Shell characteristics remain the primary form of taxonomic information for dividing snail species into different groups and in some cases, they can be used to construct a reliable phylogenetic relationship between related species[6]. Although these shell characteristics could be modified by both intrinsic and extrinsic factors in different habitat, hence, a combination of shell characteristics with other taxonomic structure like the use of radular forms a robust approach in differentiating closely related species mostly in low and middle-income countries in the tropical and sub-tropical countries. Three species that belong to the *forskalii* group were studied, two of these species (*B. senegalensis* and *B. forskalii*) have been reported in Nigeria and the report showed that they are sympatric in their existence. *B. forskalii* were not found in temporary pools habitat[15], while *B. senegalensis* was collected from temporary pools as it was abundant in such pools during the rainfall[15]. On the other hand, in Niger, *B. forskalii* have been confused with *B. senegalensis*, where *B. senegalensis* appears to live in temporary pools for less than three months in the year, during the rainy season, while *B. forskalii* is present almost all the year in the channels of the irrigated areas[16,17].

The recent occurrence of *B. camerunensis* in Nigeria and the ability of the species to serve as an intermediate host of *schistosoma* parasite have been reported. *B. camerunensis* seems to have been transported into the country, perhaps, by a human agent or migratory birds[18]. The only means of previous identification of these species was the use of shell morphology. Although the use of shell characters is one of the methods used in differentiating these species, the radula method is more reliable as observable differences occurred. Radula method has been successfully used in other countries for the identification of these species[8]. In this study, the multi cuspid found in the marginal tooth of *bulinus* agreed with similar work in South Africa[19]. The differences in the marginal cusps observed in the *forkalii* group could be explored for use in their systematics. While the marginal cusps of *B. forskalii* were intermediate with seven cusps, the marginal cusps of *B. camerunensis* were angular with six cusps and the marginal cusps of *B. senegalensis* were angular with twelve cusps on each transverse row.

Though shell characteristics alone have been proved not to be enough for the differentiating snail intermediate host, however, it is apparent that for a cross-sectional epidemiological survey of infections in snail intermediate host[20], which always involves large sample collection, the use of morphometric and soft part is largely recommended for preliminary observable differences. Recently in Niger, infections in 87 *bulinus* snails were done. Of the total snails collected, only 36 of them were confirmed through molecular method [11], while the remaining snails were primarily identified by shell characteristics. Similar occurrence was observed in N'Djamena, Chad, where more than 800 *planorbidae* snails were collected and identified through morphological method, only a small number of the collected snail samples were confirmed through molecular method[12]. This could be attributed to the high cost of molecular methodology. Therefore, combining shell morphology and radula characteristics in the identification of snail species should be encouraged because the observable differences in the radula characteristics in combination with shell morphology will aid in the preliminary identification of snail species. Although our radulae slides were well prepared and stored, the absence of electron microscope impaired the pictorial view of our radulae.

CONCLUSION

A combination of shell and radula morphology of *bulinus* species could be adopted as part of the characteristics used for the preliminary classification of these species, as observable differences occurred between the shell and radulae of *bulinus* in the *forskalii* group. These observable differences in these *bulinus* species can ensure correct preliminary species identification which will provide a guide towards control of schistosome in low and medium-income areas. Moreso, the similarity between these three snail intermediate hosts of schistosomes need quick and cost-effective means of differentiation. Thus, this study observed differences in conchological measurements and radula characteristics of these species.

SIGNIFICANCE STATEMENT

This study discovered the radula formula of *bulinus* species in the *forskalii* group and the important radula morphology which could be adopted for providing relevant characteristics for separating these snail species. Hence, this study will assist researchers in preliminary identification of *bulinus* species in the *forskalii* group in low-income and medium-income countries.

ACKNOWLEDGMENTS

The authors acknowledge welcome trust funded Institute for Infectious Disease of Poverty (IIDP) for funding this project. We are also grateful to the Head of Communities in Yewa North Local Government Area, Ogun State.

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