Species Composition and Diversity of Terrsetrial Molluscs at Mfamosing and Odukpani, Calabar Cross River State, Nigeria

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Abstract
Determination of species composition and richness on a variety of scales is a fundamental requirement for understanding both patterns of distribution and the dynamics of local communities. Two localities within the rainforest region were selected for this study**;** Mfamosing limestone hills which is a relatively pristine upland hill and Odukpani community a low land terrain. This study was aimed at addressing the knowledge gap in species composition, diversity, indices and establishes the presence of species endemism and also to draw attention to any species that are facing threats which may warrant the need for an improved resources management and habitat protection in Calabar, Cross River state. Twenty two (22) plots measuring 400 m^2areas were surveyed in each sampled area. Sampling was carried out during the rainy season between May and September 2017, using a direct search method and leaf-litter filtering techniques for two hours. The area sampled at odukpani yielded between 2 to 64 individuals per plot while Mfamosing yielded 3 to 108 individuals. A total of 857 individuals representing 31 species in 16 genera and ten families were recorded at both Mfamosing and Odukpani. Eighteen species were recorded for Odukpani whereas 26 species were found at Mfamosing. Whittakers index revealed a beta diversity of 1.12 at both sampled stations which indicated a low homogeneity in molluscan fauna. Nonparametric species richness estimators such as Chao2 and Jacknife2 gave values of 29.12 and 30.87 for Mfamosing and Odukpani respectively while Shannon Weiner index showed that richness was higher in Mfamosing(2.82) than Odukpani (2.456). The families Streptaxidae and Urocyclidaewere the most represented as regards species richness, with Streptaxidae (35.48%) being the most abundant numerically. The most abundant species was *Trochozonitescalabaricus* contributing 14.35% of the total number of individuals. Species rank abundance also showed the presence of some rare species and five species were observed as singleton and doubleton respectively. Bray- Curtis similarity index values of*R*=0.93, P=0.34, indicated that areas studied were well separated, although not significantly different.Further studies in these communities are necessary to determine the impact of anthropogenic activitieson them and to know if they pose a serious threat that may lead to species extinction.

INTRODUCTION

In recent human history, tropical rainforest are disappearing at unprecedented rates. At these rates biological diversity are threatened with extinction(Laurence 2006). The tropical forests of lowlands in north eastern part of Cross River (Nigeria) are currently exploited through logging and commercial agriculture (Laurence 2006). The need to know the species present in various habitats within these regions has become increasingly urgent for conservation purposes. Most often limestone hills with special quality of rich molluscan diversity and high level of species endemismare under the threat of commercial exploitation of lime for cement and allied industries uses(Clements *et al.,* 2006).

Species richness typically refers to the measure of the number of different kind of organisms present in a given area or geographic region. Historically, it was used more often for a variety of conservation prioritization purposes than for endemism since intuitively the more species a region contains the more worthy it is for conservation (Prendergast *et al.,* 1993; Jennings *et al.,* 2008).Global patterns of species richness and endemism correlates highly among taxa for amphibians, reptiles, birds and mammals, but are not consistent within these taxa (Lamoreux*et al.,* 2006). In the absence of fine-scale information, areas with high levels of endemism are expected to protect not only those endemic organisms for which they were selected, but also a large diversity of organisms in general, making endemism the most widely agreed upon surrogate measure for hotspot identification (Orme*et al.,* 2005).

Species diversity refers to the measure of diversity of organisms in an ecological community which is the total number of different species in a community(Condit *et al.,* 2002). Diversity patterns are of theoretical interest, providing material to test theories of why diversity varies among sites and how species turnover increases with intersite distance (Condit *et al.,* 2002).In recent human history, tropical rainforest are disappearing at unprecedented rates. At these rates biological diversity are threatened with extinction(Laurence 2006). The tropical forests of lowlands in north eastern part of Cross River (Nigeria) are currently exploited through logging and commercial agriculture (Laurence 2006). The need to know the species present in various habitats within these regions has become increasingly urgent for conservation purposes.

MATERIALS AND METHODS

Study Area

Two localities within the rainforest region were selected for this study. Although both localities were situated within the same region, they differ contrastingly in their levels of resource utilization as one site. Mfamosing limestone hills was relatively pristine and an upland (hill), while the other Odukpani community was a low land rain forest.

Mfamosing (limestone) hill

Mfamosing lies within latitudes 5° 4'37.6"N and 5° 4'58.0"Nand longitude 8° 30.00' 9" E and 8° 31'05.7" E, average elevation above sea level being 36mranging from 34 to 38 m. Its hills are dominated by deeply weathered sedimentary rocks covered with layers of shale units of the Ekenkpon formation (Petters*et al.,* 1995). The formation is characterised by minor intercalation of marks and calcareous mudstone (Petters*et al.*, 1995).

Odukpani community

The second locality was at Odukpani in the same Cross River state in Nigeria. The site was at a heterogeneous primary forest in a lime-stone cave. It is situated between latitude 5˚ 07′ 0″ N and longitude 8˚24′ 0″ E.

Climate

The hills at Mfamosing are humid tropical areas with well-marked rainy and dry seasons. The former starts in March and ends in October with an annual rainfall between 2000 mm to 2500 mm (Reijers and Petters, 2003). The latter starts in November and ends in February with an annual rainfall between 1200 mm and 1600 mm. Relative humidity remains high throughout the year, not greater than 65 percent at noon in any month of the year (Reijers and Petters, 2003).

Soil texture and geology

The top soil is rich in nutrients from decomposition of leaf litters and dead wood (Reijers and Petters, 2003).

Table 1: Geographic information and conservational statuses of the two selected localities

Study areas	Latitude	Longitude	Area (km^2)	Condition

Mfamosing	5˚ 04′ 37.6″ N	8˚ 30′ 009″ E		highly exploited
	5˚ 04′ 58.0″ N	8˚ 31′ 05.7″ E	36.62	highly exploited
Odukpani	5˚ 07′ 0″ N	8˚24′ 0″ E	85.32	protected from exploitation

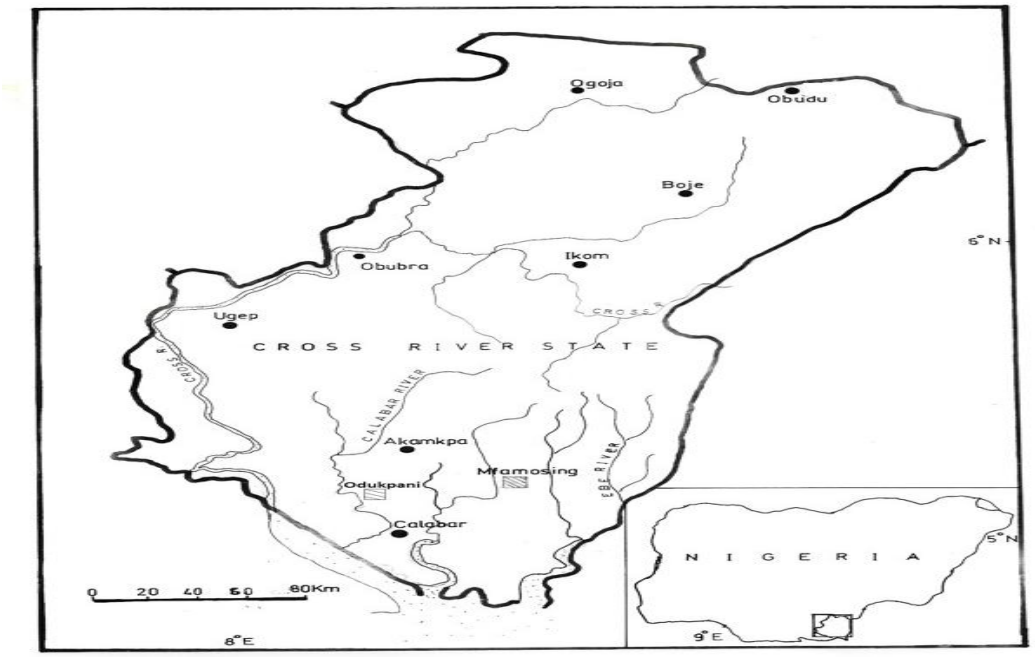


Figure 1. Study localities where samples were collected, all within Cross River state, in Nigeria

Sampling Method

Forests at Mfamosing and Odukpani were sampled during the rainy season (between May and September 2017) by a combination of direct search for 2 hrs and leaf litter sieving techniques as described by Tattersfield (1996).

Direct searching involves examining randomly selected plots of 400 m^2 all in areas all that are potentially molluscan microhabitats that could be accessed, like deep litter beds, rocks, bark and spaces between buttress roots of large trees and dead-decayed logs. Sampling areas were marked out using pegs and ropes. Each plot was intensely sampled for snails and slugs during two man-hours (i.e. two searchers active for an hour). Additionally litres of leaf litters and top-soil were

further collected from ten randomly selected sites of 1 m^2 within each plot. Both leaf litter samples and top-soils are sieved with a 0.75 mm mesh width in the field and collected into polythene bags for transportation and onward assessment in the laboratory. Samples were dried before exhaustively searching for any mollusc therein. All snails, slugs and shell fragments encountered in the *in situ* collections were preserved in 70% ethanol. The specimens obtained were subjected to further anatomical studies. To avoid overestimation of species richness, juvenile and or broken shells were excluded from the analysis, thus only underestimation was likely.

Data analysis

Species composition was tabulated and molluscs counts were summarized using appropriate statistics and tools. The diversity indices were calculated using PAST™. Indices such as alpha diversity, species richness (S) and Whittaker's index (I) [which the total number species recorded (S) divide by mean number of species per site (α), providing a measure of diversity difference between sites (Schilthuizen and Rutjes, 2001) were computed. Estimation of true diversity was carried out by calculating S using the Chao 2 and second-order Jackknife estimators in the program Estimates 7.5 (Colwell 2006). Sample-based rarefaction curves were used to produce a smooth curve that estimates the number of species that would be observed for any smaller number of samples, assuming random mixing of sample order (Colwell and Coddington, 1994; Gotelli and Colwell 2001). Sampling intensity was defined as the ratio of individuals to species while inventory completeness was defined as the percentage of observed number of species over the expected number of species as estimated by Chao2 or Jackknife2 (Coddington*et al.*,1996; Soberon*et al.*, 2007).

Data analyses were performed using the PAST software (Hammer *et al.*, 2001). Hierarchical Clustering (Bray-Curtis similarity measure) was used to identify natural groupings among the sampled points according to similarities in species abundance. Cluster analysis is the arrangement of samples into groups (clusters), so that samples within the same cluster are more similar to each other than to samples from different clusters (Gauch and Whittaker, 1981).

One-way Analysis of Similarity (ANOSIM:Clarke,1993) was used to test for statistical differences in species composition between clusters, Similarity Percentage (SIMPER:Clarke, 1993) analysis (using the Bray-Curtis similarity measure), was used to assess the taxa

responsible for any observed difference between groups of samples as previously documented (Clarke, 1993).

RESULTS

In total, 857 individuals which comprise 31 species in 16 genera that belong to ten families were recorded from 22 separately sampled stations in both Mfamosing andOdukpani. Each sampled station yielded between 2 to 64 individuals per plot in Odukpani (mean = 21± 16.29), while a range of 3 to 108 was recorded for Mfamosing (mean = 27.65±34.12, Table 1). Twenty-six (26) species were found in Mfamosing and eighteen (18) species in Odukpani (Table 1). Figure 3 showed that *Lamistes* sp. had the highest number of species in Mfamosing while *Trochozonitescalabaricus* had a high number of individuals occurring in both Mfamosing and Odukpani. *Quickia* sp. had the lowest number of species in Mfamosing and Odukpani with *Gulellapoense*soccured the most in Mfamosing.

Two families Streptaxidae and Urocyclidae were richer and abundant in both Mfamosing and Odukpani. The family Streptaxidae was represented by eleven species (*Gulellagemma, G. poenses, G. obani, G. opobonesis, G. jonkindi, G. germaini, G. ptychotrema*sp., *Ptychotremaameyi*and *P.martensi, streptosile*sp.) with 174 individuals accounting for 35.48% of total species abundance and Urocyclidae represented by five species (*Trochozonitescalabaricus, T. sutusralis, T. bifilaris, T. theeli*and *T. lystrix*) and 254 individuals with a total species abundance of 16.13%. The genus with the most individual in Mfamosing and Odukpani was *Gulella* with six species namely *Gulellagemma, G. poenses, G. obani, G. opobonesis, G. jonkindi*and*G. Germaini*(Figure 4).

The Family Streptaxidae was the most abundant in Mfamosing with an abundance of 26.92%, while Family Ailyidae, Ampularidae, Cyclophoridae, Euconulidae, Succineidae and Thiaridae were the least abundant with 3.85% recorded for each family (Figure 4; Table 2). The Urocyclidae were observed as the most abundant family in Odukpani with a percentage of 23.41% and Thiaridae with an abundance of 0.0% was the least abundant (Table 2).The most abundant species in Mfamosing was *Lamistes*sp. (19.18%) with 108 individuals belonging to the Family Ampularidae and while in Odukpani, *Trochozonitecalabaris* (21.77%) was the most abundant with 123 individuals, which belong to Succineidae (Table 3).

Using Kruskal-Wallis Test (H= 0.4401,*p*<0.4352) there were variation in species richness (S) amongst the plots in both sampled plots. The number of species collected was not significantly different between the plots in both sampled sites. Species Richness and mean number of species were significantly higher in Mfamosing (H= 05932, p= 0.4352than Odukpani (H= 0.6088, p= 0.4352) sampled plots. There was a considerable variation in species richness (S) amongst the plots in each reserve but the species diversity (Shannon-Weiner) was higher in Mfamosing with (2.82) and Odukpani (2.456).

There was a considerable variation within habitat (Beta) diversity. The rarefaction curve (Figure 6) reached an asymptote and showed variation in species richness when sampling stopped, after estimation of species richness based on Chao29.12 and Jacknife30.87 respectively. This indicated that species richness was reduced and an increase in sample location and sampling efforts may not increase species richness. Sample intensity (ratio of individuals to species) was 19.47.1 while inventory completeness was 85.02% using Chao2 estimator, while Whittakers index calculated as the overall species richness S = 31 divided by the mean number of species per plot $\alpha = 27.65$ was 1.12 in Mfamosing and Odukpani, which indicates a low homogeneity in species composition among sampled sites.

Analysis using Bray Curtis index revealed significant differences in species composition between the two clusters. Moreover, the dendrogram of similarity divided the plots into three distinct groups at 50% similarity. Analysis of similarity (ANOSIM) using Bray Curtis similarity index gave values of R = 0.1137, P = 0.0603, indicating that the sites were well separated, although not significantly different.

A common way to display abundance distribution is the rank order of species (Figure5). The species on the first ranks have the highest abundance (common species) and the tail of the curve consists of species, which were encountered only in a few samples or with few individuals (few rare species encountered in less than 2 samples or less individuals). The rank abundance curve for the Mfamosing lies above the other including most species and therefore displaying the longest tail followed by Odukpani sampled area. Species rarefaction analysis showed variation in species richness among sites (Figure 4).

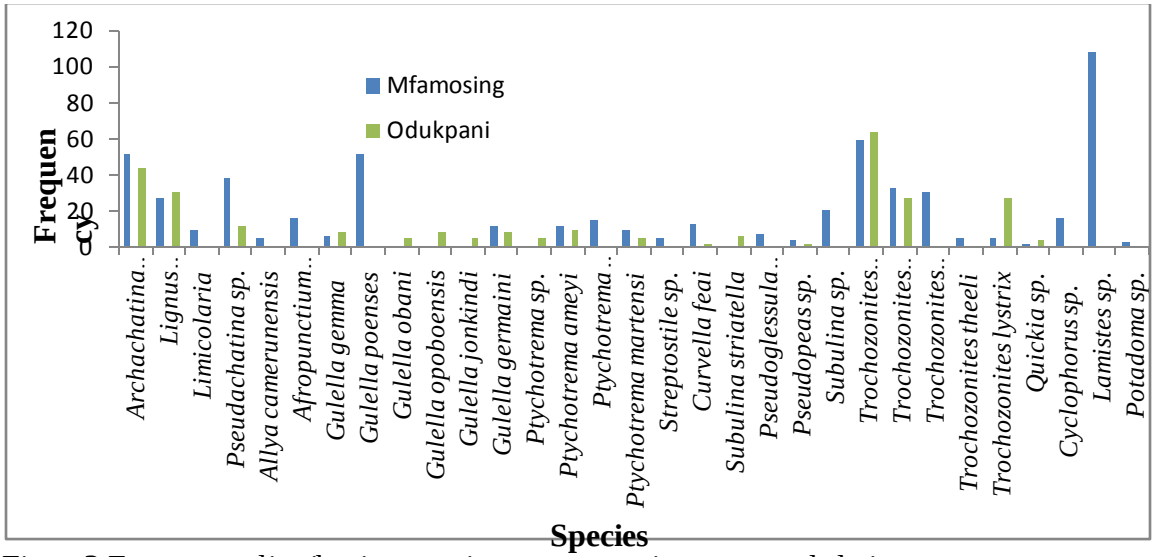


Figure**3** Frequency distribution species per genera in two sampled sites

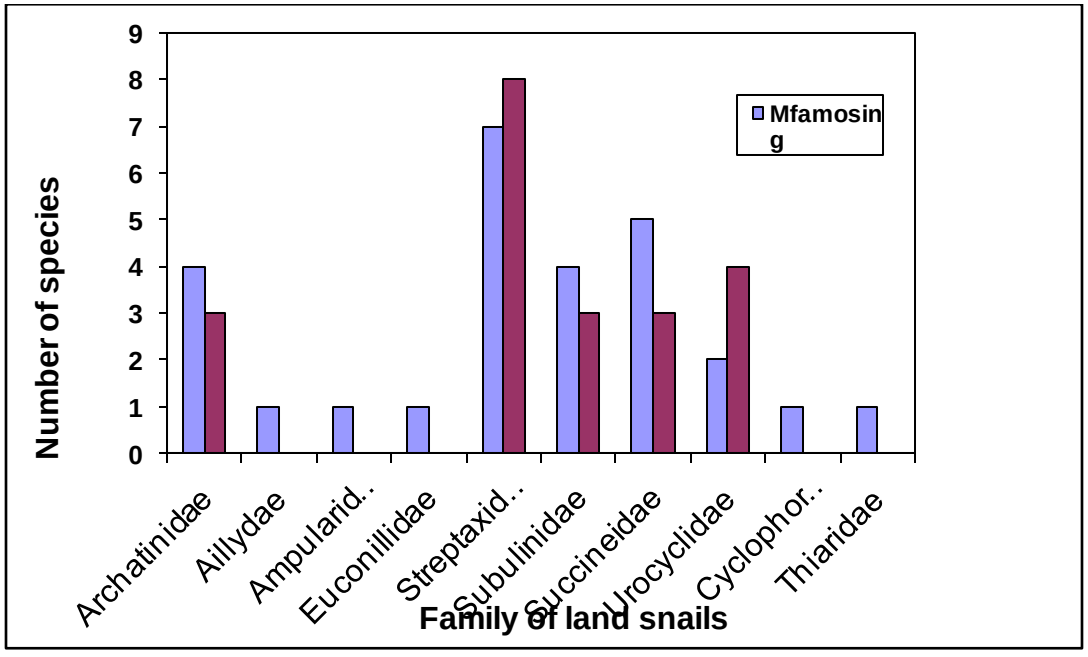


Figure 4. Individuals per family across the two sampled sites

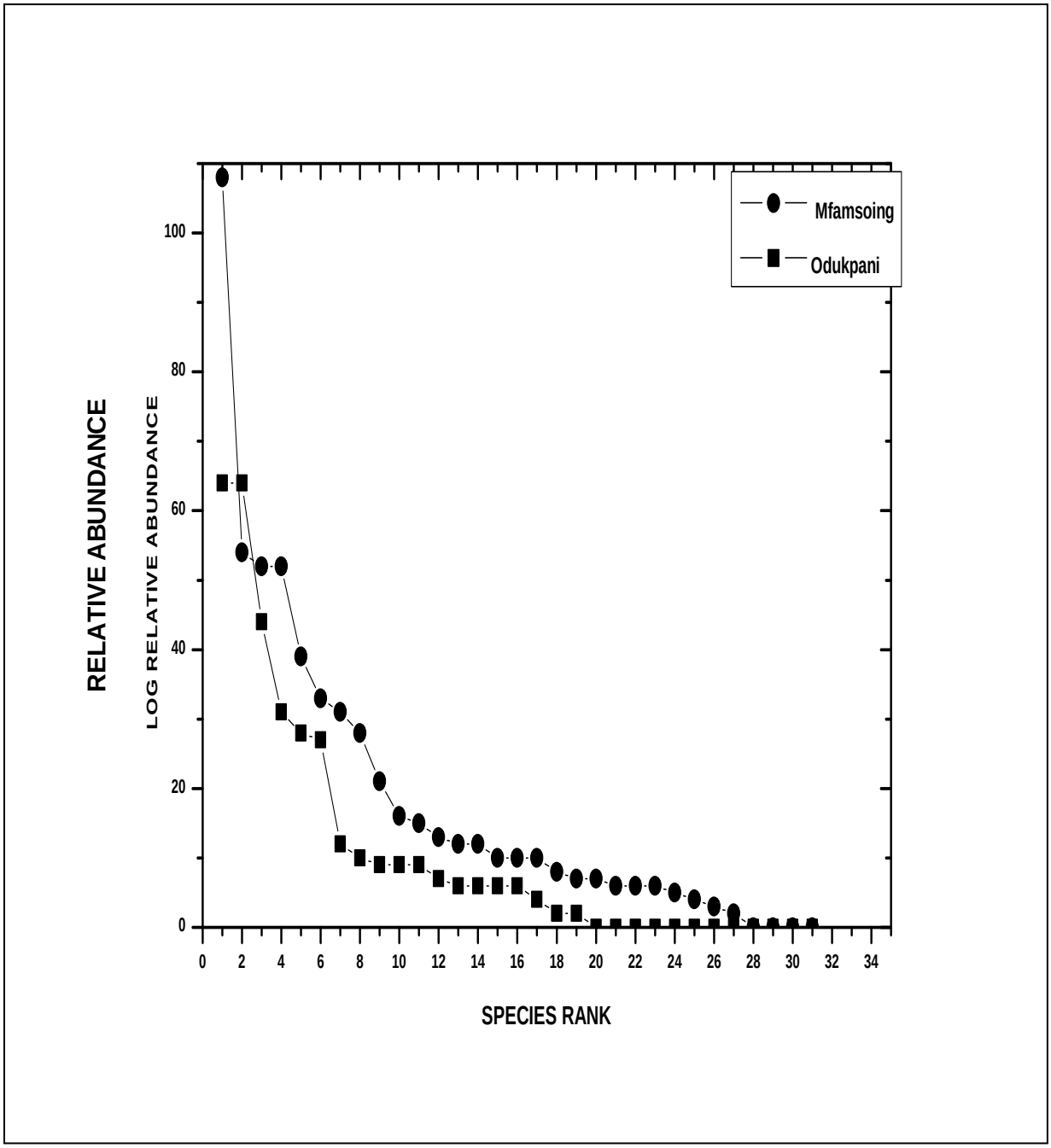


Figure 5 Species Rank abundance distribution for land molluscs in Mfamosing and Odukpani sampled sites

Table 2 Percentage abundance of species and individuals for each sampled plots

Families	Mfamosing		Odukpani	
	% Species	% Individuals	% Species	% Individuals
Ailyidae	3.85	0.00	1.02	0.00
Ampularidae	3.85	0.00	19.01	0.00
Archatinidae	15.38	16.67	22.72	30.85
Cyclophoridae	3.85	0.00	2.50	0.00
Euconulidae	3.85	0.00	3.00	0.00
Streptaxidae	26.92	44.44	20.00	21.63
Subulinidae	15.38	16.67	8.01	3.90
Succineidae	3.85	5.56	0.31	1.42
Thiaridae	3.85	0.00	0.02	0.00
Urocyclidae	19.23	16.67	23.41	42.10
Total	100.00	100.00	100.00	100.00

Table 3 Counts and relative percentage abundance of species and individuals sampled in the survey.

Family	Number (percentage)	
	Species	Individuals
Ailyidae	1 (3.23)	6 (0.70)
Ampularidae	1(3.23)	108(12.60)
Archatinidae	4(12.90)	216(25.20)
Cyclophoridae	1(3.23)	16(1.87)
Euconulidae	1(3.23)	17(1.98)
Streptaxidae	11(35.48)	174(20.30)
Subulinidae	5(16.13)	57(6.65)
Succineidae	1(3.23)	254(29.64)
Thiaridae	1(3.23)	3(0.35)
Urocyclidae	5(16.13)	6(0.70)
Total	31(100)	857(100)

DISCUSSION

The distribution and diversity of land snails in different parts of Nigeria, it has identified as well as established species in Mfamosing and Odukpani forests; however the findings herein was limited as it did not evaluate the impacts of anthropogenic activities on the communities nor did it evaluate the level of threat to species richness and diversity.The study also established species diversity/abundance and beta diversity which are comparable to some earlier studies in the country (Oke and Alohan, 2002; Oke and Alohan, 2004; Oke and Alohan, 2006; Oke*et al.*, 2007; Oke*et al.*, 2007, Ogebeide*et al.*, 2018) and beyond (Tattersfield*et al.*, 2001; Daget, 2003; Fontaine *et al.*, 2007;Wronski*et al.*, 2014).

The species richness and diversity observed at Mfamosing and Odukpani in Calabar, unveiled 857 individuals of 31 species from ten families. This was lower than that recorded from other parts of Nigeria. For instance, Oke and Alohan (2002) recorded 15 species above that at Okomu National Park in Edo State, while a later study by Oke and Alohan (2004) recorded four species at Oban hills in Cross River State. Still fewer species were reckoned in this study when compared with reports by Oke*et al.* (2000) which was higher than by 2 species at Erin-Ijesha hills, Osun state, then in another study, Oke and Alohan (2006) additional 7 species were recorded at Ehor, Edo state, compared to the findings of this current study. Varying numbers of species have been recorded elsewhere (Oke and Ugiagbe 2007; Oke et al. 2007, 2008; Oke and Choker 2009), with the latest being at Omo forest reserve at Ogun state, where 28 species were encountered (Oke 2013). Although an earlier survey at Odukpani had 21 species(Oke*et al.*, 2007), in this current study only eighteen species were observed. Nonetheless the number of species in this study was observed to be high for Mfamosing and Odukpani. The relatively short list of molluscs species in Nigeria and other parts of West Africa, suggests that many species are yet to be fully described and the taxonomic status of some remain unclear.

The species diversity of both Mfamosing and Odukpani was fourteen species lower than those recorded by Tattersfield*et al* (2001) (37 species) in Kenya, Daget (2003) at Mount Nimba in Cote d'ivore, Fontaine *et al* (2007) (43 species) in Garbon, Wronski*et al* (2014) (37species) in Equatorial Guinea. However the number of species recorded in this study was higher than those of Memel *et al* (2009) (twenty-three species) in the Pare National du Banco and Prakasa*et al* (2013) in Chittor district of Andhra Pradesh (India) (twenty-one species). The low number of

species diversity in Mfamosing and Odukpani could be as a result of the number of sampling plots in the study area. Most observed species were large and conspicuous, thus small or tiny specimens could have been overlooked which makes it possible that not all species present in the plots were detected.

There was considerable variation within habitat (Beta) diversity, which reflected considerable heterogeneity in molluscan fauna inMfamosing and Odukpani respectively. The rarefaction curve reached an asymptote and showed variation in species richness when sampling stopped and was in contrast to the studies by Oke and Alohan (2004); Oke*et al* (2007); Oke*et al* (2008); Oke and Choker (2009); Oke (2013). This indicates that even with an increase in sample location and sampling efforts species richness will not change. Variations in species richness and diversity within plots and habitats showed differences in faunal compositions and was comparable to the works of Oke (2008) andOke (2013), but did not conform with that of Oke and Chokor (2009).

Species richness was estimated based on Chao 2 and Jackknife 2 (Colwell, 2006) for Mfamosing and Odukpani combined and gave values of 29.12 and 30.87 species respectively. This did not conform to the studies of Oke*et al* (2008) (25 and 26 species); Oke and Choker (2009) (3.1 and 2.72 species), while higher Oke and Alohan (2006) observed 57 and 63 species respectively. Bray Curtis index for similarity in species composition between the two clusters (Mfamosing and Odukpani), produced R and P values of 0.11 and 0.06 respectively. This indicated that the sites were well heterogeneous, but not significantly different from each other. Given that the species accumulation curve almost reached an asymptote at the time the survey was ended, it could mean that parts of the sampling sites not visited may be plausibly inhabited by a few more species despite the higher inventory of completeness (85.02%). A complete inventory of species could be have been achieved if more samples were collected, as many of the common species found in other parts of Nigeria were missed during sampling. Some of these species were found in very low densities or may be locally extinct in the sampled areas.

Four species were found with less than 12 individuals, this suggest that more rare species remain to be discovered as highlighted previously from species accummulation curve, and these rare species are often at a greater risk of local and regional extinction. Given these outcomes Mfamosing and Odukpanishould be targeted for conservation as they contain unique species

within the rainforest ecosystem in Southern Nigeria. Mfamosingwas richer than Odukpani, with a significance difference between the sampled sites in terms of species composition and abundance.

Malacofauna was dominated by three families which are the most species-rich and abundant in both Mfamsoing and Odukpani. These arefamily Streptaxidae (carnivorious) represented by 11 species, Subulinidae (herbivorous) represented by six species and Urocyclidae (detritivorious) represented by five species. Surprisingly, the Subulinidae dominated the fauna in species richness with 254 individuals contributing to 29.64% of the total number of individuals in the sampled sites. The high abundance of Streptaxidae, Subulinidae and Urocyclidae makes the sampling area a unique site for molluscan conservation. Interesting, *Trochozonitescalabaricus* was the most abundant species, which makes it unique in that region, where it is present in both Mfamosing and Odukpani contributing about 14.35% of the total number of individuals collected. The species has been uncommonly found previously in low number elsewhere in the country (Oke and Alohan, 2006; Oke and Alohan, 2009).

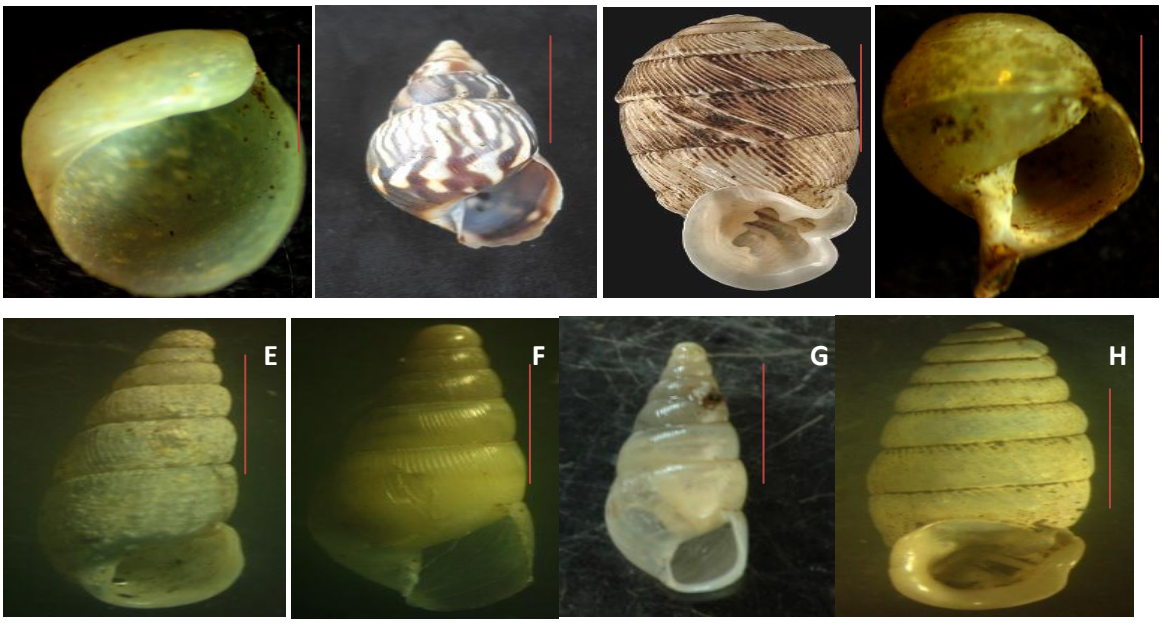
Housing/urban development was assumed to be of no significant risk as these were far off from Mfamosing and Odukpani forests. A total of 31 species were recorded for both Mfamosingand Odukpani forests in this study, and show a reduction in species. This could be due to habitat modification resulting from anthropogenic activities.

At the moment, forest reserves in Nigeria are exposed to uncontrolled exploitation of timber and wildlife poaching, land conversion to farming, bushfire, grazing, and exploitation of non-timber products together with direct and indirect impacts from extractive industries (Oke and Olisa-Emodoh, 1998; Oke, 2013). Limestone hills have been recently highlighted as a vulnerable ecosystem in the world due to surrounding forest degradation and quarrying activities (Ministry of Natural Resources and Environment Malaysia, 2016. The effects of disturbance in the forest are manifested easily at the forest floor and the species community therein (Ogbeide*et al.,* 2018). The preservation of biodiversity in tropical rainforest requires high-quality data and efficient methods for prioritizing species and sites for conservation (Ogbeide*et al.,* 2018). Land snails are often not considered in the establishment and selection of reserves and the checklist or systematics of the invertebrate species (Ponder, 1997; Oke, 2013). Due to the on-going loss of land snail biodiversity without any abatement to reverse it and give some level of protection for

species in these localities, an urgent assessment for land snail diversity is necessary.Further studies will help to determine the magnitude of species lost due to forest degradation or destruction, and also help to pin point critical sites for long-term conservation of snail fauna in Nigeria.

CONCULUSION

This study is the first inventory to compare the land molluscs in Mfamosing and Odukpaniand has provided basic data on heterogeneity and species abundance. It has provided information highlighting the species of identified snail species in Mfamosing and Odukpani which were relatively rich with Mfamosing being the richer. Since both sampled areas are not protected rainforest and with Mfamosing forest rich in limestone and Odukpani forest being used for farming both are subject to anthropogenic activities such as quarrying, bush clearing and burning, which may result in habitat loss and reduced snail diversity. Further studies in these communities are necessary to determine the impact of anthropogenic activitieson them and to know if they pose a serious threat that may lead to species extinction. This will help to acertain the extent to which these activities have changed, modified or destroyed the habitat and the possible measures needed to correct them as well as the best ways to achieve the desired results.



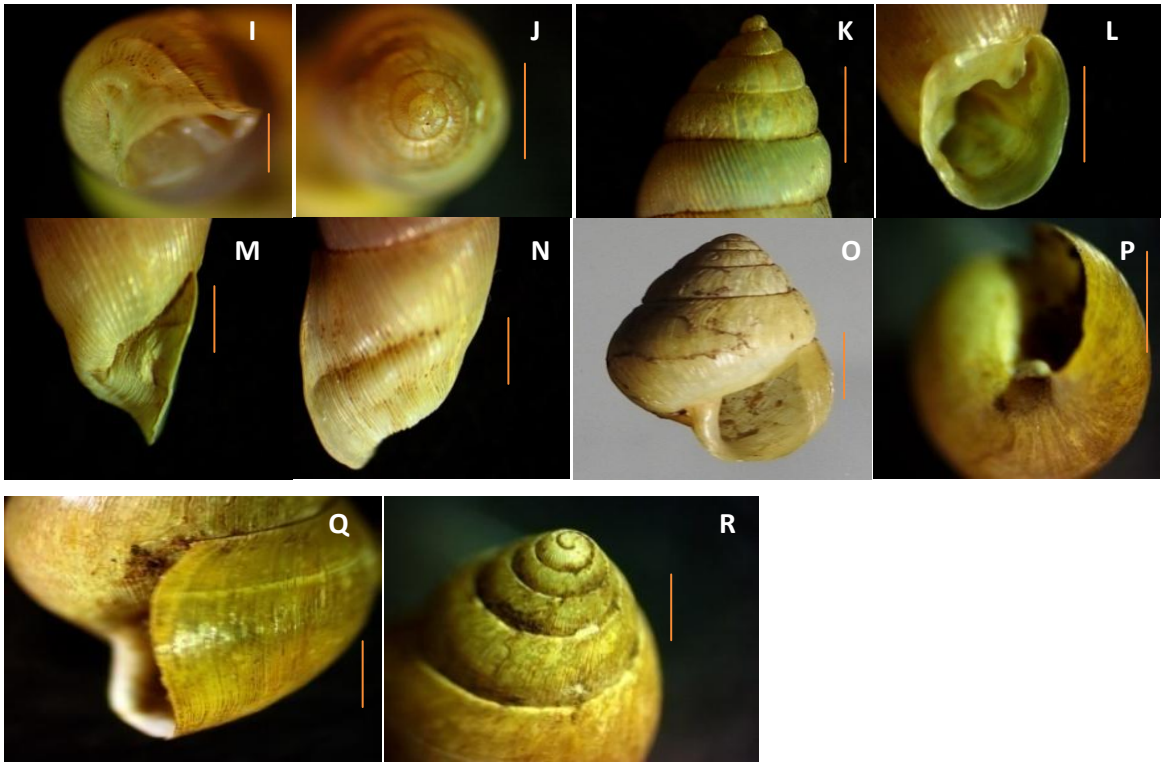
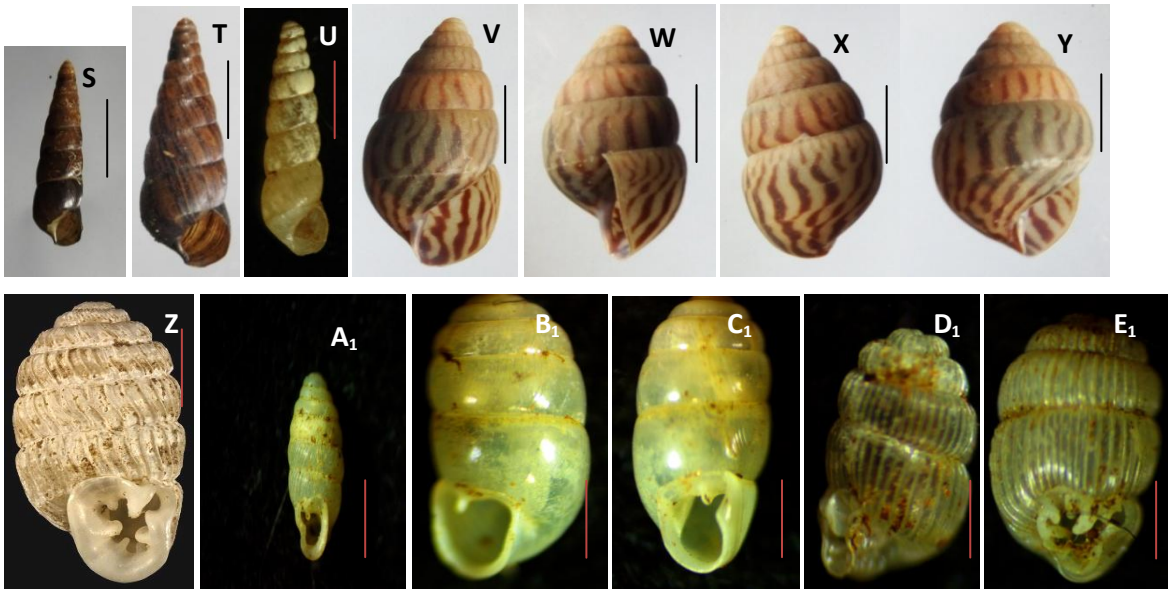


Plate: (A) Alliyae*camerunensis*H 4.5mm;(B) *lignus* sp. H 46mm; (C)*Ptychotremacollumelaris* H 9.8mm; (D) *Cyclophorus* sp.H 5.5mm;(E-G) *Subulona*or *Subulina*sp.H 23mm; (H-N) *Ptychotremacollumelaris*H 15.8mm;(O-R) *Curvella* sp.H 5.6mm. *Scale bars* = 5mm in (Figs A-D) and 1mm in (Figs E-R)



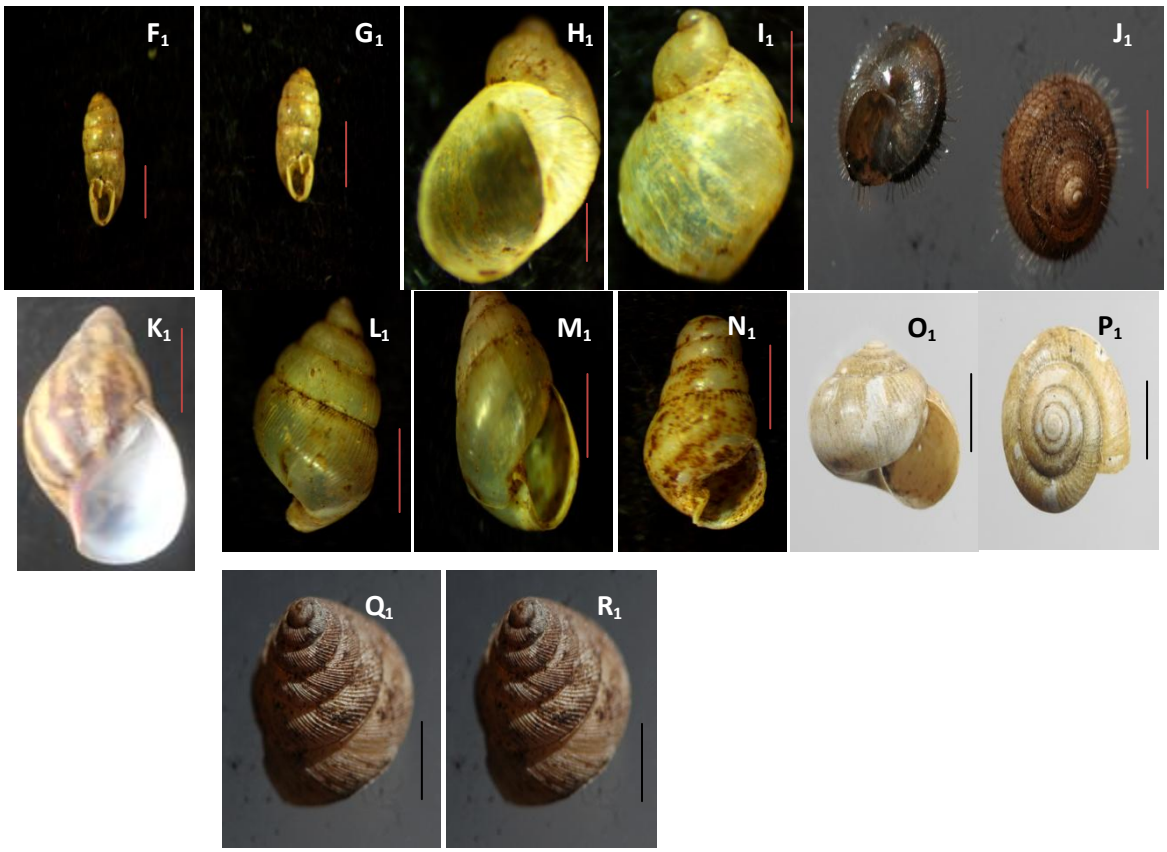


Plate: (S-U) ***Streptostele*** **sp1H 21.5mm; (V-Y)** ***Limicolaria*** **sp. H 51mm; (Z)*Gulella*spH 15.4mm; (A_1)** ***Gulellajonkindi*****H 4.08mm;(B_1-C_1)*Gulellagemma*H 4mm;(D_1-E_1)** ***Gulellaopoboensis*****,H 4.7mm;(F_1-G_1)** ***Gulellaobani*****H 3.5mm;(H_1-I_1)** ***Quickia*** **sp.H 4mm;(J_1)** ***Trochozoniteslystrix*****(Urocyclidae),H 6.1mm; (K_1-M_1)*Achatinidaemarginata*H 155mm;(N_1)** ***Pseudopeas*** **sp.H 11.3mm;(O_1-P_1)** ***Thapsia*** **sp. (Urocyclidae)H 8.5mm; (Q_1-R_1)*Trochozonites*** **sp. (Urocyclidae)H 6.1mm.*Scale bars* = 10mm in (Figs S-U, Q_1-**

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