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Research Article

Regression Analysis of Shell Morphology of a Few of Endemic Aquatic and Terrestrial Gastropods as a Prelude to their Conservation Strategy

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Abstract

Mollusks, being the second largest assemblage among invertebrates, could be used as the efficient models in prediction of shell size and shape heterogeneity by traditional morphometric analysis. The study of aquatic and terrestrial gastropods in traditional morphometric was limited in Indian scenario. Thus in our research study, we utilize traditional morphometric as a tool in estimating the correlation coefficient and multivariate regression analysis to establish the linear relation between body weight and height and breadth of shell of each species of gastropods collected from different locations in the present study. A significant correlation was observed between these variables chosen for aquatic and terrestrial snails. The observation of body weights was found in synchrony with the predicted body weights calculated through regression analysis which determined coefficient of determination (r^2) with standard error for ten snails from each family of the chosen gastropods. In our study, ANNOVA analysis was also done which showed high significant F value for the chosen samples. The output of the residuals was evaluated using the multiple regression equation $y = mX_1 + mX_2$ where X_1 is the height of the shell and X_2 is the breadth of the shell.

Keywords: Gastropods; Morphometry; Correlation; ANNOVA; Regression Analysis

Introduction

The mollusks are soft bodied and unregimented invertebrates. They occur in various habitats and could be classified into aquatic (freshwater, marine) and terrestrial forms. Mollusks shells are widely studied due to their shell texture, coiling patterns (sinistral and dextral) with geometrical functions [1]. The shell morphology can be described as protoconch which forms the apex of the spire, the coils and whorls develop as the snail reaches adulthood. The overall shape depends on the height and width ratio thus shells of snails are grouped into oblong, globose and depressed. The sculptured shell consists of ridges which could be longitudinal or transverse. Traditional morphometrics could be used as a multivariate statistical analytical tool to study shape and other variable components to determine the heterogeneity of snails' population. The snail's morphology analysis implies to mark its anatomical and biological significance which means to define adaptable shapes [2]. Two breeds of snails *A. marginata* and *A. achatina* studied for phenotypic correlations and body weights were predicted and used as morphometric traits in Nigeria. The correlation coefficient of morphometric traits and generation of predictive live body weights of snails were estimated [2]. In a different study where in morphometric traits (hatchling body shell length and body shell width) was predicted which showed a strong positive and significant correlation [3]. Shell morphometric variation was analyzed based on size and shape components by the method of principal component analysis for the land snail *Notodiscus hookeri* founded in subantartic islands [4]. It was observed largely that intraspecific variation exists in shell morphology. Further, land snails' shells could be used to provide relevant morphometric data which could be used in taxonomy in phylogenetic inference as the analyses carried entire record of ontogeny [4]. A prior work had observed that the traditional approaches by the simple measurements ratio of width to height [5] to create a 'size/shape space'. Also, there were some prior reports of morphometric analysis in variations of shell and operculum which was observed in viviparid snail, *Cipangopaludina chinensis* in Taiwan. In their study the allometric shell growth pattern was estimated using linear regression analysis where the shell and operculum characters were measured through multidimensional scaling and canonical analysis (CDA). Also, snails *Lymnaea* and *Bellamya* were used to measure the morphometric parameters namely shell width, aperture length, aperture width and total body weight which could determine the animal shell length through phenotypic correlation and multiple regression analysis [6]. In these studies, shell size and shape heterogeneity among samples were determined. From the above literature surveys it was observed that little studies are available on traditional morphometrics on species viz., *Pila globosa, Pila virens, Pomacea diffusa, Bellamya benghalensis, Nassarius olivaceus, Neritapolita, Indoplanorbis exustus, Achatina fulica* from different families of India. Thus in our research study, we utilize traditional morphometrics as a tool in estimating the correlation coefficient and multivariate regression analysis to establish the linear relation between body weight and height and breadth of shell of each species of gastropods collected from different locations in the present study.

Material and Methods

Taxon sampling

The species collected were from six families namely *Ampullariidae* (3 species), *Viviparidae*, (1 species), *Neritidae* (1 species), *Nassariidae* (1 species), *Planorbidae* (1 species) and *Achatinidae* (1 species). Out of the chosen families, *Ampullariidae*, *Viviparidae*, and *Planorbidae* were freshwater families. *Achatinidae* is a terrestrial gastropod. Families *Neritidae* and *Nassariidae* were marine gastropods. The taxa sampling done was represented in table 1. The snails sampling was carried over between months of June to August, also the pre-monsoon period of October and November for freshwater and marine gastropods.

Sample maintenance prior to study

The snails were sorted soon after bringing to the laboratory based on the regions. The collected snails were brought in polythene bags with slight amount of water inside the bag and maintained proper ventilation to prevent from dying. Each species comprising 10 sample size was taken and maintained in the laboratory. The samples of snails procured after collection was properly washed to remove the dirt and greenish algae. They were kept in watered-tubs fed with lettuce and green leaves. The observation of snails was done for a week to check for the mortality rates. The snails were sorted based on the morphological observation viz., body weight of the snails, color and shell texture.

Sl. No.	Family	Species	Habitat	Location (India)	Lat-Long.
1	*Ampullariidae*	*Pila globosa*	Freshwater	Berhampore (WB)	24.09ºN, 88.26ºE
2	*Ampullariidae*	*Pila globosa*	Freshwater	Kolkata (WB)	22.53ºN, 88.39ºE
3	*Ampullariidae*	*Pila globosa*	Freshwater	Birbhum (WB)	23.84º N, 87.61ºE
4	*Ampullariidae*	*Pila globosa*	Freshwater	Kokhrajhar (Assam)	26.14ºN, 91.77ºE
5	*Ampullariidae*	*Pila virens*	Freshwater	Vijaywada (A.P)	16.50ºN 80.64ºE
6	*Viviparidae*	*Bellamya benghalensis*	Freshwater	Berhampore (WB)	24.09ºN 88.26ºE
7	*Viviparidae*	*Bellamya benghalensis*	Freshwater	Bhubaneshwar (Odisha)	20.27ºN, 85.84ºE
8	*Ampullariidae*	*Pomacea diffusa*	Freshwater	DumDum (WB)	22º.37'N, 88º25'E
9.	*Ampullariidae*	*Pomacea diffusa*	Freshwater	Kolkata (Wb)	22.53ºN, 88.39ºE
10.	*Ampullariidae*	*Pomacea diffusa*	Freshwater	Kalyani (Wb)	22º.58'30ºN, 88º.26'04'E
11.	*Ampullariidae*	*Pomacea diffusa*	Freshwater	Berhampore (Wb)	24.09º N, 88.26 ºE
12.	*Achatinidae*	*Achatina fulica*	Terrestrial	Bhubaneshwar (Odisha)	20.27ºN, 85.84ºE
13.	*Achatinidae*	*Achatina fulica*	Terrestrial	Berhampore (WB)	24.09ºN, 88.26ºE
14.	*Neritidae*	*Nassarius olivaceus*	Marine	Diglipur, South Andaman	13º 16'N, 93º 00' E
15.	*Neritidae*	*Nerita polita*	Marine	Diglipur, South Andaman	13º 16'N, 93º 00' E
16.	*Planorbidae*	*Indoplanorbis exustus*	Freshwater	Diglipur, South Andaman	13º 16'N, 93º 00' E

Table 1: *Gastropoda* species chosen in the present study along with their assigned family, habitat and location.

Taxonomic identification of the species

For, a proper classification it was mandatory that the snails must be identified. Hence, the collected freshwater snails kept in a plastic jar labeled properly filled with water and then was given to the Molluscan section of Zoological Survey of India (ZSI), Kolkata for identification. For terrestrial snails, they were put on a plastic jar labeled and then given in ZSI for identification.

Habitat of taxon sampled in the present study

According to Bouchet and Rocroi 2005 [7] classification, the class *Gastropoda* comprises of 24 clades, 4 informal group and 129 super families and reports of 409 families are mentioned.

Traditional morphometric analysis

The parameters measured in traditional morphometrics were body weight (BW) of the snails, the linear distances which included height of the shell(H), breadth of the shell(B), length of operculum (LO) and breadth of the operculum (BO). The shell measurements of each species were determined by Vernier calipers. Statistical analyses done for morphometrics study were Correlation, Analysis of Variance (ANNOVA) and Regression analysis. These analysis were done through software package of Microsoft Excel 2010. Coefficient of determination (r^2) showed the degree of association between body weight of the snail, height of the snail and breadth of the snail.

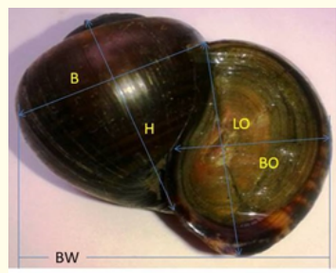

Figure 1: *Pila globosa* representing morphometric parameters to measure.

H: Height of Shell; B: Breadth of Shell; LO: Length of Operculum; BO: Breadth of Operculum; BW: Total body weight of the snail.

Results

Pila species (Ampullariidae)	Correlation Coefficient (r)
Pilaglobosa (Berhampore) and *Pilaglobosa* (Kolkata)	0.9422
Pilaglobosa (Berhampore) and *Pilaglobosa* (Birbhum)	0.7628
Pilaglobosa (Berhampore) and *Pilaglobosa* (Assam)	- 0.3260
Pilavirens (Vijaywada) and *Pilaglobosa* (Kolkata)	0.4037
Pilavirens (Vijaywada) and *Pilaglobosa* (Birbhum)	0.3119
Pilavirens (Vijaywada) and *Pilaglobosa* (Assam)	0.7534
Pilaglobosa (Kolkata) and *Pilaglobosa* (Birbhum)	0.7314
Pilaglobosa (Kolkata) and *Pilaglobosa* (Assam)	- 0.3838
Pilaglobosa (Birbhum) and *Pilaglobosa* (Assam)	-0.0418

Table 2: Correlation Coefficient between Body weights of *Pila* species from different locations.

Species (Families)	Correlation Coefficient (r)
Pilaglobosa (Berhampore) and *Pomaceadiffusa* (Dumdum)	-0.7048
Pilaglobosa (Berhampore) and *Bellamyabenghalensis* (Bhp)	-0.1060
Pilaglobosa (Berhampore) and *Nassariusolivaceus* (S. Andaman)	- 0.4855
Pilaglobosa (Berhampore) and *Neritapolita* (S. Andaman)	-0.7900
Pilaglobosa (Berhampore) and *Indoplanorbisexustus* (S. Andaman)	0.4820
Pilaglobosa (Berhampore) and *Achatinafulica* (Berhampore)	-0.6558

Table 3: Correlation Coefficient between Body weights of *Pila* (Ampullariidae), *Pomacea* (Ampullariidae), *Bellamya,* (*Viviparidae*), *Nassarius* (*Nassaridae*), *Nerita* (*Neritidae*), *Indoplanorbis*, (*Planorbidae*) and *Achatina* (*Achatinidae*).

Pila species (Ampullariidae)	Correlation Coefficient (r)
Pilaglobosa (Berhampore) and *Pilavirens* (Vij)	-0.5841
Pilaglobosa (Berhampore) and *Pilaglobosa* (Kolkata)	0.1167
Pilaglobosa (Berhampore) and *Pilaglobosa* (Birbhum)	0.0976
Pilaglobosa (Berhampore) and *Pilaglobosa* (Assam)	0.2873
Pilavirens (Vijaywada) and *Pilaglobosa* (Kolkata)	0.1403
Pilavirens (Vijaywada) and *Pilaglobosa* (Birbhum)	0.2818
Pilavirens (Vijaywada) and *Pilaglobosa* (Assam)	- 0.5244
Pilaglobosa (Kolkata) and *Pilaglobosa* (Birbhum)	0.5003
Pilaglobosa (Kolkata) and *Pilaglobosa* (Assam)	-0.2642
Pilaglobosa (Birbhum) and *Pilaglobosa* (Assam)	-0.2045

Table 4: Correlation Coefficient between measured Length of Operculum (mm) of *Pila* species from different locations.

Pila species (Ampullariidae)	Correlation Coefficient (r)
Pilaglobosa (Berhampore) and *Pilavirens* (Vijaywada)	0.3057
Pilaglobosa (Berhampore) and *Pilaglobosa* (Kolkata)	0.3438
Pilaglobosa (Berhampore) and *Pilaglobosa* (Birbhum)	0.2524
Pilaglobosa (Berhampore) and *Pilaglobosa* (Assam)	-0.0783
Pilavirens (Vijaywada) and *Pilaglobosa* (Kolkata)	0.2193
Pilavirens (Vijaywada) and *Pilaglobosa* (Birbhum)	0.3916
Pilavirens (Vijaywada) and *Pilaglobosa* (Assam)	-0.6158
Pilaglobosa (Kolkata) and *Pilaglobosa* (Birbhum)	0.5647
Pilaglobosa (Kolkata) and *Pilaglobosa* (Assam)	-0.2739
Pilaglobosa (Birbhum) and *Pilaglobosa* (Assam)	-0.1077

Table 5: Correlation Coefficient between measured Breadth of Operculum (mm) of *Pila* species from different locations.

Species	Family	Observation	r^2	Adjusted r^2	Standard Error
Pila globosa	*Ampullariidae*	10 (samples)	0.9995	0.8745	0.7655
Pila virens	*Ampullariidae*	10 (samples)	0.9996	0.8746	0.5220
Pomacea diffusa	*Ampullariidae*	10 (samples)	0.9992	0.8742	0.7135
Bellamya benghalensis	*Viviparidae*	10 (samples)	0.9994	0.8744	0.4449
Nassarius olivaceus	*Nassariidae*	10 (samples)	0.9998	0.8748	0.6584
Nerita polita	*Neritidae*	10 (samples)	0.9994	0.8743	0.2764
Indoplanorbis exustus	*Planorbidae*	10 (samples)	0.9998	0.8748	0.1253
Achatina fulica	*Achatinidae*	10 (samples)	0.9999	0.8749	0.4811

Table 6: Calculation of r^2, Adjusted r^2 and Standard Error of the species by Regression analysis.

Species	Analysis	Degrees of Freedom (df)	Sum of Squares (SS)	Mean of Square MS= SS/Df	Significance F
Pila globosa (Bhp)	Regression	2	1143272	5716.36	8.74E-13
	Residual	8	4.6879	0.58599	-
	Total	10	11437	-	-
Pila virens (Vijaywada)	Regression	2	6424.406	3212.203	4.508E-13
	Residual	8	2.180333	0.272542	-
	Total	10	6426.586	-	-
Pomacea diffusa (Dumdum)	Regression	2	5445.997	2722.999	7.15E-12
	Residual	8	4.072588	0.509073	-
	Total	10	5450.07	-	-
Bellamya benghalensis (Bhp)	Regression	2	2824.213	1412.107	2.61E-12
	Residual	8	1.583588	0.197949	-
	Total	10	2825.797	-	-
Nassarius olivaceus (S. Andaman)	Regression	2	16775.4096	8387.7048	7.96E-14
	Residual	8	3.46862	0.43357	-
	Total	10	16778.878	-	-
Nerita polita (S. Andaman)	Regression	2	1001.6842	500.842107	3.50965E-12
	Residual	8	0.6111851	0.076398	-
	Total	10	1002.2954	-	-
Indoplanorbis exustus (S. Andaman)	Regression	2	582.4626	291.2313	9.25E-14
	Residual	8	0.125697	0.015712	-
	Total	10	582.5883	-	-
Achatina fulica (Odisha)	Regression	2	13888.08	6944.041	1.71E-14
	Residual	8	1.851857	0.231482	-
	Total	10	13889.93	-	-

Table 7: ANOVA analysis for sample species.

Species	Variables of snail	Coefficients	Standard Error	Lower 95%	Upper 95%
Pil globosa (Bhp)	Height of shell	0.1523	0.4969	-0.9935	1.2982
	Breadth of shell	0.8244	0.4975	-0.3228	1.9728
Pila virens (Vijaywada)	Height of shell	-0.1062	0.0656	- 0.2575	0.0452
	Breadth of shell	1.1218	0.0889	0.9190	1.3255
Pomacea diffusa (Dumdum)	Height of shell	0.9249	0.3027	0.2270	1.6249
	Breadth of shell	0.0357	0.3051	-0.6689	0.7393
Bellamya benghalensis (Bhp)	Height of shell	-1.0029	1.0915	- 3.5198	1.5140
	Breadth of shell	1.9317	1.1055	- 0.61745	4.4809
Nassarius olivaceus (S. And)	Height of shell	0.9531	0.7997	0.8887	2.7948
	Breadth of shell	0.0041	0.8148	-1.8726	1.8808
Neritapolita (S. And)	Height of shell	0.8291	0.3007	0.135595115	1.5226
	Breadth of shell	0.1539	0.3221	-0.58989729	0.8957
Indoplanorbis exustus(S. And)	Height of shell	1.0389	0.3039	0.339284	1.7365
	Breadth of shell	-0.0495	0.3277	-0.7822	0.6832
Achatina fulica (Odisha)	Height of shell	1.4201	0.5622	0.123739	2.7165
	Breadth of shell	-0.4565	0.5762	-1.7853	0.8723

Table 8: Confidence Interval estimate of slope height of the shell and breadth of the shell for sample species.

Species	Observation	Predicted Body Weight (g)	Residuals
Pila globosa	1	30.364	-1.8047
	2	32.0555	0.0044
	3	33.9239	0.1270
	4	35.1553	0.0957
	5	29.0390	0.8309
	6	34.9288	-0.3688
	7	35.9641	0.6758
	8	36.9565	0.0734
	9	34.8936	0.1363
	10	34.0060	0.3139
Pila virens	1	25.8332	-0.2732
	2	26.4122	0.3277
	3	24.1775	-0.8675
	4	21.9960	-0.1660
	5	22.6106	-0.5806
	6	27.3185	0.3314
	7	26.3679	0.4520
	8	27.7568	0.0032
	9	23.9747	0.7453
	10	26.3254	-0.1054
Pomacea diffusa (Dumdum)	1	26.4458	0.8741
	2	27.3485	0.6815
	3	26.9301	0.3998
	4	25.0435	-0.0235
	5	24.0450	0.0749
	6	21.8376	-0.6176
	7	20.1690	-0.9390
	8	18.1862	-1.1662
	9	21.0806	0.1594
	10	20.2309	-0.1709

Table 9: Residual Output for 10 observations of sample species of Ampullariidaeas calculated through regression analysis.

Discussion

In this study we measured the morphometric parameters such as total body weight (BW), height of the shell (H), breadth of the shell (B), length of the operculum (LO), breadth of the operculum (BO), from different snails belonging to various taxonomic families collected from a varied regions as shown in table 1 [8]. A significant correlation was observed between these variables chosen for aquatic and terrestrial snails. In this study, the result of correlation coefficient analyses depended on values which ranged from -1 to +1. Correlation coefficient in body weights between *Pila globosa* species ranged from 0.9422 to -0.0418 as observed from table 2. A strong positive correlation of 0.9422 was observed between *Pila globosa* (Berhampore) and *Pila globos*a (Kolkata). A negative correlation was observed between *Pila globosa* (Berhampore, Birbhum, Kolkata) and *Pila globosa*(Assam). A positive correlation of 0.7534 in body weights was observed between *Pila virens* and *Pila globosa* (Assam). Further, the correlation coefficient between body weights of *Pila* (*Ampullariidae*) along with *Pomacea* (*Ampullariidae*), *Bellamya*, (*Viviparidae*), *Nassarius* (*Nassariidae*), *Nerita* (*Neritidae*), *Indoplanorbis*, (*Planorbidae*), *Achatina* (*Achatinidae*) were measured which showed significantly negative correlation except *Indoplanorbis exustus* which only showed positive correlation of 0.4820 as seen in table 3. Also, correlation coefficient was measured between length of operculum (mm) of *Pila* species which was sampled from different locations which ranged from 0.0976 to -0.5841 as seen from the table 4. Correlation coefficient between measured Breadth of Operculum (mm) of *Pila* species from different locations was also given in table 5. Table 6 reveals the calculated regression statistics coefficient of determination r^2, adjusted r^2 with standard error of estimate. The observations were done for 10 data points of each chosen species. As revealed from our study data, we observed a very good r^2 ranged from 0.9992 to 0.9999 which shows a perfect significant linear relationship among the chosen species of aquatic and terrestrial snails. In multiple linear regression model, apart from calculating r^2, adjusted value of r^2 was also calculated which can be defined if the added explanatory variable (X_1, X_2) such as height and breadth of the shell explained the variation in variable Y which was the body weight of the snails. The adjusted r^2 was also evaluated as shown in the table 6. Adjusted r^2 was used to evaluate the goodness of fitted model. Standard error of the estimate was also evaluated for the multiple linear regression model as observed from table 6. In our study, ANOVA analysis was also done which showed high significant value for the chosen samples as shown in table 7. The significance of F-test for ANOVA which was an alternative test for t test for linearity showed an evidence of linear relationship among the chosen gastropods as observed in table 7. Confidence interval at 95% of height and breadth of the shell for sample species, least square estimate and standard error of least square estimates were computed in ANOVA as shown in table 8.The body weight was depended on explanatory variables height and breadth of the shell. Thus, the above model was a multiple linear regression model $y = m_1X_1 + m_2X_2 + c$ where "c" is the intercept or constant term and "X_1" and "X_2" were the height and breadth of the shell in snails 'm' was the regression slopes or regression coefficients [9]. In order to fit the model we estimated the c, m_1 and m_2 by the method of least squares. The good estimates of m_1, m_2 and c were obtained by the method of least squares. In the best fit model, error or residuals had to be minimum. The goodness of the fitted model was evaluated when adjusted r^2 is closer to 1. By output of the residuals a multiple regression model was evaluated using the regression equation $y = m_1X_1 + m_2X_2$ where X_1 is the height of the shell and X_2 is the breadth of the shell and m is the slope [10]. The observation of body weights was found in synchrony with the predicted body weights of *Ampullariidae* as calculated through regression analysis equation shown in table 9. A significant correlation was observed between the variables chosen in the present study for aquatic and terrestrial snails.

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Conflict of Interest

There is no conflict of interest.

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