



## **Mirrored chirality hypothesis test: A case study on shells of African snail *Achatina fulica* (Férussac, 1821) (Gastropoda, Achatinidae)**

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This study aimed to compare the morphometry of dextral and sinistral shells of *Achatina fulica*, and to test the hypothesis of mirrored chirality between the shells, using morphometric measures. Principal component analysis indicated that the width/length and height/length ratios were the main variables that differentiated the dextral and sinistral shells, confirming the absence of mirrored chirality. Testing the hypothesis for other species and gastropods is recommended.

**Keywords:** Mollusks. Gastropods. Morphology. Dextral. Sinistral.

**Teste de hipótese de quiralidade espelhada: Um estudo de caso em conchas do caramujo-africano *Achatina fulica* (Férussac, 1821) (Gastropoda, Achatinidae).** O presente estudo tem como objetivo comparar a morfometria de conchas dextrógiras e sinistrógira de *Achatina fulica*, e testar a hipótese de quiralidade espelhada entre as conchas, utilizando as respectivas medidas morfométricas do gastrópode. A Análise de Componentes Principais (PCA) efetuada, indicaram as razões largura/comprimento e altura/comprimento como principais variáveis que diferenciam conchas dextrógira e sinistrógira, confirmando a hipótese da não existência de quiralidade espelhada. Recomenda-se o teste da hipótese em outras espécies e gastrópodes.

**Palavras-chave:** Moluscos. Gastrópodes. Morfologia. Dextrógira. Sinistrógira.

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Determination of the characteristics of gastropod shells is essential for the correct taxonomy of the species (LEAL, 2002). In this sense, it is important to know that the majority of gastropods (more than 90%) have a dextral shell, a term used for shells that have an opening facing the right side (SCHILTHUIZEN; DAVISON, 2005). However, in some species, it is possible to find individuals with inverted shells, i.e., those facing the left side, called sinister (CAMACHO; DEL RÍO, 2011; SCHILTHUIZEN et al., 2005).

Conchology, a science that studies the variations in shape and color in shells, analyzes several morphological characteristics to obtain a general pattern/characteristic of the shell of the species, including chirality, i.e., the direction of the twist of the shell (SCHILTHUIZEN et al., 2005). According to Brettingham (1990), morphological characteristics analogous to those identified as standard can occur within a family or species of gastropods.

*Achatina fulica* (Férussac, 1821), known as the "African snail," is one of the species with records of individuals with a sinistral shell (ASAMI et al., 1998; SANTOS et al., 2019; SCHILTHUIZEN et al., 2005). However, Santos et al. (2019) cite that the records of sinistral shells in *A. fulica* are generally found on websites whose purpose is the disclosure/commercialization of shells.

Chirality has recently been cited as one of the major questions in science (KENNEDY; NORMAN, 2005). Most studies only record the occurrence of a sinistral shell without analyzing/comparing the symmetry with that of dextral shells. Schilthuisen et al. (2005) mentioned that the dextral and sinistral shells of gastropods are characterized by mirrored chirality, and

this hypothesis is currently not contested.

The present study aimed to compare the morphometry of dextral and sinistral shells of *A. fulica*. Our hypothesis is that there is no mirrored chirality between the shells, and therefore, sinistral shells have a different morphometry than that of dextral shells.

An example of a sinistral shell (Figure 1), registered by Santos et al. (2019), was used along with 25 specimens of dextral shells (Figure 2), all preserved and available in the malacological collection of the Zoology Museum of the Federal Rural University of the Amazon (MZUFRA). Of note, the dextral shells were randomly selected from those available in the malacological collection MZUFRA; however, we sought to obtain the largest possible class of total length of *A. fulica*.

In order to verify if the number of shells used was sufficient to test the proposed hypothesis, correlation analysis of the morphometric measurements (total length (L), width (W), height (H), and length and width of the opening) of the shells of *A. fulica* was performed (Figure 3). Verification was performed using linear regression analysis and through interpretation of the correlation coefficients ( $R^2$ ) and Pearson's correlation ( $r$ ); finally, the  $t$ -test was performed. Thus, it was evident that the morphometric measurements were highly correlated (Table 1). These results are similar to those found by Santos et al. (2020), and it was confirmed that sample sufficiency was achieved.



Figure 1 – *Achatina fulica* sinistral shell used in this study and available for consultation at the Malacological Collection of the Zoology Museum of the Federal Rural

University of the Amazon (MZUFRA). Source: Chagas et al. (2019).

To test the proposed hypothesis, the area of the opening of the shell ( $Sh_{area}$ ) of *A. fulica* was previously measured using Inkscape 1.0 (<https://inkscape.org/about/>) (HIITOLA, 2010). Later, we calculated the following morphometric relationships:  $W/L$ ,  $H/L$ ,  $H/W$ ,  $L/Sh_{area}$ ,  $W/Sh_{area}$ , and  $H/Sh_{area}$ . Prior to the analyses, the normality of the data was verified using the Levene test ( $p < 0.05$ ). Subsequently, a principal component analysis (PCA) was applied with the measured morphometric ratios in order to highlight the main determinant variable in the difference between the sinistral and dextral shells.

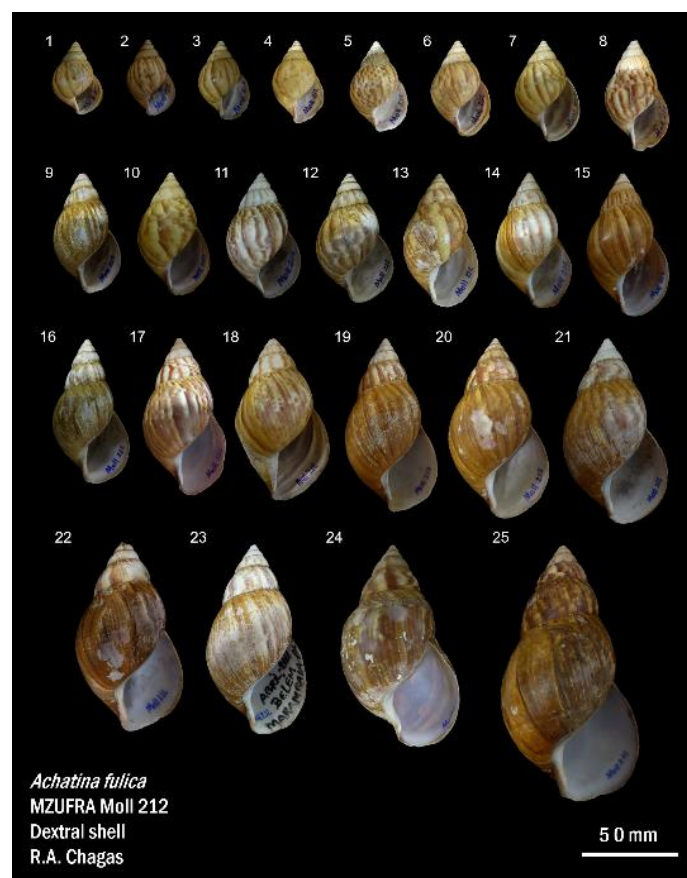


Figure 2 – List of 25 dextral shells of *Achatina fulica* used in this study and available for consultation in the collection of the Malacological Collection of the Zoology Museum of the Federal Rural University of the Amazon (MZUFRA). Scale: 50 mm. Source:Chagas et al. (2019).

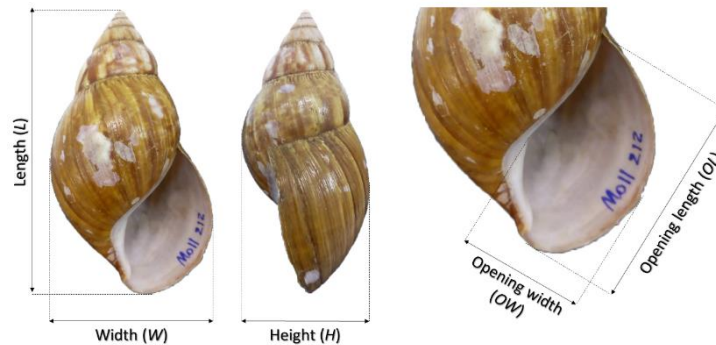


Figure 3 – Shell of *Achatina fulica* (MZUFRA Moll 212), with indication of the morphometric measurements used. Sample available in the collection of the Malacological Collection of the Zoology Museum of the Federal Rural University of the Amazon (MZUFRA), Source: Santos et al. (2020).

Table 1 – Morphometric relationships of the dextral shells of *Achatina fulica*, where: Total length (L), width (W), Height (H), opening length (OL), opening width (OW), correlation coefficients ( $R^2$ ), correlation of Pearson ( $r$ ) and statistical significance ( $p$ ), with  $** p < 0.001$ .

Morphometric relationship	$R^2$	$r$	$p$
L X W	0.976	0.988	**
L X H	0.981	0.990	**
W X H	0.984	0.992	**
OL X L	0.970	0.985	**
OL X W	0.977	0.989	**
OL X H	0.961	0.980	**
OW X L	0.966	0.983	**
OW X W	0.989	0.994	**
OW X H	0.968	0.983	**

All statistical analyses were performed using PAST - Paleontological Statistics (version 4.0) (HAMMER, 2020), using a 95% significance level.

The PCA relating the morphometric ratios of *A. fulica* dextral and sinistral shells described 83.91% of the relationship between the variables. The results indicated that only the first main component (PC1) was statistically responsible for the order of the variables. In addition, it indicated the W/L and H/L ratios as the main variables in determining the order (Figure 4).

Thus, we observed that the sinistral shell of *A. fulica* (orange square in

Figure 4) is outside the group of those originating from the dextral shell, and therefore, presents a morphometric difference. Thus, the hypothesis of nonexistence of mirrored chirality between the dextral and sinistral shells of *A. fulica* is confirmed.

Sinistral samples are extremely rare, and considered a very intriguing phenomenon whose explanations are remain speculative; however, studies indicate that it is determined by the maternal genetic contribution (GERNET et al., 2018b; SCHILTHUIZEN et al., 2005). Schilthuizen et al. (2005) mention that gastropod chirality has important

implications for the study of evolution, genetics, and development, and may be a path to reproductive isolation, and consequently, the emergence of new species.

Studies indicate that it is indisputable that the inversion of chirality occurred independently and evolutionarily in gastropods (ASAMI et al., 1998). Furthermore, it was recently

cited as one of the major questions to be resolved (KENNEDY et al., 2005). However, most studies address only the registration of species with sinistral shells (GERNET et al., 2018a, 2018b; SANTOS et al., 2019), without considering an analysis of the asymmetry of the shell, i.e., the existence of a mirrored chirality.

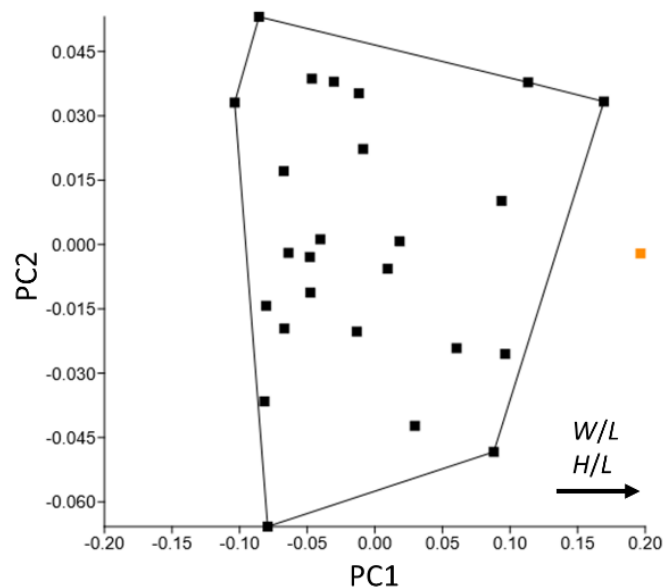


Figure 4 – Principal Component Analysis (PCA) carried out with the morphometric ratios of the dextral (■) and sinistral (■) shells of *Achatina fulica*, showing the absence of mirrored chirality.

In the present study, we compared the morphometry of dextral and sinistral shells of the African snail *A. fulica* and confirmed the hypothesis that there was no mirrored chirality. As this study is a case report, and therefore, it is not possible to extrapolate such results, we recommend the same study with the use of other species with the occurrence of sinister shells.

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