

Original Research Paper

## Heat stress and new evidence on land snails' biodiversity in Algeria.

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### Abstract

Understanding both the genetic diversity and geographical distribution of animal genetic resources is essential for their sustainable utilization, improvement, and conservation. Land snails that have adapted to arid conditions confront imminent threats such as habitat loss stemming from urbanization, agriculture, pollution, and the ever-growing impacts of environmental and climate change. The present study was undertaken to explore the current morphological variability within the land snail's population in Algeria. From November 2022 to June 2023, 4 quantitative body measurements (shell size and live weight) and 2 qualitative traits (shell and flesh color) were recorded on 211 adult land snails from the 4 geographic provinces that comprise different climate zones of Algeria. The collected data were first analyzed using multiple comparisons of least-square means (LSmeans), followed by generalized linear model (GLM) procedures, to explore the relationships among the measured morphometric traits and the 4 geographic zones. Univariate analyses indicated that all quantitative linear body measurements varied significantly ( $P < 0.05$ ) across the phytogeographical zones. The covariance analysis using the sampling month to illustrate the heat stress effect shows a significant difference of body weight among land snails' population from 4 regions. In addition, multivariate analysis shows that individuals from Ain Temouchent province are distinguished by their small shell size and low weight. These results need to be investigated further to clarify this biodiversity among land snails and their environmental adaptation.

**Keywords:** Biodiversity, Algeria, heat, land snail.

### Introduction

Biodiversity, or biological diversity, refers to the diverse array of life forms originating from various environments, whether they are terrestrial, aquatic, or marine. It encompasses variation within populations of the same species (genetic diversity), differentiation between different species (organism diversity), and the variety of ecosystems that harbor them (ecological diversity) (FAO, 2015).

It is nearly 100 years since the first studies on variation in the shell patterns of land snails. In the meantime, snails have come to play a crucial role in our understanding of natural selection in the wild (Clarke et al.1978). The hot and dry summers of the Mediterranean region pose an enormous challenge for many animals (Dittbrenner et al., 2009). The skin of terrestrial snails is strongly water permeable, and they therefore easily run the risk of desiccation in hot and dry habitats. They can lose up to 40% of their body mass through evaporation of water in 2 hours (Prior, 1989). In addition, thermal death by overheating poses a danger for these animals in hot regions (Staikou, 1999). Land snails, therefore, have evolved into many adaptations to cope with hot and dry conditions including adaptations at morphological, physiological, and behavioral levels.

The snail is an adaptable species found in various environments, soils, and climates (Benguedouar, 2016). Its soft and not fully segmented body lacks articulated appendages. The visceral mass undergoes a 180° torsion relative to the foot, resulting in asymmetry in certain organs such as a single kidney, lung, and one atrium (Larba and soltani, 2014). Morphological adaptations may include variations in shell color, shell aperture area and shell size. Snails with brighter colored shells will not heat up as rapidly as snails with

darker colored shells due to the difference in sunlight reflectance (Yom-Tov, 1971). Land snails with smaller shell aperture areas (relative to their shell size) suffer less water loss during periods of inactivity (Goodfriend, 1986). Furthermore, land snails, to avoid being dissected, are typically more active at night or in humid weather. Their outer covering ensures survival by maintaining dry conditions and protecting them from evaporation. Shell-less slugs can burrow deep underground to escape heat (Karas, 2009).

Several studies on land snail inventories have been carried out in several biotopes of Algeria, notably in the northwestern (Damerdji, 2008, 2013), and northeastern regions (Larbaa and Soltani, 2013; Douafer and Soltani, 2014; Belhiouani et al., 2019 and Zaidi et al. 2021). Recently, a survey of gastropods was conducted in many areas of northeastern, western Algeria (Bouchiba et al., 2021).

Snail farming in Algeria has experienced recent growth due to the increasing demand for seafood products both domestically and internationally. Snails are considered a healthy food, making them popular among local Algerian consumers. However, snail farming is still a relatively young sector and faces challenges, such as a lack of knowledge regarding best farming practices, limited availability of snail varieties, and restricted access to the necessary equipment and facilities for expanding breeding activities. The present study aims to identify the abundance and diversity of land snails' species in northwestern Algeria and to investigate the relationship between their morphology and experiencing a period of drought using statistical analysis (modeling).

## Material and Methods

### *Animals and sampling areas*

For this study, we collected 211 samples of land snails from 9 western regions of Algeria. The samples were obtained either from breeders or by purchasing them from the markets. The sampling started from November 2022 to June 2023 with an 8-month interval and that covered 4 seasons (Table 1).

**Table 1.** Distribution of sampling regions with annual temperature and rainfall levels for each province

Provinces	Regions	Coordinates	Samples size	Sampling date
Tlemcen	Remchi	35°03'40"N 1°25'51"W	111	June 17 <sup>th</sup> 2023
	Ouzidane	34°56'10"N 1°17'13"W		Feb. 11 <sup>th</sup> 2023
	Bab el assa	34°57'51"N 2°01'51"W		Nov. 4 <sup>th</sup> 2022
	Ghazaouat	35°05'40"N 1°51'33"W		Jan. 6 <sup>th</sup> 2023
	Maghnia	34°51'20"N 1°44'15"W		Nov. 11 <sup>th</sup> 2022
Oran	Labasti market	35°42'10"N 0°38'02"W	26	March 10 <sup>th</sup> 2023
Ain-Temouchent	Beni saf	35°18'13"N 1°22'59"W	32	Feb.18 <sup>th</sup> 2023
	El malah	35°23'17"N 1°05'52"W		
Mascara	Ain - fekan	35°13'38"N 0°00'07"E	42	Feb.3 <sup>rd</sup> 2023



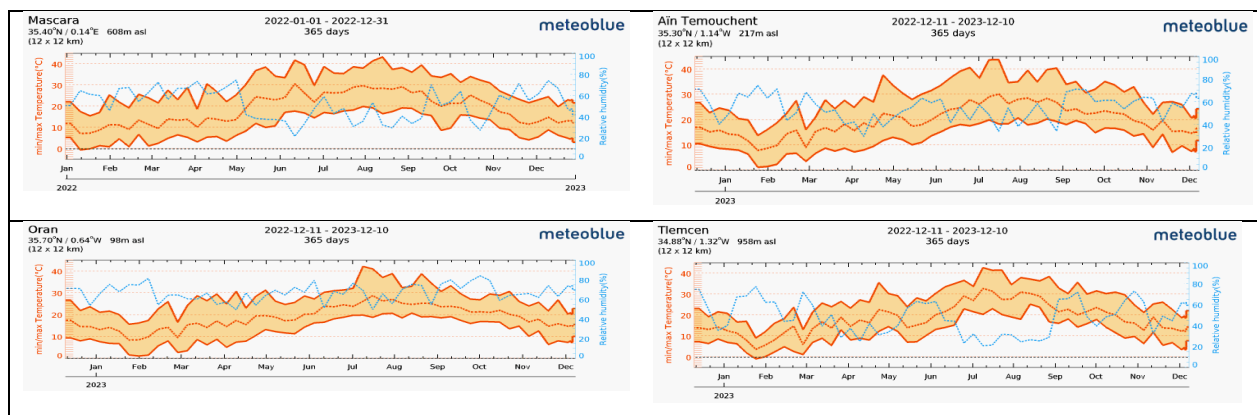
**Figure 1:** Land snails at local markets in Algeria



**Figure 2:** Some phenotypes of land snails from Tlemcen regions (northwestern Algeria)

We initiated our study with an exhaustive literature review aimed at delineating and defining terrestrial snails, gathering all their characteristics. Subsequently, we carried out a field investigation by interviewing a professional terrestrial snail farmer. This method facilitated the acquisition of detailed insights while observing the interviewee's working environment. Such an approach proved instrumental in gathering supplementary data, enriching our study (figure 1 and 2).

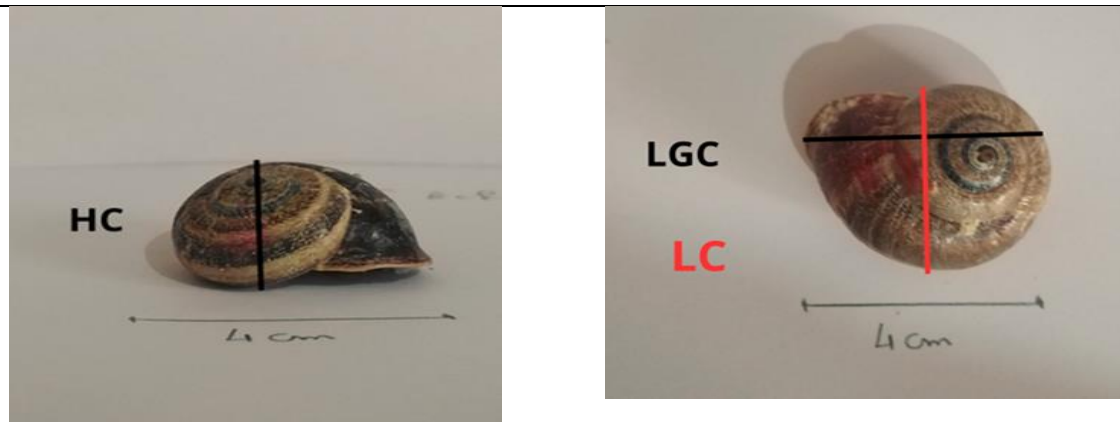
The climate of all four provinces is Mediterranean with influences from continental and semi-arid regions. Winter is quite cold there, while summer is very hot. Additionally, the cities experience periods of cold spells and snowfall during winter. The average temperatures and rainfall are as follows: Tlemcen 15.4°C and 454 mm, Oran 18.4°C and 305.9 mm, Ain-Temouchent 19.1°C and 316.2 mm, and Mascara 17.2°C and 378 mm, respectively. (figure 3).



**Figure 3:** Ombrothermic diagrams for the four study region localities (mascara, ain Temouchent, Tlemcen and Oran, (Meteoblue, 2023))

### *Morphological parameters.*

Morphological study is divided into 2 steps: the first step is for quantitative characteristics using ImageJ software (Schneider et al 2012). After photographing everyone in two dimensions - vertically for Shell Height (HC) and horizontally for Shell Width (LGC) and Shell Length (LC)(figure 4), we proceeded to measure the live weight of the animals using a balance (g). The subsequent step involved assessing qualitative characteristics, such as Shell Color (CC) and Flesh Color (CCH).



**Figure.4** Measurement of the shell height (in-left) and the shell width and length (in-right) (original. 2023). *Shell Height (HC); Shell Width (LGC); Shell Length (LC);*

#### Statistical Analysis

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics for the quantitative and qualitative traits were obtained using the procedures PROC UNIVARIATE and PROC FREQ, respectively. The frequencies and Pearson chi-square ( $\chi^2$ ) tests were used for qualitative physical traits to explore the relationships among qualitative variables. The least-square means (LSmeans), their standard errors (SEs), and the coefficients of variation (CVs) of the morphometric traits were calculated for each geographic province (all the quantitative traits are normally distributed using Shapiro-Wilk Test). The comparison of LSmeans between geographic provinces was performed using Tukey's test multiple mean comparison tests. Subsequently, the general linear model procedure (PROC GLM) followed by MANOVA, was used to analyze the relationship between geographic provinces and morphometric traits, also the month of sampling to refer to climate impact on land snail diversity.

**Model:**  $Y_{ij} = \mu + \text{province}_i + \text{month of collect}_j + e_{ij}$ , where:

- $Y_{ijk}$ : our dependent variable: shell height shell length shell width and weight for  $i^{\text{th}}$  province to  $j^{\text{th}}$  and month of collect.
- $\mu$  = is overall mean.
- $e_{ijk}$  = is the residual.

Further analyses were undertaken using the canonical discriminant analysis (CDA) function (PROC CANDISC) to perform univariate and multivariate one-way analyses, derive canonical functions and linear combinations of the quantitative variables that summarize variation between species, and calculate the associated Mahalanobis distances.

Finally, The Shannon–Weaver index ( $H'$ ) index is calculated by the following equation (Shannon & Weaver, 1963): where  $P_i$  is the relative frequency ( $n_i/N$ ) and  $R$  is the total number of species.

The equitability index  
diversity (Ramade,

$$H' = - \sum_{i=1}^R [p_i \cdot \log_2 p_i]$$

(E) constitutes a second fundamental dimension of 1984). The equitability is expressed as follows:

$$E = H'/H_{\max} = H'/\ln S$$

## Results and discussion

### Quantitative and qualitative traits using univariate analyses.

The result of the univariate analysis showed significant differences ( $P < 0.05$ ) among the 4 geographic provinces for all measured quantitative morphometric variables (Table 2). Overall, within the 4 geographic provinces the body weight is the most quantitative trait, had relatively high CVs, whereas the quantitative variables Shell height (HC), Shell length (LC); and Shell width (LGC), had relatively low CVs. However, between provinces, Mascara region recorded the lowest shell height compared to the 3 other provinces, the

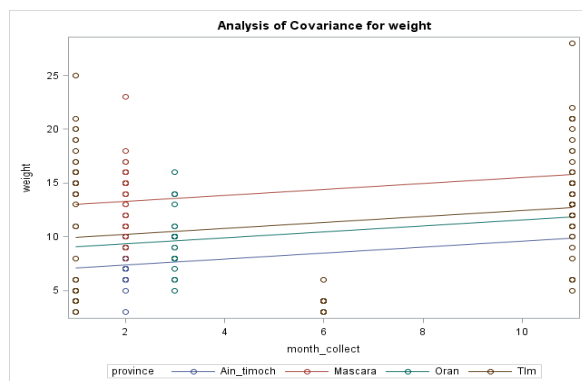


highest mean values were observed in the littoral Ain Temouchent zone. The lowest mean values recorded for both Shell length and width (cm) were respectively 3.58 and 2.95 and the highest means were 4.51 and 3.63 respectively belonging to the Ain temouchent province (Table2). However, with this later province, the body weight trait, are recorded as the lowest, in which is an opposite result comparing with the others morphological traits, in fact the same province shows a relatively big shell but have low weight, this can be explained by the low flesh weight, or further research can be recommended to answer at this stage.

**Table 2:** Least squares means (LSmeans) and standard errors (SEs) coefficients of variation (CVs) of morphological measurements (cm) across 4 geographic provinces with univariate and multivariate analysis test.

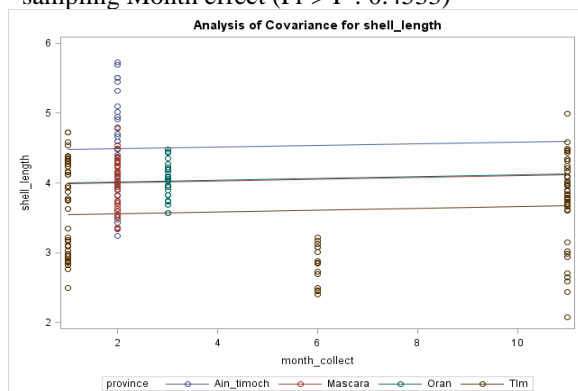
Morphological traits	Provinces				Cv	ANOVA Pr > F	MANOVA Pr > F
	Ain Tim	Mascara	Oran	Tlemcen			
	LSMEAN±SE						
Shell height(cm)	2.22±0.05a	1.81±0.04b	2.11±0.05a	1.83±0.03c	15.67	<.0001	
Shell length(cm)	4.51±0.10a	4.03±0.09b	4.04±0.11b	3.58±0.06c	15.05	<.0001	
Shell width(cm)	3.63±0.08a	3.53±0.07a	3.28±0.09a	2.95±0.04b	14.33	<.0001	<.0001
Weight (g)	8.07±0.85a	13.98±0.75b	10.05±0.92a	10.92±0.48a	43.32	<.0001	

a,b,c,d: LSmeans with different letters in rows are significantly different at  $P \leq 0.001$ ; multiple mean comparison test tukey, *Ain Tim*: Ain temouchent



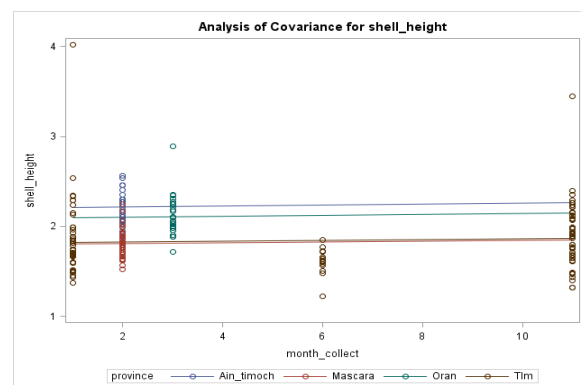
Tlm: Tlemcen, Ain\_timoch: Ain temouchent

Province effect (Pr > F <.0001)  
sampling Month effect (Pr > F : 0.4533)



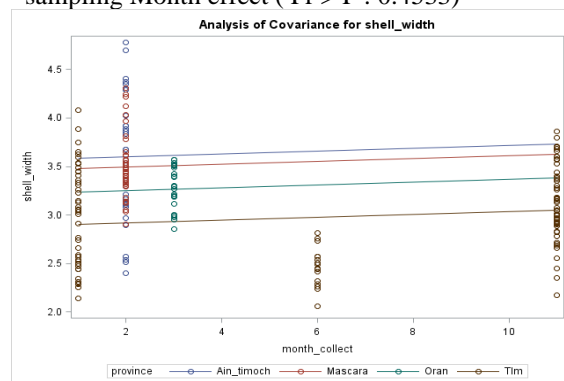
Tlm: Tlemcen, Ain\_timoch: Ain temouchent

Province effect (Pr > F <.0001)  
sampling Month effect (Pr > F : 0.3001)



Tlm: Tlemcen, Ain\_timoch: Ain temouchent

Province effect (Pr > F <.0001)  
sampling Month effect (Pr > F : 0.4533)



Tlm: Tlemcen, Ain\_timoch: Ain temouchent

Province effect (Pr > F <.0001)  
sampling Month effect (Pr > F : 0.1189)

**Figure 5:** graphics showing the covariance analysis results for quantitative traits across all 4 geographic provinces.

Univariate and multivariate analyses revealed significant differences among geographic provinces for all measured morphological traits, suggesting the possible influence of areas of origin of the snails on their evolutionary adaptation in terms of these morphological traits. This result is in line with the finding of a previous study (Bouchiba et al., 2021), who reported a significant impact of the environmental and climate status on morphological traits in the land snail's population from 14 provinces extending from east to west of Algeria. However, this result suggests that the land snail in the aforementioned zones is closer to each other in terms of morphology. In addition, the quality of soil can also influence the characteristics of the shell because it is part of the snail's diet and constitutes an essential calcium supply for the formation of its shell and influences its growth (Gomot et al., 1989; Dallinger et al., 2001).

Regarding the heat stress effect on different morphological traits, we performed a model in which the month of sampling is considered as the season effect. To illustrate this effect, we needed to add sampling month as class effect but unfortunately the data don't allow, so, we used instead a covariance model between the 4 geographic provinces and the sampling month, and the results are showed in figure 7. Indeed, the only significant difference observed among monthly samplings is in body weight (<.0001SIGNIFICANCE). This disparity can be attributed to climate change and the availability of diverse food sources across different months. Bouchiba et al. (2021) noted a proliferation of empty-shelled land snails during the hottest days, suggesting the prevalence of more resilient species within the studied populations. Elkarmi and Ismail (2007) also highlighted the impact of temperature variation on the growth and morphometrics of snail populations. Similarly, Dupouy (1993) identified a correlation between shell morphology and ecophysiological adaptation in *Biomphalaria pfeifferi* populations across Africa.

**Table 3.** Frequency (%) of qualitative traits in land snails' population of the 4 geographic provinces of Algeria.

Province	Ain Témouchent	Mascara	Oran	Tlemcen	Total	Chi- Square	p
<b>shell color</b>	n= 32	n= 42	n=26	n=111	n=211	144.3370	<.0001
Brown_beige	<b>11.85</b>	5.69	<b>6.64</b>	8.53	33.17		
Brown_white	0.00	0.00	0.00	5.21	5.21		
Black_beige	0.00	0.00	0.00	0.47	0.47		
Beige	0.47	3.32	1.90	0.00	5.69		
Brown	0.95	<b>10.90</b>	3.32	<b>18.48</b>	<b>33.65</b>		
Brown_black	0.00	0.00	0.00	7.58	7.58		
White	0.00	0.00	0.00	12.32	12.32		
White_brown	0.95	0.00	0.00	0.00	0.95		
White_brown_beige	0.95	0.00	0.00	0.00	0.95		
<b>Flesh color</b>							
Beige_black	2.84	0.00	1.42	0.00	4.64	86.9243	<.0001
Black_gray	11.85	19.91	10.43	28.44	<b>70.62</b>		
Gray	0.47	0.00	0.00	4.27	4.74		
Gray_black	0.00	0.00	0.00	7.11	7.11		
Gray_beige	0.00	0.00	0.00	1.42	1.42		
Beige	0.00	0.00	0.00	9.00	9.00		
Beige_gray	0.00	0.00	0.00	0.47	0.47		
Black_beige	0.00	0.00	0.00	0.95	0.95		
Brown	0.00	0.00	0.00	0.95	0.95		

*Bold values: are the most representative colors in the population of land snails.*

Significant differences in the frequencies ( $P < 0.0001$ ) were observed among the 4 geographic provinces for certain qualitative traits, such as shell and color (Table 3). A composite flesh color (70.62%) was more frequently observed regardless of the geographic zone, with a dominance of Black\_ gray (70.62%), Beige (9.00%) and Gray with black (7.11%) colors. In other hand the predominate color of shell is Brown (33.65) followed by the Brown with beige (32.70%) and White (12.32%) colors. Taking the 4 provinces, the predominant shell color is brown for both Tlemcen and Mascara are brown, and it is brown with beige for

Ain Temouchent and Oran those are coastal regions. Secondly, the predominant flesh color black with gray is the same between the 4 provinces.

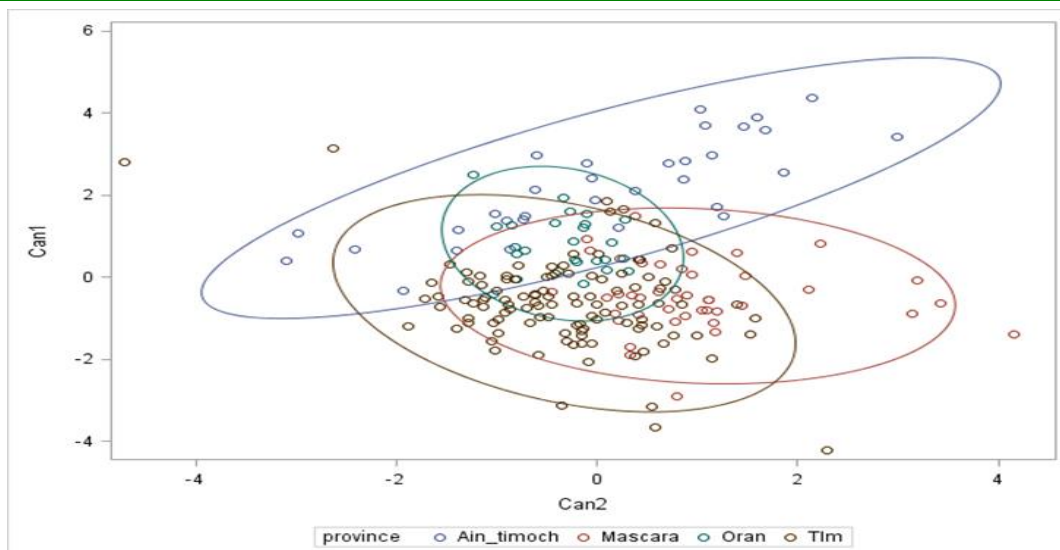
These colors of shell and flesh are the most popular in the market and appreciated by the socio-cultural state of consumers (Bouchiba et al. 2021, Bouchiba and metahri 2020). Land Snails animals can change their color to adapt to the heat waves and climate change; such colored shells will not heat up as rapidly as snails with darker colored shells due to the difference in sunlight reflectance (Yom-Tov, 1971).

#### **Identification of land snails' sub-populations using multivariate analyses**

The results of the canonical discriminant (CDA) analysis table (5), the use of the 4 significant ( $P < 0.0001$  for column  $Pr > F$ ) quantitative variables in the CDA generated two significant ( $P < 0.0001$ ) canonical variables (CAN 1 and CAN 2) that explain 99% of the total variation, as revealed by the standardized coefficients for the discriminant function, the canonical correlation, the eigenvalue, and the share of total variance taken into account (Table 5). Canonical loadings that measure the simple linear correlations between each independent variable and canonical variables are reported in Table 5. CAN 1 was dominated by positive loadings of shell height, shell length and shell width and negative loadings of body weight. In contrast, CAN 2 was dominated by positive loadings of shell width, shell length and weight. In addition, the plot of the centroid values of the first two canonical discriminant functions (CAN1 and CAN2) unfortunately don't show a distinct and homogenous land snail sub-population with many overlapping events (Figure 8). This can be explained by the geographic proximity and exchange of animals between these provinces. However, we can say that the province of Ain Temouchent has some individuals are distinct from the other provinces, this can refer to the morphology differences.

**Table 5:** Total canonical coefficients for the canonical function, the adjusted canonical correlation, the eigenvalue, the approximate standard error of the canonical correlations and the percentage total variance accounted for obtained from the canonical discriminant analysis performed on 4 morphometric variables.

Variable	Can1	Can2
Shell height (mm)	0.613	-0.190
Shell length(mm)	0.605	0.432
Shell width(mm)	0.477	0.762
Weight (g)	-0.452	0.337
Adjusted Canonical Correlation	0.464	0.718
Approximate Standard Error	0.033	0.054
Eigenvalue	1.0656	0.2754
Proportion	79%	20%



**Figure 8:** Scatterplot visualizing the 211 individual land snails sampled from the 4 geographic provinces of Algeria on the first two canonical discriminant functions.

### ***Mahalanobis distances***

The Mahalanobis distances among the 4 geographic provinces and 3 species are presented in Table (6) and table (7) respectively. All pairwise distances were significant ( $P < 0.0001$ ). According to the provinces, the two largest measured squared Mahalanobis distances were between the Ain temouchent and Tlemcen provinces (7.98) and between also the Ain temouchent and Mascara provinces (7.77). The closest distance (1.79) was between the mascara and Tlemcen provinces. About species, we found that predominate species in the area *Helix aspersa* is so far in term morphological than *Sphincterochil boissieri* and in contrast the same species is morphological comparable in with *Otala punctata*. In addition, in term of taxonomy both *Helix aspersa* and *Otala punctata*: Commonly known as the garden snail, are belonging in the same the family *Helicidae* in which also are both *Native* to the Mediterranean region (Capinera and White. 2011). These findings may be supported by that these two species are some demanding in market and by consumers. The canonical discriminant functions accurately classified a relatively high proportion of the individual land snails are belonging into their *a priori* group (3 provinces Tlemcen, Oran and Mascara) (Table 7).

**Table 6:** Mahalanobis distances between 4 geographic provinces (n = 211) obtained from the canonical discriminant analysis.

From province	Ain temochent	Mascara	Oran	Tlemcen
Ain temouchent	0			
Mascara	7.77***	0		
Oran	2.05***	3.42***	0	
Tlemcen	7.98***	1.79***	2.14***	0

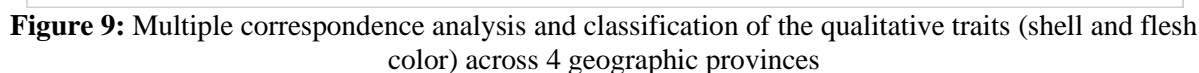
**Table 7:** Mahalanobis distances between land snail species obtained from the canonical discriminant analysis.

From species	<i>Helix aspersa</i>	<i>Otala punctata</i>	<i>Sphincterochil boissieri</i>
<i>Helix aspersa</i>	0		
<i>Otala punctata</i>	3.21296***	0	
<i>Sphincterochil boissieri</i>	17.28983***	6.98724***	0

### ***Multiple correspondence analysis of land snails' qualitative traits***

Multiple correspondence analysis (MCA) highlighted the association between the different qualitative physical traits and geographic provinces (Figure 5). The first two dimensions (Dim 1 and Dim 2) explained 68.12% and 25.03% of the total variation, respectively. Two groups were classified as the best similarity scale between land snail animals using the phenotype color for the shell and flesh. In fact, the 3 provinces Tlemcen, Oran and Mascara are clustered in the first group (Color blue) and the Ain Temouchent was represented as alone cluster. Moreover, we can look closely that the 3 provinces from first cluster shared some phenotypic similarities like gray, white and beige shell, and flesh color, this may be explained by the high temperature that the animals adopt for the light colors to reflect outside the heat period (Yom-Tov, 1971 Benguedouar, 2016). Furthermore, these regions are considered as interior-located zones, in which the climate is more arid. On the other hand, the province of Ain Temouchent is predominantly by some land snail's animal with dark color especially the black mixed with gray and brown, this province is a coastal region still have a mild climate (figure 4). These results are also supported by Bouchiba et al (2021).





After calculating the Shannon-Weaver and Pielou H diversity index in Excel, we obtained a rate equal to 0.88 for the studied population. This index indicates relatively high genetic diversity, likely reflecting a diverse gene pool within the population. Moreover, the variability observed in the Shannon index may be due to various factors, including adaptation to local conditions and the distinct attributes of the exploited regions, such as altitude, precipitation, vegetation, and temperature (Bouchiba et al., 2021).

Variable	H
Weight (g)	0.93679
Shell height	0.85321
Shell length	0.87329
Shell width	0.86126
Average	0.88113

In the context of the evaluation of animal genetic resources in general, particularly focusing on land snail diversity, our investigation reveals the substantial genetic wealth inherent in these animals. Despite this, research in this domain remains scarce, failing to adequately address these critical aspects. Our study highlights significant differences between individuals about all quantitative morphometric characteristics measured on the shell as well as the physical exterior trait like color. There is a high level of genetic diversity in the population studied, with a Shannon-Weaver index of 0.88113. Additionally, we observed a positive correlation between some of the studied traits, which can be explained by the sensible of the land snails' influence by the environment status especially the long dry and hot climate in the regions. The same way can also refer to the traits are controlled by several common genes.

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