

MORPHOMETRIC CHARACTERIZATION OF SOME SPECIES OF BATS IN WESTERN ALGERIA.

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Abstract

Our research work is divided into two parts. The first is based on the identification of specimens captured in the study area and the second part consists in characterizing these animals morphometrically and applying the bioinformatics tools for data processing obtained. During this original study, we contributed to the morphometric study (20 measurements) of two bat species from western Algeria (*Rhinolophus ferrumquinum* in the Wilaya of Tlemcen and *Asellia tridens* in the Wilaya of Bechar). The analysis of the diversity of the studied species was carried out starting from 20 parameters by the calculation of the index of Shannon and Weaver. The study was conducted on a sample of 50 individuals belonging to each species studied. The data collected is analyzed by a principal component (PCA) in order to know the different correlations that exist between the characters studied and a hierarchical ascending group classification by the HCA function. These statistical analyzes were carried out by the R version 2.15.2 software, Excel and the XLSTAT 2016 software. Our results showed that there is an average rate of genetic diversity in the species studied; the Shannon and Weaver index is between 0.43 for the species *Rhinolophus ferrumquinum* and 0.59 for the species *Asellia tridens*. We also observed a positive correlation between some traits studied which can be explained by the influence of the same group of genes on these characters. The HFA separated the two species into two major groups.

Key words: Algeria, *Rhinolophus ferrumquinum*, *Asellia tridens*, Bats, Morphometric measurement.

Introduction

Among mammals, chiroptera form a relatively old group, which appeared on our planet about 65 million years ago (Teeling E. et al, 2005). They can be found almost anywhere in the world, in desert oases, in tropical and subtropical forests, in coastal plains or in relatively high mountains, in isolated islands, in temperate regions, and in boreal regions (Serra -Coba J. et al, 2009). Algeria, which is the largest country in Africa (2,381,741 km²), offers a wide variety of biotopes that make possible the existence of a great diversity of bat species. There are, in fact, Mediterranean regions, desert regions, mountainous regions, high plateau areas and ancient volcanic regions. Each of these areas can potentially host a particular bat fauna. At present, while bats through a large number of studies have been shown to be of great importance from an ecological point of view; unfortunately no study in western Algeria has been recorded on this heritage, that is why we have started this study whose general objective is to contribute to the study of bats in this region at the level of two biotopes

different ; the Tell (Tlemcen) and the Sahara (Bechar). Chiroptera are therefore poorly known (especially in Algeria).

Materials and Methods:

Field part:

Choice of study stations: Preliminary investigations during the spring of 2017 allowed us to list cottages to examine the most representative stations. The choice of these stations was made according to the presence of species of bats. For the recognition of the cottages sheltering bats we based ourselves mainly on the K. Kowalski and A.L. Ruprecht, guide, (1981), the previous studies (Allegrini B., 2007) as well as on investigations near the hunters and riparians. After recognition and verification of the existence of the species, the study stations were located.

Choice of species studied: During our sampling we found several species of bats, but we chose the two most abundant species for this study (*Rhinolophus ferrumquinum* in the Wilaya of Tlemcen and *Asellia tridens* in the Wilaya of Béchar).

Techniques for catching and measuring chiroptera:

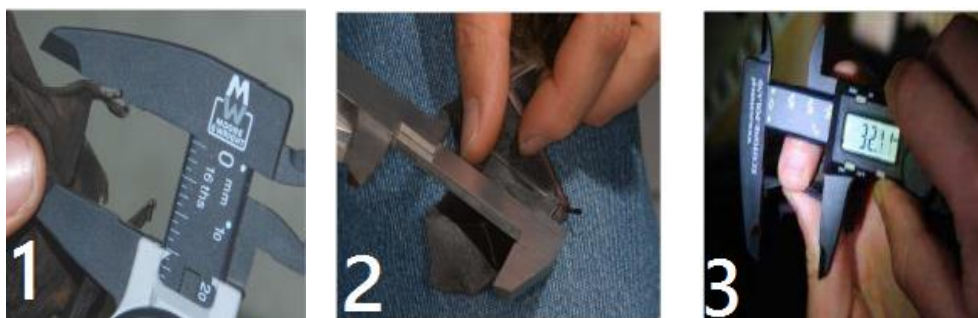
a. Japanese net catch:

A Japanese net with 12 m long and 2 m high, (16 mm mesh) was placed between two poles and was deployed between 16h and 22h. The net is tightly placed either near a water point or on the potential flight paths of animals leaving a cottage (dormitory site of bats). Individuals caught in the net are gently handled with gloves, taking care not to injure them. The catches taking place in the evening so we used headlamps to enlighten us. Captured specimens are placed in 45x45x45 cm wire mesh cages to take biometric parameters for identification. The animals are quickly released near their natural lodgings. Morphometric measurements were performed on 50 animals for each species.

b. Morphometric measurements:

The biometric parameters (20 measurements) were recorded thanks to a vernier caliper. AB: Forearm, Abd: Length of the abdomen, C: Length of Elbow, D1: Length of Inch, D2: Length of Second finger, D3: Length of Third finger, D4: Length of Fourth finger, D5: Length of Fifth finger, G: Length of Kneeling, Lail: Length of garlic (patagium), LO: Length of Ear, LP: Length of Feet, LT: Length of Tragus, LTt: Total length, M: Length of Jaw, Ne: Length of Nose, Q: Length of Tail, R: Length of Radius, Tè: Length of Head, Ure: Length of Urotagium (Table n ° 1).

Table n ° 1: Photos showing the taking of some measurements on the bats. (Original, 2017).





[1]. Knee length, [2]. Length of the 5th finger, D5, [3]. Length of the forearm (AB). [4]. Thumb length, 1st finger (D1) [5]. Tail length (Q) [6]. Total length (LTt).

Determination of the species caught:

The determination and confirmation of the captured species was conducted according to the guidelines of Hurst J. et al. (2016).

Statistical analysis :

Statistical tools in population genetics make it possible to quickly process a series of data. To get the software R (Chambers J., 2008) and XLSTAT for Microsoft Excel® and software Microsoft® Excel were used for this study. The measurements were used to develop a matrix that was used to perform various descriptive and analytical statistical tests.

Descriptive statistics:

From the animal measurements, averages were calculated with the XLSTAT software and distributed in class by the software R by the "summary" function. From these classes the Shanon and Weaver index was calculated according to the following formula:

$$H' = - \sum p_i \log_2 p_i$$

H' : is the diversity index expressed in bit units.

P_i : is the probability of meeting species i .

$$P_i = n_i / N$$

n_i : is the number of individuals of species i .

N : is the total number of individuals of all species combined.

This index gives an idea of the specific diversity of a population.

Principal Component Analysis (PCA):

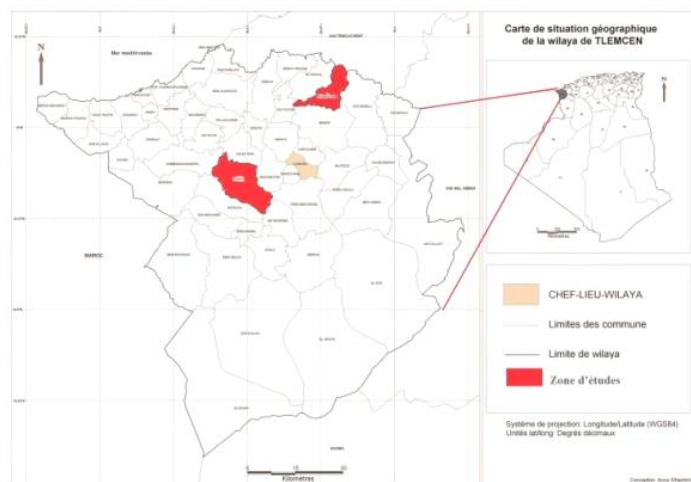
Principal Component Analysis (PCA) is a method of data analysis (Kouani.A. et al., 2007) more generally of multivariate statistics, which consists in transforming linked variables (called "correlated" statistics) into new variables uncorrelated from each other. These new variables are called "principal components", or main axes. It allows the practitioner to reduce the number of variables and make the information less redundant.

Hierarchical cluster analysis (HCA):

The aim of the HCA is to obtain a simple schematic representation of a rectangular array of data whose columns, according to usage, are descriptors of all the observations, placed in rows (Roux M., 1987).

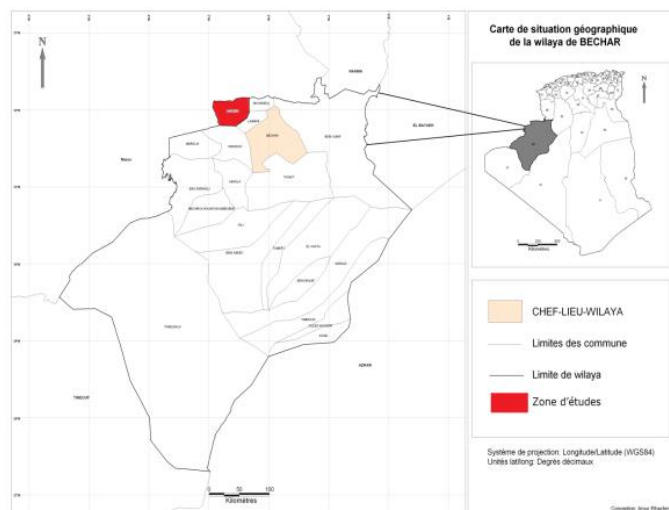
Result and discussion

In Tlemcen (Map 1), the chosen study stations (02) are located at the Benskrane locality (this is an artificial cave) and the other one is located in the locality from Sabraa elbabeda (this is a natural cave).

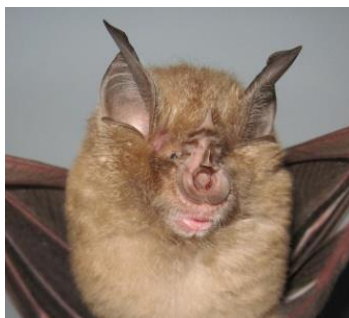


Map n ° 1: Location of the Tlemcen wilaya in Algeria (Geological Service Of Algeria, 1996).

At Bechar (Map No. 2) also two stations were chosen for the study. The station Boukayisse 1 (natural cave) is located 40 km from the city of Bechar. The second station (Boukayisse 2) is located 300 m in the north of Boukayisse station 1.



Map n ° 2: Location of the Béchar wilaya in Algeria (Geological Service De L'Algérie, 1996). The two selected species are described above: **Rhinolophus ferrumquinum** (Schreber, 1774) (The Greater Horseshoe Bat): picture n ° 1



Picture n ° 1: *Rhinolophus ferrumquinum* (Original, 2017).

It is a large species (length of the body is 5,7 to 7 cm), with a rather dark dorsal coat, a lighter head, belly and chest are beige. The lancet of the nasal leaf narrows regularly to the tip while the saddle is compressed at its center. The ears are large, broad, with a strongly convex outer edge, the wings are broad, and can envelop the whole body. **Asellia tridens** (Geoffroy, 1813): picture n ° 2



Picture n ° 2: *Asellia tridens* (Original, 2017)

The trident is a bat of rather large size (length of the body from 6.6 to 9 cm), with the fur of variable color, light brown to red, characterized by a nasal leaf whose enlarged lancet carries three square tips, the caudal vertebrae are welded into a rigid rod, except for the last two.

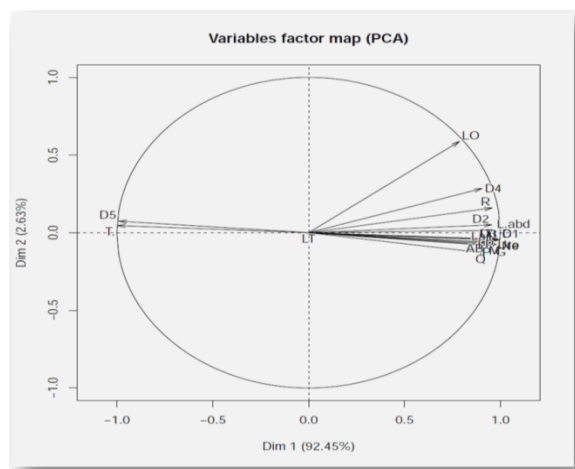
We obtained a computation rate of 0.43 for the species *Rhinolophus ferrumquinum* and 0.59 for the species *Asellia tridens* following Shannon and Weaver H's diversity index on Excel. The difference between these two values is probably due to a bottleneck effect suffered by *Rhinolophus ferrumquinum* due to probably abiotic stress. This index is relatively high for both species, which is probably a reflection of significant genetic diversity.

Concerning the 20 characters to be studied at the level of each species, there are 7 characters Forearm ((AB), Inch (D1), Head (Té), Nose (Ne), Knee (G), Tail (Q), Radius (R)) which represents a diversity index which varies between 0 (for *Rhinolophus ferrumquinum*) and 0.79 (maximum value) for *Asellia tridens*. LTt "Total Length" has a diversity index that ranges from 0 for *Asellia tridens* to 0.41 for *Rhinolophus ferrumquinum*. The character length of the tragus (LT), presents a diversity index H 'equal to 0 and it at the level of the two regions and for both species. This is probably due to the fact that the gene controlling this trait also controls characters of significant physiological interest.

Table 2: Comparison of the Shannon-Weaver Diversity Index between the two species studied.

Characters	Tlemcen <i>Rhinolophus ferrumquinum</i>	Béchar <i>Asellia tridens</i>	Total Average
H' _{AB}	0	0.48	0.24
H' _{D1}	0	0.47	0.23
H' _{D2}	0.73	0.52	0.62
H' _{D3}	0.33	0.76	0.04
H' _{D4}	0.32	0.76	0.54
H' _{D5}	0.43	0.41	0.42
H' _{LO}	0.46	0.36	0.41
H' _{LT}	0	0	0
H' _{Té}	0	0.21	0.1
H' _{Ne}	0	0.9	0.45
H' _M	1	0.93	0.96
H' _G	0	0.79	0.39
H' _{LP}	0.96	0.94	0.95
H' _Q	0	0.48	0.24
H' _{LTt}	0.41	0	0.2
H' _{Labd}	0.8	0.03	0.91
H' _{Lail}	0.43	0.47	0.45
H' _{Ure}	0.78	0.66	0.72
H' _C	0.79	0.77	0.78
H' _R	0	0.68	0.34
Moyenne total	0.43	0.59	0.51

It is noted at the level of the ACP of Figure No. 1 that the characters to study in the species *Rhinolophus ferrumquinum* in the Tlemcen region is closer in their majorities of the circle (apart from the LT character) which reflects a significant level of significance from a statistical point of view. It is also noted that the ACP in question represents 95.08% of information used for statistical processing which is very significant. We distinguish the formation of three groups of characters with positive, zero and negative correlations between these parameters.

**Figure 1:** PCA parameters studied for all individuals of the species *Rhinolophus ferrumquinum*.

The first group includes the parameters (AB, D1, D2, D3, D4, LO, NE, G, LP, Q, LTt, Labd, Lail, Ure, C, R and M). The second group, has a negative correlation with the first is includes the parameters (D5, Té). Finally, the third group (LT character only) has zero correlation with all other

characters as it is fixed ($LT = 0$). The positive correlation of these characters between them can be explained either by the influence of the genes, that is to say that these characters are controlled by a number of genes in common or these characters react in the same way vis-a-vis environmental conditions (adaptation). The null correlation of the LT character with all the other characters is due to the fact that the latter has no polymorphism. This trait is important for adaptation or controlled by genes that code for important physiologically functions.

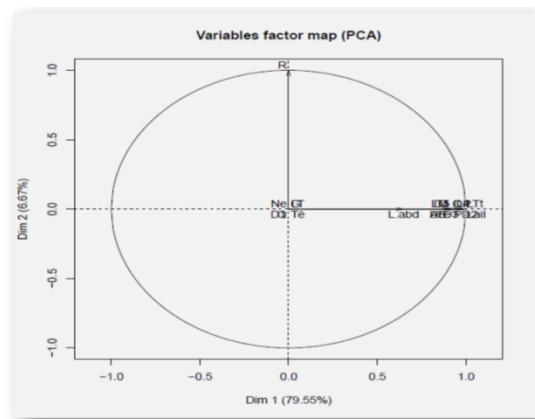


Figure n ° 2: PCA parameters studied for all individuals of the species *Asellia tridens*.

Note at the level of the ACP of Figure 2 that the characters to study in the species *Asellia tridens* in the region of Bechar is closer in their majorities of the circle which reflects a level of significance significant statistically . It is also noted that the ACP in question represents 86.22% of the information used which is statistically significant. We distinguish the formation of two groups of characters with positive and zero correlations between these parameters. The first group, includes the parameters (D2, D3, D4, D5, LO, M, LP, LTt, Lail, Labd, Ure, C, R), the second group presents with the first a zero correlation is includes the characters (LT, Te, Ne, Q, G, R, D1). The positive correlation of these characters between them can be explained either by the influence of the genes, that is to say that these characters are controlled by a certain number of genes in common, or these characters react in the same manner environmental conditions (adaptation). The correlation None of the characters Te, Ne, G, Q, R and D1 with all the other characters is due to the fact that the latter characters have no polymorphism. These traits are either important for adaptation or controlled by genes that code for important physiologically functions.

Concerning hierarchical cluster analysis (HCA) :

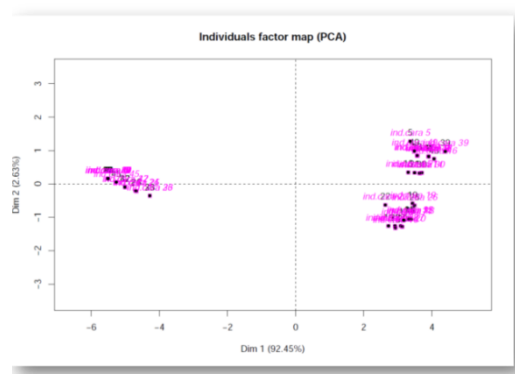


Figure 3: PCA representing the distribution of animals of the species *Rhinolophus ferrumquinum*.

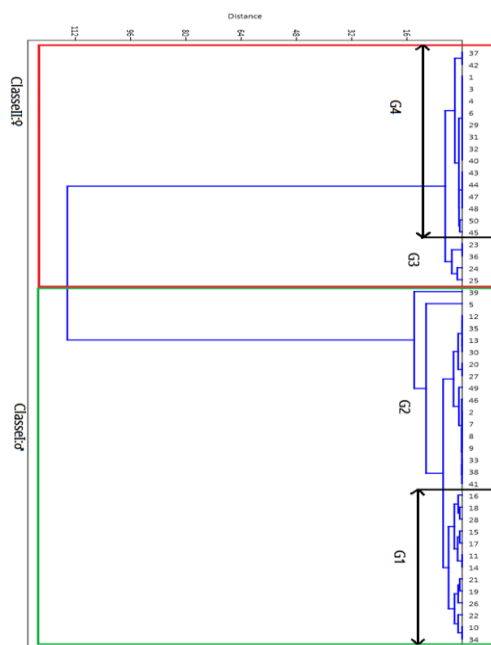


Figure 4: Ascending hierarchical tree HCA for animals of the species *Rhinolophus ferrumquinum*.

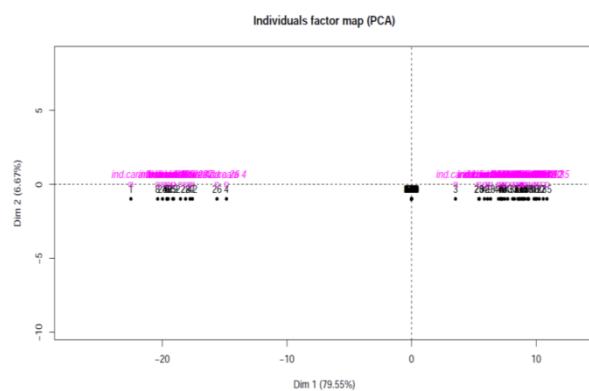


Figure 5: PCA representing the distribution of animals of the species *Asellia tridens*.

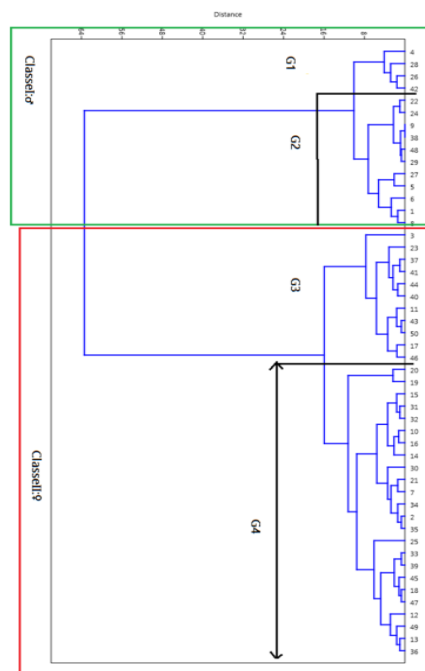


Figure 6: Ascending hierarchical tree (HCA) for animals of the species *Asellia tridens*.

It can be seen from Figures 3, 4, 5 and 6 that the two species studied present a subpopulation structure. It is also clear that sexual dimorphism exists in these species.

Conclusion:

About ten years ago, animals such as bats were relatively little studied in the Mediterranean basin. In Algeria, the number of works is very small. If at the level of Western Algeria, we engaged in this study, it is to deepen our knowledge of this faunal group whose ecological importance is proven. Indeed, through this work we have helped to explore sites, previously unknown, represented by natural caves, an old abandoned house (Wilaya de Tlemcen) and the palm grove of Boukaysse (Wilaya de Bechar). Animal genetic resources are an important element in the economic, food, environmental and sociocultural domains of a country. During this original study, we contributed to the morphometric study (20 measurements) of two bat species, the Greater Horseshoe Bat and Desert Trident in western Algeria. The analysis of the diversity of the two species studied, from 20 parameters by the calculation of the Shannon and Weaver index, revealed a greater diversity in the desert Trident compared to the Greater Horseshoe Bat, probably due to genetic drift imposed by an important abiotic stress. The study was performed on a sample of 50 individuals of each species studied (with blood samples on EDTA tubes). The data collected is analyzed by a principal component (PCA) in order to know the different correlations that exist between the studied characters and a hierarchical ascending group classification by the HCA function for give us an idea about their population structuration. These statistical analyzes were carried out by the software R version 3.0.3, Exel and the software XLSTAT 2016. We observed a positive correlation between some traits studied which can be explained by the influence of the same gene group on these characters and that sexual dimorphism exists for both species. HCA shown a population substruction in the two species.

It would be interesting to look for inter and intra-specific relationships, and what kind of anti-predator strategies are used by these mammals. In addition, it would be important to further study other aspects of the biology and eco-ethology of bats to identify their movement and behavior throughout the year, in order to better know the species and contribute to their preservation. However, it should be noted that a more serious threat is being exerted on bats populations in Algeria, it is mainly due to the human pressure in the environments of their distribution, causing impacts on their way of life, for that it

would be essential to create protected areas, with a view to safeguarding our biodiversity and our biological heritage for future generations, and to deepen our inventory through genetic studies to confirm their taxonomy. During this study an important first step in the pathway of genetic characterization was carried out. 50 blood samples were collected from two species (50 samples of the Greater Horseshoe bat species and 50 samples of the Desert Trident species), and another step was to be taken, ie the molecular study by microsatellite markers or SNP.

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