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The Morphometric Geometry of the wing venation in Culicidfauna (*Culiseta longiareolata*)

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Dedication

I dedicate this thesis to

The most cherished person my niece Ania
My dearest parents, sister and my brother-in-law who have been always there for me through all my struggles
My partner Djeddou Chaima
All my family.
A special dedication to my friends Yasmine,Rayane,Nada,Sara,Malak and Rania who shared with me good and bad memories.

Hadil

I dedicate this modest work to my dearest parents, as a token of my deep respect, my great love and my gratitude for their unfailing support throughout my life, for having encouraged and supported me during all these years and it is thanks to them that I support it today. May this work be for them a source of pride and a testimony of my affection and my gratitude.

May God give them health, happiness and long life.

To all my family and my partner Djaber Hadil.

To my best friends: Manel, Jihane, Nada,Nour, Sara and Rayane.

To my study colleagues and to all those who taught me

All along my school life.

Chaima

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List of abreviations

A: Altitude

ANOVA: Analysis of variation

***Cs. longiareolata*:** *Culiseta longiareolata*

CS: Centroid size

E: East

GM: Geometric Morphometric

LM: Landmark

N: North

PCA: Principal component axes

SL: Semi landmarks

T: Temperature

°C : Degree celsius

% : Percent

Introduction

Introduction

Arthropod embranchment is the most successful on our planet, with the most species and individuals of any animal kingdom. Arthropods abound in all ecosystems, from snow-capped mountains to abyssal gorges, and deserts to tropical forests (Morin, 2002). The majority of tropical and subtropical African countries provide an ideal environment for the development of harmful arthropods and disease vectors, which are severe constraints to food production (Icipe, 1994; Ben malek, 2010). Insects are the first arthropods to have colonized the earth (Lecointre, 2001).

The Diptera, specifically mosquitoes, are the most important group of vectors in human public health among the several orders that make up the insect class. Malaria, yellow fever, dengue fever, a variety of arboviral encephalitis, and lymphatic filariasis are all transmitted by them. They are to blame for the spread of a variety of diseases to both humans and animals (Rodhain & Perez, 1985). To this must be added the annoyance caused by the bites, as well as the significant financial cost of these health issues (Hadjoudj, 2012).

Mosquitoes are a valuable research resource for entomologists (Boulkenafet, 2006). In Algeria; (Merabti and *al*., 2021) makes an update checklist of Culicidae based on record published (1903-2021) presented with 53 species. The behavior of most Culicidal species varies from one location to the next in their distribution area, influencing their vector role (Hassaine, 2002).

The morphological characteristics of the Culicidae family are generally apparent, allowing for easy identification and a clear description. Their classification into subfamilies, genera, and subgenus, on the other hand, is significantly more difficult (Wilkerson, 2021). Culicidae systematics can be examined with Dichtomic keys, which allow species to be identified using a set of criteria and very precise microscopic descriptors (Aïssaoui, 2014).

Techniques were established in a rigorous quantitative discipline termed "morphometry" (Adams and *al*., 2013) to investigate the form, which constantly raises issues. Morphometrics is the quantitative statistical description of variation in biological shape (Rohlf, 1990; Zelditch and *al*., 2012; Cardini, 2013).

In Algeria, Barour (2012) was the first to use this method in his doctoral thesis to analyze the biodiversity of Honey Bee *(Apis mellifera)* populations, followed by Lateb (2014) who used it on the oaks of Akfadou. The latter's findings are highly intriguing. He was able to identify three probable hybrids in addition to the two previously mentioned in the literature, namely *x Quercus kabylica* and *x Quercus numidica*

Mosquito wing geometric morphometric (GM) is a well-established, low-cost, and reliable mosquito identification approach (Louise and *al*., 2015; Dujardin & Slice, 2006). It can be used to identify epidemiologically important vector mosquitoes, sibling species, cryptic species, and females in some species that have proven difficult to identify using other methods (Börstler and *al*., 2014; Vidal and *al*., 2011; Lorenz , 2012). The aim of this work was to use wing morphometric to properly identify mosquito species from the three most epidemiologically relevant mosquito genera.

Our research intends to investigate the wings geometry morphometric within this context. To examine inter-individual and inter-stational morphological diversity by using the approach and contemporary morphometric method on the females of *Culiseta longiareolata* (*Cs*.*longiareolata*) according to the effects of the different factors (Altitude (A) and temperature (T)) in a three different region.

This manuscript is structured in three parts: an introduction in the first part. The second part collects generalities on the family of Culicidae (morphological criteria, bio-ecology), a generality on the Geometry Morphometric; the method was based on the collection of *Cs.longiareolata* wings from three different regions. The third part contains the results and discussion of our work, and finally a conclusion.

Material and Methods

I.Material and Methods

I.1.Mosquitoes

I.1.1. Systematic

Culicidae, or mosquitos, belong to the Dipterea order and the Nematoceres suborder. Mosquitoes can be recognized from other Nematoceres by their lengthy proboscis and the presence of scales on the wing veins, according to (Seguy, 1951).

Subdomain: Animal

Subrange: Metazoan

Phylum: Arthropods

Subphylum: Antennates

Class: Insects

Subclass: Pterygotes

Order: Diptere (Linne, 1758)

Suborder: Nematoceres (Latreille, 1825)

Family: Culicidae (Latreille, 1907)

Subfamily: Culicinae

Genus: Culiseta (Neveu-Lemaire, 1902)

Species: *Culiseta longiareolata*

I.1.2. Morphology

Mosquitoes have two stages of development: the aerial phase, which includes adults, and the aquatic phase, which includes eggs, larvae, and pupae. This family, which comprises roughly 3546 species (Inventory, 2020) includes mosquitoes in the strict sense. Anopheles (400 species), Culex (800 species), and Aedes are the three most important medical genera (1200 species).

I.1.2.1. The adult

Figure 01: Morphology of *Culiseta longiareolata* (Original 2022).

I.1.2.1.a. The head

The head houses a large number of sensory organs: antennae and eyes that take up the majority of the antero-lateral region of the head and mouth parts. The median eclypeus limits the head to its anterior part. Vulnerable parts are protected in a labium or proboscis. The length of the maxillary palps varies depending on the species (Gopfert and al., 1999).

I.1.2.1.b. The thorax

The prothorax, mesothorax, and metathorax are three stiff segments that come together to form the thorax (Rioux, 1958). It is coated in elongated hairs, and the second segment, which is the most developed, has a pair of scale-covered wings with a simple venation. The third segment, which is less noticeable, lacks wings but is equipped with a complex neural system that serves a sensory function. It is necessary for flight: the loss of only one equilibrium renders this one impossible (Merabti, 2016).

I.1.2.1.c. The wing

Longitudinal and transverse ribs support the translucent wing membrane, which delimiting cells between them. Furthermore, these ribs transport scales, and the wing's posterior edge is adorned with a fringe of scales of various forms, colors, and arrangements, which also coat the thoracic segments and legs (Rodhain & Perez, 1985; Hadjoudj, 2012).The wings are made up of broad lines called veins and membranes called cells that span between them. The mosquito's wings have six longitudinal veins with scale-carrying branches.

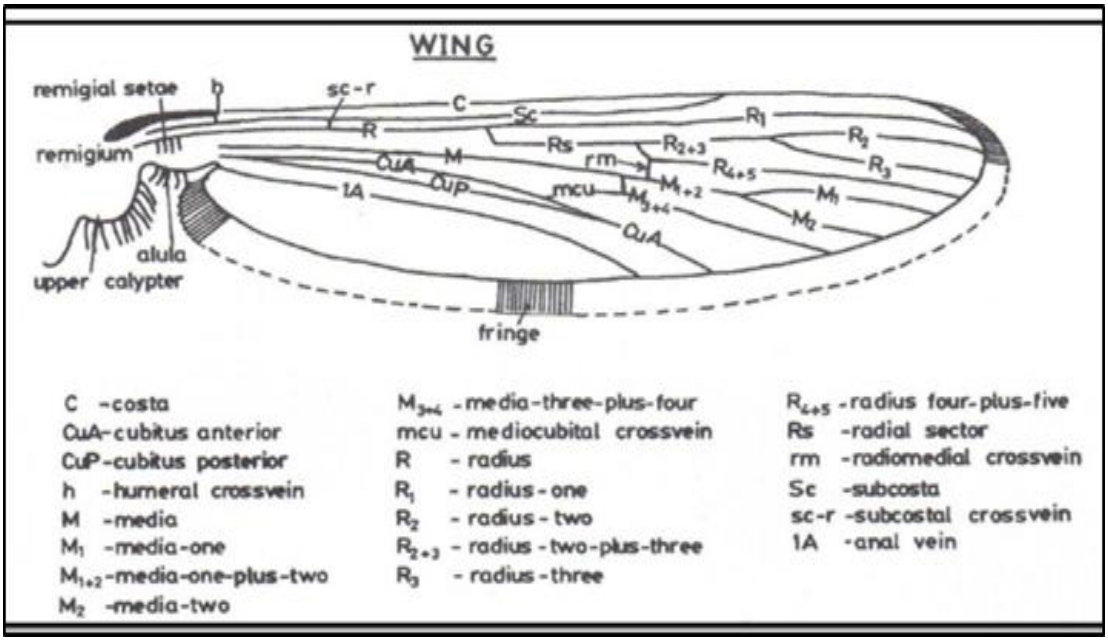

Figure 02: Wing venation in mosquitoes (Reuben and *al*., 1994).

I.1.2.1.d. The leg

The hip or coxa, the trochanter, the femur, the tibia, and a tarsus of five pieces, the last of which bears two claws and sometimes an empodium and two pulvilli, comprise each leg from its base to its distal end (Rodhain, 1985). The relative length of the five tarsi, the presence or lack of pulvilli, and the ornamentation owing to the scales are all taxonomic features seen on the legs (Himmi and *al*., 1995; Hadjoudj, 2012).

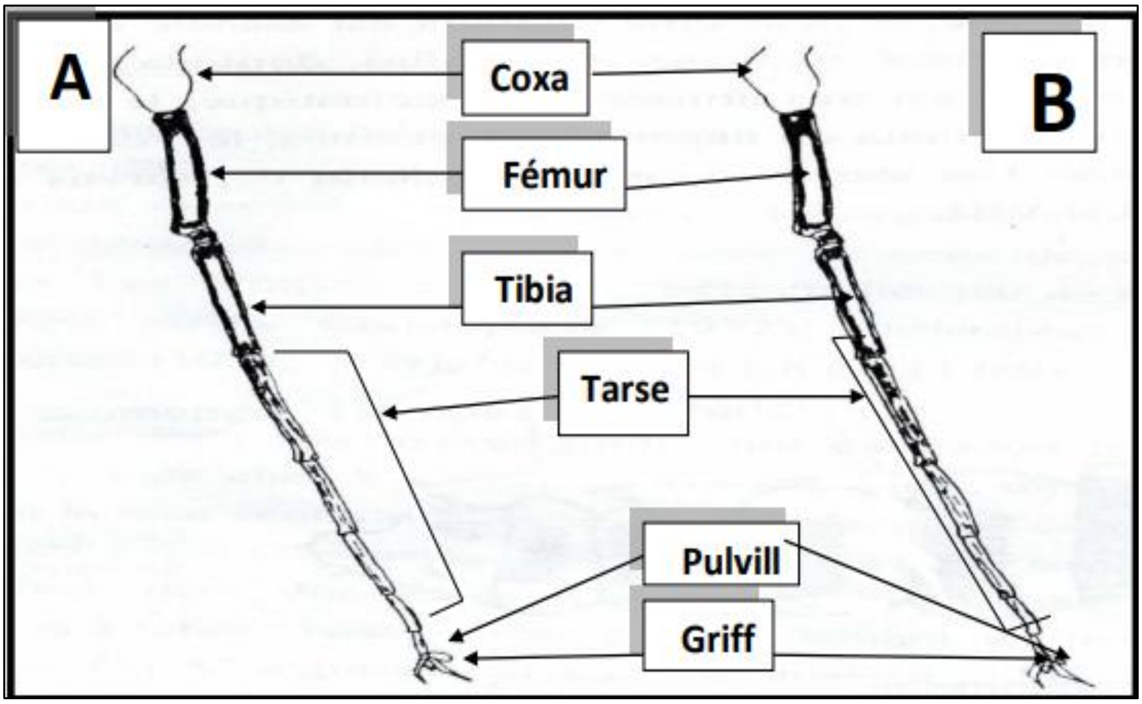

Figure 03: Morphology of the legs: **A**: anterior **B**: posterior (Bendali-Saoudi, 2006).

I.1.2.1.e. The abdomen

It is slender and lengthy (Himmi and *al*., 1995). Each segment has a dorsal (tergite) and ventral (sternite) component joined by a lateral flexible membrane, with bristles and scales. The genital appendages (genitalia) are essential in systematics, and the morphology of the last abdominal segments (9th and 10th) is particularly complicated, especially in males (Rodhain & Perez, 1985). The Phallosome (or penis) can be equipped with blades that are often separated into pointed teeth (leaflets) that are extremely useful for species identification (Himmi and *al*., 1995) (Hadjoudj, 2012).

I.1.2.2. The nymphe

The transformations that allow the mosquito to move from an aquatic to a terrestrial environment begin with the lysis of the muscles at the end of the larval stage and continue in the nymph stage with the development of a completely new system (Senevet, 1941).The nymph is shaped like a comma. It doesn't feed; instead, it draws on the stores built up during the larval stage. Two trumpets on the cephalo-thorax allow it to breathe (Himmi and *al*., 1995).

I.1.2.3. The larvae

This stage is aquatic aquatic and breathes by a siphon on the 9th segment of the abdomen. The lack of legs distinguishes Culicidae larvae from those of other aquatic insects. The moults separate the four larval phases L1, L2, L3, and L4 (Arbaoui, 2017).They consist of a hard head (due to chitinization), a thorax, and a less chitinized, softer abdomen (Tahraoui, 2012).

I.1.2.3.a. The head

A thick integument forms a sub-spherical capsule around this one. Laterally, two eye spots as well as two antennas with different features depending on the group but always carrying characteristic bristles can be distinguished. The chewing mouthparts are ventral (Berchi, 2000).

I.1.2.3.b. The thorax

Prothorax, mesothorax, and metathorax are the three divisions. Setae cover the thorax. The arrangement of the prothoracic setae enables species identification (Senevet, 1955 ; Rioux, 1958).

I.1.2.3.c. The abdomen

Elongated sub-cylindrical is made up of nine separate segments, the eighth of which is particularly interested in taxonomy. The abdomen generally ends with two important structures: the comb, located on the lateral surface and which is constituted by a set of spines, and the respiratory siphon which includes a certain number of taxonomic criteria, such as the subapical hook, the ventral siphonic comb and the siphonic setae (Hadjoudj, 2012).

I.1.2.4. The egg

The eggs measure around 1 mm in length (Arbaoui, 2017). They are usually laid on the water's surface, either individually or in pod-like masses, or on wet substrates, where they may hatch after a period of desiccation. Surface tension phenomena or the presence of lateral (Anopheles) or apical (Culex) floats cause the eggs to float on the water's surface (Rhodain & Perez, 1985; Ben malek, 2010), From the inside out, the egg contains the embryo, the pellucid vitelline membrane, a thick endo-chorion, and a more or less colored and decorated exo-chorion (Tahraoui, 2012).

I.1.3. Life cycle

Mosquito life cycles differ greatly between species. All are holometabolous, or fully metamorphosing insects. From mating until emergence, the cycle includes stages it goes through oviposition, hatching, post embryonic development, and pupation (Tabti, 2015).

I.1.3.1. Mating

Mosquito mating occurs in flight or in the foliage. The male Culicidae apply themselves to the females belly to belly in the solitary forms of Nematocerans at night, and the two insects continue to fly together. Fertilization is quick; however a temperature of at least 20°C is required. A single male can mate with multiple females at different times. They leave once fertilized in search of a blood meal. Males live for only a few days, feeding on the nectar of the flowers, which contains carbohydrates that give them with energy (Seguy, 1950).

I.1.3.2. Oviposition

After absorbing blood, the female seeks refuge in a safe location to digest her food. She deposits her eggs in various watery habitats or on the damp ground a few days later, depending on the species. Depending on the species, 50 to 300 eggs can be laid in a matter of hours or days (Rioux, 1958).

I.1.3.3. Hatching

The hatching can take a few hours or be more or less delayed depending on the species and the time of year. By several months at times some Culicide eggs can tolerate three to five years of drought (Anonyme, 2003). Early in the spring, when the eggs are exposed to water, they hatch (Seguy, 1950).

I.1.3.4. Growth and nutrition

The fat body is the most developed during development. It conserves energy reserves for use during metamorphosis. The larvae's growth is influenced by the type of food they eat, the humidity and temperature in which they live, as well as the water's composition. The larva of the Diptera is a little agile worm with opaline and transparent teguments at its first age, which lasts only a few days. The larval life comes to a close during the fourth stage which lasts longer (Seguy, 1950).

I.1.3.5. Nymphosis

The larva becomes less active once has completed its growing. It develops into a pupa or nymph. This one is shaped like a comma and is much stockier than the larva. The nymph is active, yet it can also be seen quiescent just beneath the water's surface, absorbing air through its breathing tubes. It dives to the bottom to avoid predators if disturbed. Although active the Culicidae nymph does not feed (Pihan, 1986).

I.1.3.6. Emergence

The adult mosquitoes emerge from the water's surface and lasts around 15 minutes (Roubaud, 1933).The nymph extends, the tegument divides dorsally, and the imago emerges from the exuvia very slowly. The newly emerged adult is quite soft. It usually stays on the surface until its wings and body have dried and hardened before flying away. Males often emerge before females because their sex glands require more time to mature (Reyes-Hernández & Perez-Stapels, 2017).

I.1.4. Ecological role

Mosquito larvae and adults are part of a variety of food chains. In both aquatic and terrestrial habitats, they represent an abundant source of energy for many predator species. Insects (dragonfly larvae, dytic larvae) and fish eat the immature stages in water. Insects, amphibians, reptiles, birds, and bats prey on adults. Mosquito larvae feed on very small particles of dead organic matter in stagnant water before maturing into adult mosquitoes that are eaten by a variety of terrestrial predators; they are detritivores that intervene in the chain of saprophages and also play an important role in the functioning of stagnant water of the aquatic ecosystems (Bourassa & Jean-Pierre, 2000).

I.1.5. Medical and veterinary interest of Culicidae

Culicidae are extremely important in veterinary medicine. Malaria, yellow fever, and dengue fever, as well as several arbiviral encephalitis and filariasis, are transmitted by them (Foster & Walker, 2019). The infectious agent (virus, bacteria, protozoan, or helminth) is transmitted from one infected individual to another, primarily by a hematophagous arthropod (insect or mite). Unlike a transmission that occurs in a simple pathogen transport, it is a biological or active transmission since the infectious agent completes a cycle of amplification or development in the arthropod vector. According to the OMS research, vectorial diseases such as dengue fever, chikungunya, Japanese encephalitis, West Nile virus, and malaria were responsible for around 17% of infectious disorders globally in 2004 (Elise, 2011).

I.2. Geometric morphometric

I.2.1. Historical

According to (Bookstein, 1998) the morphological analyses changed from descriptive to quantitative over the year. In 1971, a book entitled "Multivariate morphometrics" was launched, which treats morphometric using distance variables analyzed in a multivariate way (Blackith & Reyment, 1971).

However, it was not based on landmarks (LM), but it was a revolutionary step in the field of morphometry (Adams and *al.*, 2004). With the development of more elaborated statistical methods such as correlation coefficient (Pearson, 1895), analysis of variance (Fisher, 1935) and principal component analysis (Pearson, 1901; Hotelling, 1933), It has become possible to describe the biological variations from a quantitative point of view. Although such reliable statistical tests are available, distance-based morphometric still has serious problems. For example,

(a) there was no general deal between the different methods used for size correction;

(B) The measured linear distances were not always homologous identical or similar, which made the comparison of structures difficult

(C) At the beginning, it was impossible to graphically represent a shape based on linear distances; therefore, many aspects of the original shape were lost (Adams and *al.*, 2013).

These obstacles led to the development of a new technique to evaluate phenotypic variations while keeping the original characteristics of the form. In the 1980s, the field of morphometric saw another revolution with the invention of coordinate-based methods, the proposal of the "statistical theory of shape" (Mitteroecker & Gunz, 2009 ; Bookstein, 1998 ; Rohlf & Marcus, 1993) and the spline with thin plate (Duchon, 1976).

This new morphometric path has been named Geometric Morphometric (Rohlf & Marcus, 1993) because it allows to keep the original geometry of the shape and to translate the statistical results directly into the shape of the structure (Mitteroecker & Gunz, 2009).

The studies elaborated in the GM field have largely been originated from the studies of Kendall (1989) who suggested suitable mathematical and statistical approaches to use landmarks and highlighted the particular importance of the Procrustes distance.

Afterwards, (Bookstein, 1996) pointed out the usefulness of these approaches to biologists. It should be noted that the use of GM has become important in the biological and medical field because it solves the problem of difference within and between species and also sexual dimorphism. This method has been progressively applied to mosquitoes, more precisely to compare their wings. Numerous GM methods also have been developed in recent decades. Among the methods that study mosquitoes is the Procrustes method (Bookstein, 1998; Small, 1996; Dryden & Mardia, 1998). It combines landmark configurations using least squares estimates for scale, translation and rotation parameters.

Another used method is Fourier analysis (Lestrel, 1981; Rohlf & Archie, 1984) which scans points on contours and lines and then compares the curves using the function coefficients as shape variables in multivariate analyses (Adams and *al*., 2004).

I.2.2. Definition of geometric morphometrics

With morphometric we try to evaluate the variation of the shape and the covariation of the shape with other variables (Bookstein, 1991; Dryden & Mardia, 1998). The traditional morphometric approach consists of univariate or multivariate analysis of variables such as distance measurements, angles and/or ratios (Marcus, 1990; Reyment, 1991; Rohlf & Marcus, 1993).

While they are considered a mainstay in many fields (including biological anthropology), these methods do not characterize the entire form of an organism and the measurements are often considered independent of each other, although they are part of a larger structure and may therefore covary. This inability to preserve the geometric shape of the object or region under study has opened the door to the development of new morphometric techniques that preserve the geometric shape of the specimens to be analyzed: geometric morphometric. By using specifically defined homologous landmarks one can capture the shape of two-dimensional (2D) structures (Lorenz et *al.*, 2017).

This same period also saw the progression of the so-called "Procrustes paradigm" (Adams et *al.*, 2013), a set of approaches to GM that stem from the statistical theory of shape originally defined by Kendall (Kendall, 1984, 1985; Slice, 2001), and the relative decrease in the use of other morphometric approaches (e.g., Euclidean Distance Matrix Analysis) (Lele, 1993).

I.2.3. The use of Geometric Morphometric

It’s a technique developed to combine geometry and biology (Bookstein, 1982), GM allows studying the shape of biological structures in two or three spatial dimensions, to realize many statistical evaluations and to graphically represent the shape and size. This technique conserves the physical intactness of the shape and prevents its reduction to linear measurements that do not show the entire structure (Richtsmeier, 2002).

So the GM is based on the coordinates of identifiable landmarks (LMs) that are corresponding (homologous) from the evolutionary point of view. There is another type of labeling used for morphometric analysis that is not represented by the LMs: the position of the points placed on the curves, which are called semi landmarks (SL). When the location of a landmark on a smooth curve or surface cannot be clearly identified, it may be treated as a SL that is allowed to subjectively slide along its curvature. In those cases, only the position perpendicular to the curved surface bears a biological signal (Mitteroecker & Gunz, 2009).

The two-dimensional shapes of the wings and its veins that include natural anatomical landmarks have made the wings a key parameter in morphometric comparison in the study of mosquitoes. In addition, most veins present conspicuous landmarks and are homologous, so that they can be found in all representatives of the Culicidae family. With the GM it is possible to detect precisely where the variation is located and it can be quantified to use it in phylogenetic or biogeographic comparisons (Rohlf & Marcus, 1993).

The digitization of the LM coordinates needs knowledge of the organization but not necessarily a great technical experience and this makes the GM a simple, fast and not expensive technique. Shapes can be compared with minimal interference due to different sizes when the allometric effect (residual) is eliminated by multivariate regression analysis; this can be considered an advantage of GM (Adams and *al*., 2004).

I.2.4. The application of Geometric morphometric in mosquitoes

The shapes of organisms may present either similarities or differences, depending on gender, geographical location, phylogenetic relationship, ecological relationships and types of treatments suffered. The GM can determine morphological variations and their causes, both within and between populations (Lawing & Polly, 2010).

I.2.5. Problems in geometric morphometric analysis

Although GM has a simple execution technique, but it is necessary to be careful not to fall into the error during the execution and the analysis. Overestimation of the variation between samples can occur when we do incorrect recognition and marking of LMs (Lorenz et *al*., 2017).

In the face of the obstacle of dark spots or scales on the wing of mosquitoes Lorenz & Suesdek (2013) evaluated the effectiveness of wing marking before and after scale controls and observed that physical or chemical treatment effectively increases the visualization of LMs in Anopheles.

Physical and/or chemical scales removal treatments have been widely used in studies of mosquitoes of the genus

Aedes (Morales-Vargas et al., 2010; Kuclu et al., 2011; Vidal and Suesdek, 2012; Vidal et al., 2012; Louise et al., 2015), Anopheles (Aytekin et al., 2009; Vicente et al., 2011; Lorenz et al., 2012; Lorenz et al., 2014; Lorenz et al., 2015a; Virginio et al., 2015), Culex and Mansonia (Ruangsittichai and *al*., 2011).

Allometry, the change in shape as a function of differences in size also requires attention in GM studies. Although size and shape are not independent indicators, allometry can be evaluated using linear regression techniques (Lorenz et *al*., 2017).

Dujardin (2008) Explained that, in studies of insects belonging to the same species, the observed variation in shape may be a consequence of variation in size, and the latter is often induced by the environment. Although the allometric residue acts as a bias in shape analyses, According to (Virginio and *al*., 2015); to make a specific distinction between the sexes in studies of sexual dimorphism, it is necessary to maintain the variation in size. Another consideration involves the use of both left and right wings in the same analysis.).

Environmental stress can cause bilateral asymmetry, fluctuating asymmetry or even directional asymmetry for mosquitoes during their development (Peruzin, 2009; Galbo & Tabugo, 2014).

An asymmetry test should be performed before starting the GM analyses when symmetry is important for the study under development, to see if it is practical to work with the left and right wings in the same analysis. It is not recommended to use the right and left wings that are highly correlated and redundant in the same analysis in order not to distort the results; because the information will be duplicated (Lorenz et *al*., 2017).

I.3. Study Area

Algeria is located in the north of Arica with a surface of 2,382 million km2. Collection of mosquito samples was conducted in three cities namely, Annaba (36°46'19N, 07°53'50E), Chetma (34°50′60N, 5°48′35E) and Djemina (34.95°66′87N, 6.40°25′52E).

I.3.1. Annaba

Is located in the northeast of Algeria. Annaba has an average temperature of 18.4°C throughout the year and precipitation averages 712 mm (650 and 1000 mm/yr) per year. The probability of rainfall is very high during the period January– March and during October–December (Chemam et al., 2018). With an altitude of 3-1,009 m.

Figure 04: The region of Sidi Ammar (Annaba) (Google Earth, 2022).

The study was performed on the region of Sidi Ammar, Annaba.

I.3.2. Chetma

On the other hand, Chetma hasan average annual temperature of 19.7 C. The mean annual precipitation reaches 672.3 mm. It is located 8 km east of the capital of the wilaya of Biskra. With an altitude of 92m.

Figure 05: The region of Chetma (Biskra) (Google Earth, 2022).

I.3.3.Djemina (Tajmint)

With an altitude of 1031 m, this stronghold is located on the territory of the commune of Tkout, about 40 kilometers from the main town of the commune of Biskra. Over the year, the average temperature in Djemina is 22.4°C, with an annual rainfall of 195.9 mm.

Figure 06: The region of Djemina (Google Earth, 2022).

Table 01 : Characterization of the three regions**.**

District	A (m)	Geographic coordinates		Climate	T(°)	Month	Number of wing
		North	East				
Annaba	139	36°46'19	7°53'50	Sub-humid	16°	February	30
Chetma	92	34°50'60	5°48'35	aride	18°	March	31
Djemina	1031	34.95°66.87'	6.40°25.52'	Semi-aride	9°	December	6

I.4. Mosquitoes sampling and identification

- Mosquitoes were captured in their immature stages (larvae and pupae).
- Collected specimens were stored in a laboratory under standard temperature and humidity settings (25°C; 80% relative humidity) with a photoperiod of 12:12 (light/darkness).
- They were raised in the laboratory until they reached adulthood.
- Species identification and sex determination of the specimens were performed using the Mediterranean African Culicidae software (Brunhes, 1999) and dichotomous key (Himmi and *al*., 1995).

I.4. Wing preparation

- The right wing of female mosquito was removed from the thorax
- Following the protocol described by (Lorenz, 2013); the right wing of each specimen was removed and bathed 20min in a solution containing 3% sodium hypochlorite (NaClO) to remove scales using cotton swabs.
- The wings were washed using ethanol (99.5%).
- After that, the wings were bathed in a solution containing acid fuchsin for 1 hour and washed afterwards with 70% ethanol twice.
- The wings were mounted between a slide and a cover slip (15x15 mm), with a drop of Eugenol®.

I.6. Data analysis

I.6.1. Imagery

-The mounted wings were photographed with a camera (OPTIKA C-B5) with a binocular magnifying glass.

-A scale bar was used with millimeter paper taped with a magnifying glass.

I.6.2. Programs

-Jim Rohlf's set of tps include all of the essential programs we used:

I.6.2.1. TpsUtil

This program gave us the ability to create tps files.

The result of this step was to have a tps file that is essentially a list of our specimens, which we can open in **tpsDig** to collect data.

What the tps file looks like (opened in a text editor like Notepad):

```
LM=0
IMAGE=file/number of the sample/Region.JPG
```

Our specimens (given by the IMAGE names) currently have no landmarks (LM=0).

I.6.2.2. TpsDig

This program made it easy for us to locate our specimens by placing landmarks on our images and to record scale factors, all while saving this data in the tps file.

13 landmarks were identified and digitized using **tpsDig (V8.81)** software in order to generate cartesian coordinates in two dimensions for each mosquito individual.

Consistency in the placement of landmarks is essential, so it is important to spend a lot of time preparing and thinking about the placement of landmarks.

Our tps file should now look like this:

```
LM=13
563.00000 732.00000
432.00000 749.00000
435.00000 678.00000
572.00000 685.00000
563.00000 732.00000
432.00000 749.00000
435.00000 678.00000
572.00000 685.00000
563.00000 732.00000
432.00000 749.00000
435.00000 678.00000
572.00000 685.00000
254.00000 654.00000
IMAGE=/file/number of the sample/Region.JPG
ID=1
SCALE=0.007664
```

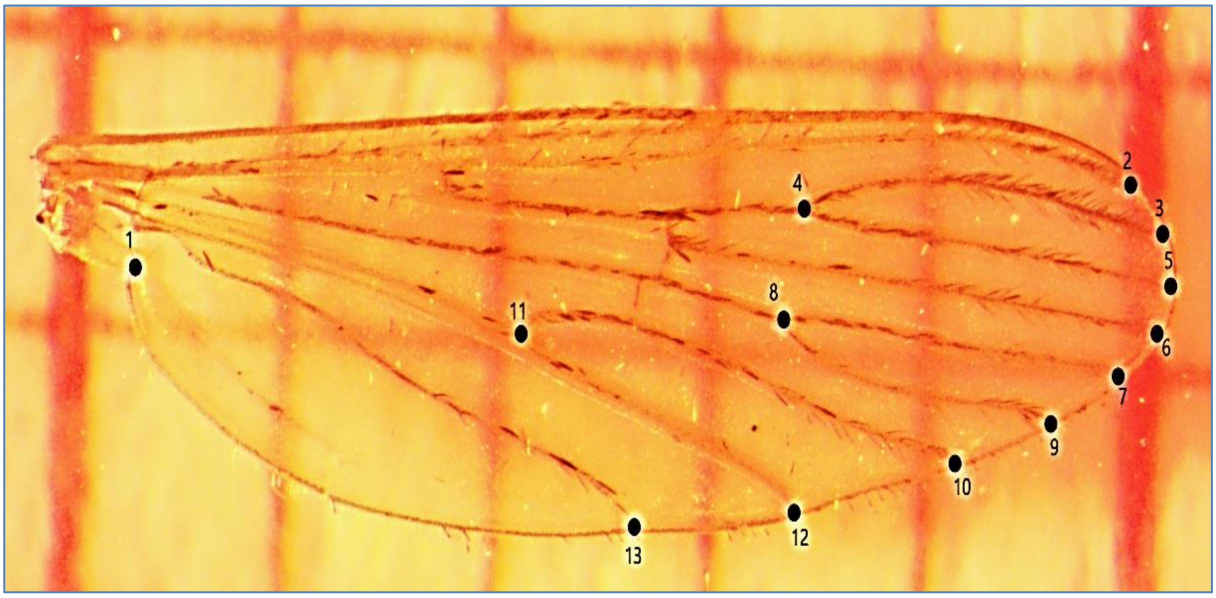

Figure 07: Right wing of *Culiseta longiareolata* numbers represent the position of landmarks. Scale1mm (Original, 2022).

Table 02 : Description of the landmarks (Original).

Landmarks	Description of the landmarks
1	Anal A1
2	Distal end of radius
3	Radial branch 2
4	Origin of radius branches 2 and 3
5	Radial branch 3
6	Distal end of radial branches 4 and 5

7	Distal end of media 1 and 2
8	Radio-sectoral vein
9	Distal end of media 3 and 4
10	Distal end of cubital vein 1
11	Origin of cubital 1
12	Distal end of cubital vein 2
13	Anal vein

I.6.2.3. MorphJ

This software package is integrated to perform geometric morphometry analysis. The main objective of MorphoJ was to provide a unique and integrated environment for geometric morphometry to focus on the biological and statistical aspects of the analysis (Klingenberg, 2011).

I.6.3. Centroid size and shape

To compare the size of the wing between the different groups (altitude and temperature), the centroid size (**CS**) derived from Cartesian coordinate data, was used as an isometric size estimator. The CS is defined as the square root of the sum of the square of the distances between the center of the configuration of the LM (or centroid) and each LM. Since the CS is based on Cartesian coordinates (XY), the results of the calculation mentioned above are a one-dimensional scale.

Results and Discussion

II. Results

II.1. *Cs. longiareolata* morphometric measurements analysis

The results show that there is a lot of variation in the characteristics of different regions depending on two factors: altitude (A) and temperature (T).

II.1.1. Checking for outliers

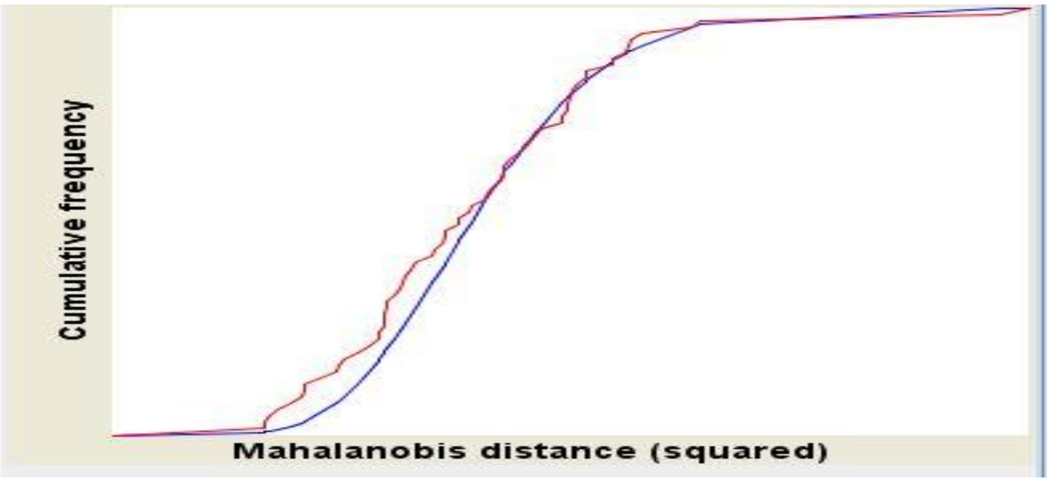

Figure 08: Cumulative distribution diagram of specimen distances and mean shape of average shapes of the complete sample (Original, 2022).

Because the data "in red" on (Figure 8) do not deviate from the normal distribution shown by the data "in blue," we can conclude that there are no outlier values and that the data follow a normal distribution.

II.1.2. Procruste's Superposition

Following Procruste's superposition in MorphoJ, we will show the graph illustrated by (Figure 9).

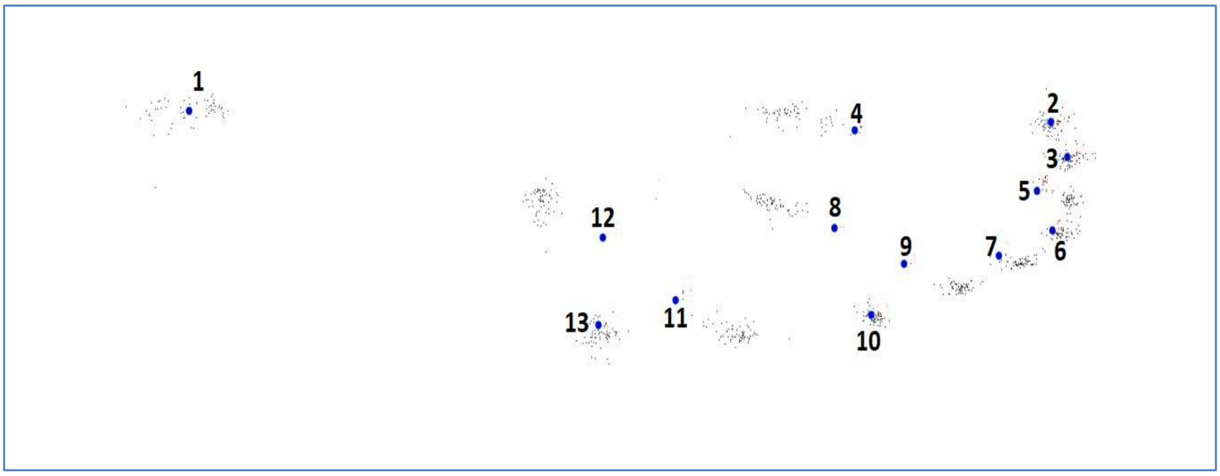

Figure09: The result of Procruste's Superposition (Original, 2022).

The results show that the distribution of LM used is located continuously. The consensus landmarks, shown in blue in (Figure 9), are each surrounded by a cloud of points representing the different coordinates of all specimens. Points with a compact, dark cloud inform us that they have coordinates close together, while a spread, diffuse cloud tells us that the points are moving away from the consensus.

II.1.3. ANOVA analysis for the centroid size for the females wings of the three regions

To analyze the morphological variation of *Cs. longiareolata* mosquito wings from different regions between altitudes and temperatures, we used a hierarchical analysis of variance (**ANOVA**). The results are shown in (Figure 10).

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Statistix 8.0                                               12/06/2022, 19:28:59

One-Way AOV for CZ by REGION

Source     DF        SS        MS        F        P
REGION      2    124212   62105.8     6.05   0.0028
Error     206   2115688   10270.3
Total     208   2239900

Grand Mean 134.62    CV 75.28
                                    Chi-Sq   DF       P
Bartlett's Test of Equal Variances     73.9    2   0.0000
Cochran's Q                      0.5727
Largest Var / Smallest Var       9.5699

Component of variance for between groups   744.068
Effective cell size                           69.7

 REGION    N    Mean      SE
 ANNABA   70  104.71  12.113
 CHETMA   69  134.87  12.200
DJEMINA   70  164.28  12.113
```

Figure10: Result of hierarchical analysis of conformation variance for the three regions (ANOVA) (Original, 2022).

From (Figure 10), we note that the difference between specimens from different regions statistically is highly significant (p- value< 0.0028). Regarding the conformational variation the results are mostly in agreement with those obtained for the centroid size.

The ANOVA analysis shows that there are morphological variations in the wings of mosquitoes from one region to another depending on the environmental factors, the nature of breeding sites, alimentation factors. The Temperature or the altitude factors maybe one of those factors that could affect the size or the shape of the females' wings.

II.1.4. PCA result for the wings of the different regions

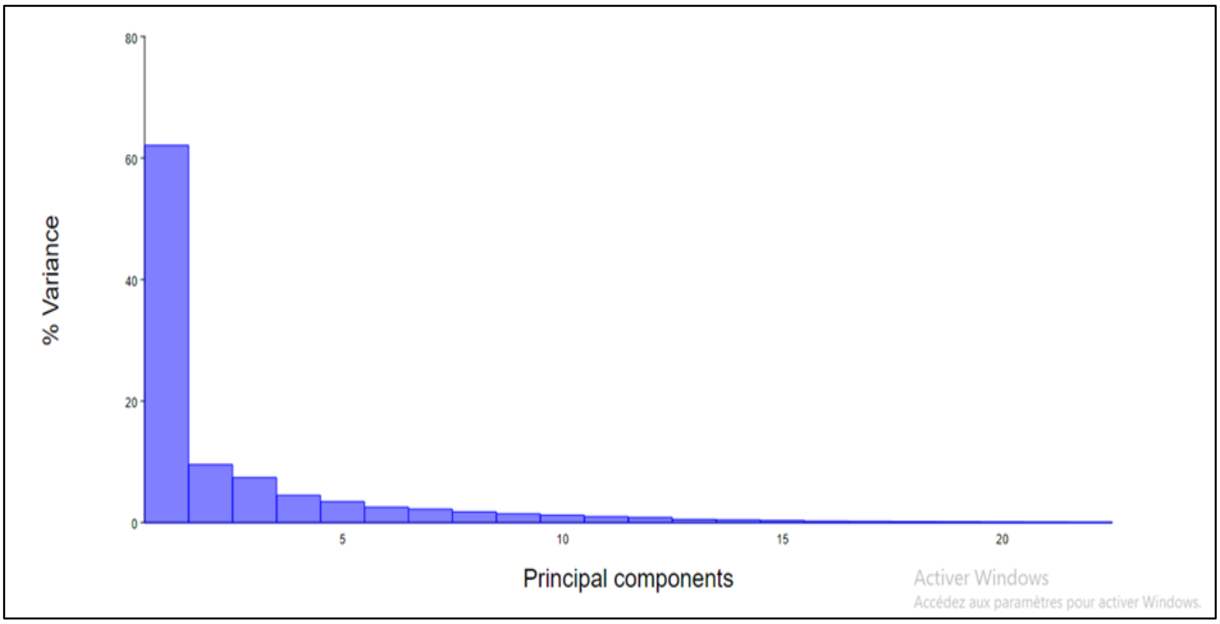

Figure 11: Percentage change in principal component axes (Original, 2022).

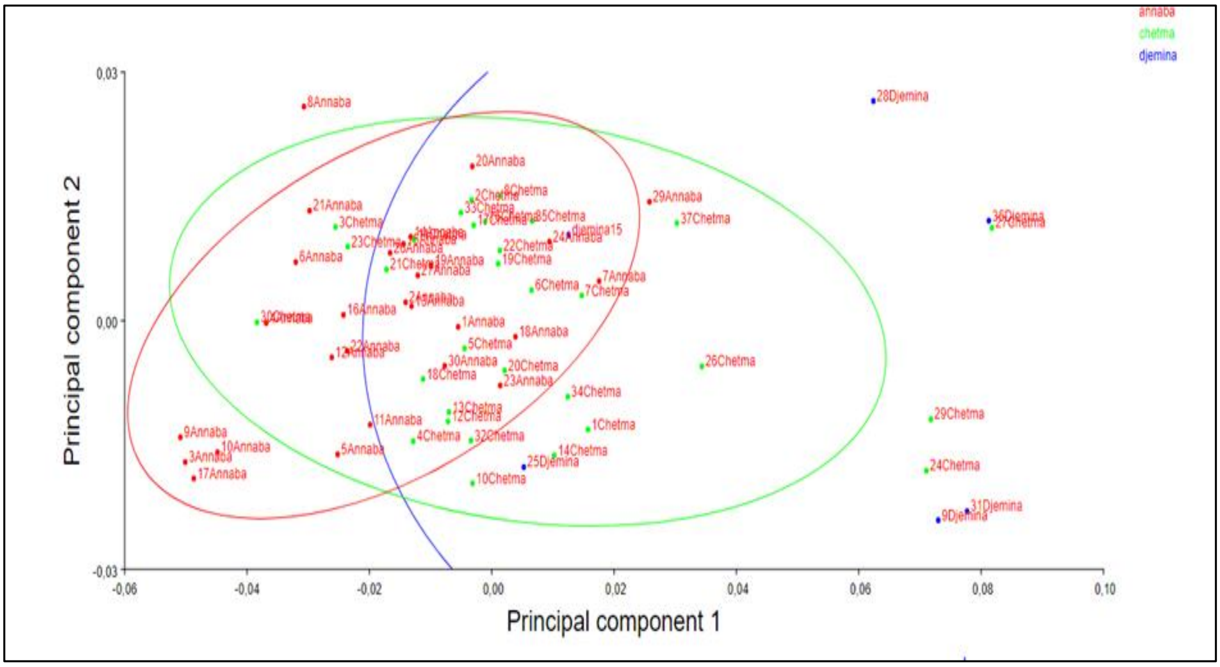

Figure 12: Projection of the specimens according to the regions (Original, 2022).

(Figure 11) shows the results obtained from the PCA. When a PCA was conducted on the 13 wing landmarks, the first two PC's summarized 60.61 and 33.30%; the first PC value suggests relative differences in the relative positions of the landmarks regarding the middle and the left of the wing. Main deformations centred on the medial of the wings of the landmarks 1, 4, 8 and 11 (Figure 13). Therefore, only the latter will be taken into account for the analyses because they explain the morphological deformation (conformation).

II.1.5. Conformation change

The following results highlight a variation of the conformation which is given by several visualization modes: A « transformation grid », B «transformation lolipop» et B «wireframe graph».

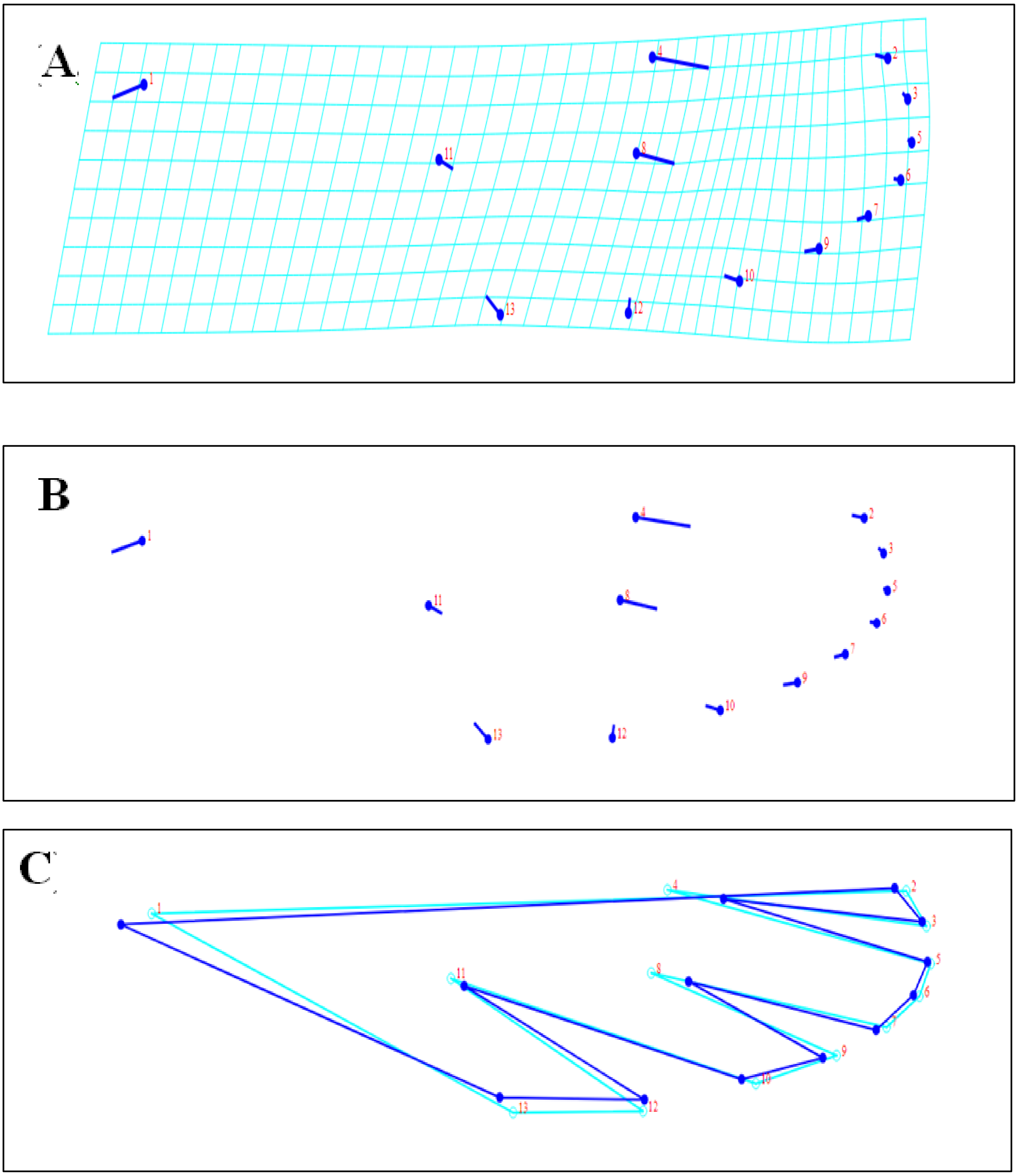

Figure 13: Visualisation of the change of conformation of the wings (A,B and C) (Original, 2022).

Generally speaking, we can see that there is a morphological difference that can be explained by a shift within the landmarks. Precisely, we can divide them into three levels (Figure 13 A and B):

Level 1: we observed a large-scale displacement around the landmarks 1, 4,8 and 11.

Level 2: a medium scale displacement were observed on the landmarks 9,10,11and 13.

Level 3: a very small displacement were observed on the landmarks 2,7and 12.

In (Figure 13 C) we present the mean annual configurations of the 13 *Cs.longiareolata* wing landmarks with connecting lines for the Procruste analyses. Comparison of these landmarks and their connecting lines indicate the displacement among the 13 wing landmark.

II.1.6. Analysis of variance of centroid size

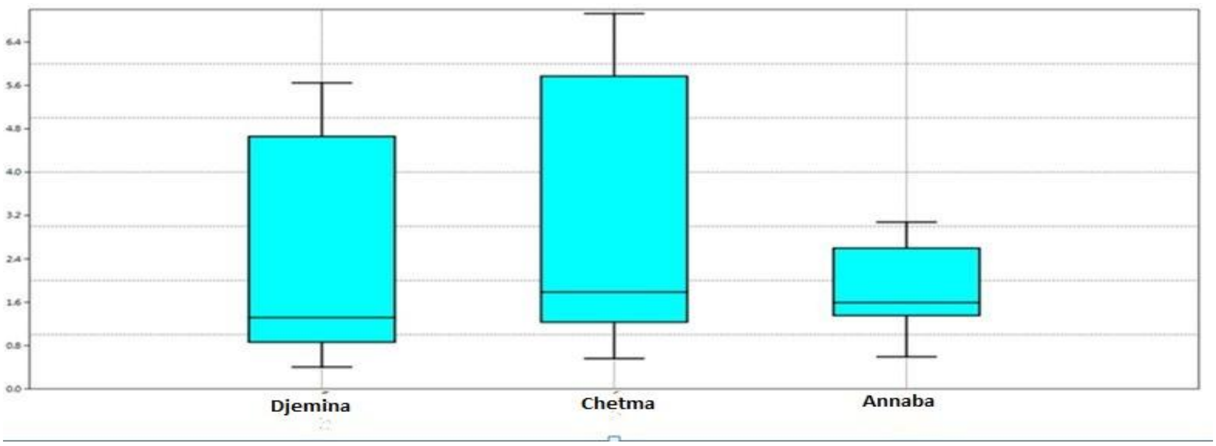

Figure 14: Box diagram representing *Cs.longiareolata* wing centroid size variation of different region (Original, 2022).

Centroid sizes were used as measures of overall wing size differences among different regions for each individual. From the results obtained, it can be seen that there is a significant difference in the centoid size of the wings between the different regions studied.

According to the box figure (Figure 14), centroid size showed a linear association with temperature and altitude, indicating that: at medium temperature and altitude smaller wing were observed in Annaba, at low temperature and high altitude medium wings were presented in Djemina and at high temperature and low altitude bigger wings were observed in Chetma.

These differences observed regarding wing shape or size which is significant could be attributed to variables like altitude and temperature, or a combined effect of both factors.

III. Discussion

The objective of our work was to confirm the effect of some envirronemental factors on the size and shape of the wings mosquitoes. To obtain this goal, we compared the right wings of female *Cs.longiareolata* mosquitoes collected from three regions (Annaba, Chetma and Djemina). These regions differ from each other in several ecological factors. The bioclimatic stage, altitude, climatic factors, nature and types of breeding sites....etc. we base in our initiative, on the temperature and the altitude of these sampling sites, the two factors can interpret the morphological difference of the wing of this species.

To begin with geometric morphometric analyses on the wings of specimens captured along the regions, the GM results obtained indicate that there is a morphological difference such as size and shape in the right wing of the female between the populations of the three regions, this difference may be due either to altitude or temperature or to the combined effect of environmental factors, a common phenomenon in many insect species. The results obtained from the ANOVA test assured us that there is a really significant difference in the right wings of *Cs. longiareolata* females in the three regions Annaba, Chetma and Djemina.

According to these morphological variations in the wings in different geographical locations, our study has found that there is a relationship between morphology (both size and shape) and environmental factors we tested: temperature and altitude. As already mentioned in a study; that coastal habitats are strongly influenced by weather and other seasonal environmental factors throughout the year (Adhami & Rieter, 1998). Other work has also identified temperature as a factor related to the wing size and shape of mosquitoes (Klingenberg, 2011). Similarly, significant results were found for *Anopheles funestus* in Africa for elevation testing rainfall and temperature and other variables (Ayala et *al*., 2011).

The displacement of the landmarks as seen from the results of the MorphoJ conformation, assures us the significant effect of all environmental variables studied within the specimens. The main deformation on the wing centered on anal A1, origin of radius branches 2 and 3 and radio-sectoral vein. Followed by an average deformationsat the level on the distal end of media 3 and 4, distal end of cubital vein 1, the origin of cubital 1 and the anal vein. Then a slight deformation in distal end of radius, distal end of media 1 and 2 and distal end of cubital vein 2 (Table 2).

According to the diagram box, centroid size was used as a measure of overall wing size differences among *Cs.longiareolata* populations, so our results highlight that the CS of the wings differed significantly from region to another, which demonstrate a significant impacts of environmental factors. So depending on the altitude and temperature we can: say bigger wings were observed in Chetma with 92m and 18°C, smaller wings were presented in Annaba with 139m and 16°C and between them a medium wing were observed in Djemina with 1031m and 9°C.

So in our case, we can indicate that there is a meaningful relationship between temperature and/or altitude with the size and the shape of wings; therefore the female mosquitoes ensure a morphological change in the wings so that they can play their biological role in different altitudes and temperatures. We can conclude that mosquitoes with bigger wing occurred at low altitudes and high temperature. We suggest that this variation is caused by the combined effect of the environmental factors.

However in a study about *Aedes albopictus* in a region of the Colombian Central Andes, they presented results agree with our results, that smaller wings were observed at altitudes between 1,200m and 1,500m for males and females. Bigger wings were found females at 1,400m and for males between 1,400and 1,700m (Leyton and *al*., 2020).

This does not consistent with the results of (Demirci and *al*., 2012) which indicate that size differences were significant between most populations with samples from populations at an elevation of 1,768 m and 1,876 m having relatively larger wings. So the centroid size is associated with altitude and showing a positive correlation between size and altitude; indicating that individuals with larger body sizes occurred at higher altitudes.

In addition , in another study about *Aedes albopictus* in Albania highlighted different results from ours, they show the presence of size differences between altitude groups with, at higher altitude, females showing larger wing, and they observed also that group of insects at low altitude display larger wings than expected (Prudhomme and *al*., 2019).

In turn, according to (Mohammed, 2011) in a study about *Aedes aegypti* , the wing shape and size observed may be due to the influence of temperature so their results do not corresponded to our own results. In this species under laboratory conditions, it has been noted that larvae subjected to temperature between 24 and 35°c, have generated males and females with larger wing size in temperature between 24 to 25°c , while at temperature from to 34 to 35°c, males and females have been obtained with smaller wing size.

On the other hand, a study about Aede*s*; their result was different from ours; they indicated that individuals reared at higher temperatures are expected to have smaller wings (Phanitchat and *al*., 2019; Ray, 1960).

It is generally known that populations located along environmental gradients caused by factors like latitude or altitude have spatially varied selective pressures (Cheng et *al*., 2012), as observed for several Dipteran species (Hoffmann, 2007; Huey and *al*., 2000; Hoffmann & Weeks, 2007; Huey et *al*., 2000). Although the relationship between wing shape and environment is still unknown, one explanation is that this phenotypic characteristic, influenced by a variety of genetic variables (Birdsall and *al*., 2000; Zimmerman et *al*., 2000), possibly vulnerable to environmental stresses when developing (Klingenberg, 2010). Additionally, seasonal and temporal variations can affect the wing size and shape of insects (Francuski and *al*., 2011; Schachter-Broide and *al*., 2009; Vidal and *al*., 2012)

According to a study that examines the impact of altitude on mosquito phenotypes, females' size and shape analyses revealed notable variations among altitudinal groups. This result is most likely related to biological (Gojkovic and *al*., 2019) and/or environmental factors such as different blood sources (different host populations available between stations), distinct microhabitats (Parker and *al*., 2019), varied ecosystem vegetation (Darriet, 2016), and climatic effects, such as temperature changes between altitude groups (Briegel & Temmarman, 2001; Morales and *al*., 2013; Phanitchat and *al*., 2019).

However, despite a lack of genetic differentiation among populations of *Cs.longiareolata*, the wing size and shape data indicate that there are significant phenotypic differences among them from region to another. The size and shape variation analysis of wings showed that there is a positive correlation between wing (body) size/shape and altitude/temperature and may be a combination of the environmental factors. This observed positive relationship between combined effect of environmental factors and body size/shape may indicate a response of the female of *Cs.longiareolata* to changes in environmental factors to adapt to the conditions for the continuation of its major role.

Conclusion

Conclusion

This work had for ambition to highlight the effects of altitude and temperature on the wings (size and shape) of Culicidae by the geometric morphometric technic to verify morphological variability in *Cs.longiareolata* wings. Differences in wing size and shape maybe corresponded to differences in weather conditions, particularly altitude and temperature. On a small scale and across an altitudinal gradient of region, we found that geometric morphometry can identify phenotypic variation for *Cs.Longiareolata* wing size and shape. The results obtained from our study; we find that the bigger wing were presented in low altitude and a high temperature, we suggest that there is a combination between environmental factors on *Cs.Longiareolata* wing, this results of variation in wing size and shape open perspectives for more specific research on other mosquitoes species.

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Abstracts

الملخص

Culicidae(عائلة البعوضيات) هي الحشرات المعروفة باسم البعوض، مصنفة في رتبة ثنائية الاجنحة ورتيبة خيطيات القرن. كان الهدف الرئيسي من هذا العمل هو دراسة التباين الظاهري لأجنحة إناث *Culiseta longiareolata* التي تم أخذ عينات منها من ثلاث مناطق مختلفة (عنابة,شتمة و جمينة). تم إجراء تحليل الأنماط الظاهرية لجناح *Cs.longiareolata* باستخدام نهج الشكل الهندسي (GM) على الجناح الأيمن. لاحظنا الاختلافات في الشكل والحجم بين مجموعات الارتفاع ودرجة الحرارة لـ*Cs.longiareolata* . تم توضيح الاختلافات في حجم الجناح وشكله بين مجموعات الارتفاع ودرجة الحرارة للأجنحة الخاصة بالحشرة الانثى. تشير هذه الاختلافات بين المجموعات البعوض انهم يتعرضون لضغوط بيئية. تشير هذه النتائج إلى أن مجموعة من العوامل البيئية هي المسؤولة عن هذا الاختلاف في الجناح.

الكلمات المفتاحية : Culicidae ; *Culiseta longiareolata* ; الهندسة الشكلية ; الارتفاع ; درجة الحرارة ; الجناح .

Abstract

The Culicidae are the insects commonly called mosquitoes, classified in order of Diptera and the sub order Nematocera. The main objective of this work was to study the phenotypic variation of *Culiseta longiareolata* female wings sampled from three different regions (Annaba, Chetma and Djemina). The analysis of *Cs.longiareolata* wing phenotypes was performed using a Geometric Morphometric (GM) approach on the right wing. We observed variations in female's wings (shape and size) among the altitudinal and temperature populations of *Cs.longiareolata*. This difference between groupes indicates that these populations are exposed to environmental pressures. These results suggest that there is a combination of environmental factors that cause this wing variation.

Keyword: Culicidae ; *Culiseta longiareolata* ; Geometric Morphometric ; altitude ; temperature ; wing .

Résumé

Les Culicidae sont les insectes communément appelés moustiques, classés dans l'ordre des Diptères et le sous-ordre des Nématocères. L'objectif principal de ce travail était d'étudier la variation phénotypique des ailes des femelles de *Culiseta Longiareolata* échantillonnées dans trois régions différentes (Annaba, Chetma et Djemina). L'analyse des phénotypes des ailes de *Cs. longiareolata* a été réalisée en utilisant l'approche de la géométrie morphométrique (GM) sur l'aile droite. Nous avons observé des variations des ailes de femelles (forme et taille) parmi les populations altitudinales et de température de *Cs.longiareolata*. Ces différences entre les groupes indiquent que ces populations sont exposées à des pressions environnementales. Ces résultats suggèrent qu'une combinaison de facteurs environnementaux est à l'origine de cette variation des ailes.

Mots clés : Culicidae ; *Culiseta longiareolata* ; Géométrie Morphométrique ; altitude ; température ; aile.