CRANIOMORPHOLOGICAL VARIATION IN THE MEXICAN POPULATION:

A COMPARISON OF THE NORTHERN, CENTRAL

AND SOUTHERN REGIONS

by

Paulina Dominguez Acosta, B.S.

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Committee Members:

Kate Spradley, Chair

Nicholas Herrmann

Ana Juarez

DEDICATIONS

“The dead cannot cry out for justice. It is a duty of the living to do so for them.”
-Louis McMaster Bujold.

To my wonderful family and friends for their unconditional love and support.

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ABSTRACT

Due to the current migrant crisis along the US-México border, numerous human remains remain unidentified due to the lack of proper data or information for the identification on the Hispanic population. Craniometric comparison between the regions in México has previously been done by Humphries (2015), and by Hughes (2013). Both researchers looked at cranial morphological variation in México while comparing the variation to the parental groups (European and Native American) and to geographical regions (Hughes et al., 2013; Humphries et al., 2015). The individuals analyzed are known Mexican nationals from UNAM, PCOME, OpID and XOCLAN-UADY. For the shape differences, discriminant function analysis and wireframe graphs were used. The size differences were analyzed through a one-way ANOVA and a follow up post-hoc test to see where there was significant variation.

The discriminant function analysis accurately classified 76.09% of the Northern region, 75.0% of the Central and 78.08% of the Southern region. Mahalanobis distances showed major differences between the Northern and the Southern region, while the Northern and the Central regions were more similar. The centroid size analysis showed violation on the assumption of equal variances ($p= 0.001$). Size differences between the regions showed statistical significance between the Northern and the Southern region ($p= 0.001$), and between the Central and Southern regions ($p= 0.013$). Overall, both shape and size differences were highly variable between the three regions.

I. INTRODUCTION

Trying to find the identity of unknown human remains poses many problems for forensic anthropologists. Being able to narrow down the list of possible matches can be challenging when the only information available are the human skeletal remains. For forensic anthropologists, estimating the biological profile can help to narrow down the pool of likely individuals. When looking at the cranium alone, forensic anthropologists can estimate ancestry and sex. The cranial variation from one individual can be traced down to different biological affinities groups, depending on the ancestry of the person (Algee-Hewitt, 2017a; Hughes et al., 2013; Martínez-Cortés et al., 2012a; Stull et al., 2014). Being able to further evaluate cranial variation to a specific region could help expedite a potential association between a missing person and an unknown person. Due to the current crisis at the border between México and the United States, many migrants who are coming from México or other central American countries are dying along the México territory or along the border (Anderson, 2008; Spradley, Stull, et al., 2016; Vogelsberg, 2018). The ability to narrow down a region of origin within Latin America would be beneficial in the identification of individuals perishing along the US-México border or in México's territory. For my proposed research, I will be looking at the cranial variation in shape and size within the Mexican population, to see if it is possible to estimate region of origin of unidentified individuals who are believed to be Mexican natives.

I will analyze and compare the geometric morphometric differences between the northern, central, and southern regions of México, in order to see if there are any differences that could help distinguish the region of origin. By determining if there are any shape and size differences between three regions, medicolegal and non-governmental agencies that work on identifying unknown human remains could greatly benefit by reducing the pool of possible missing persons matches per region.

Migrant Crisis

Recently, there has been a push to create and improve current biological profile estimation methods for Hispanic populations due to the high number of migrants perishing when crossing the U.S. border (Anderson, 2008; Hughes, Algee-Hewitt, et al., 2017; Spradley et al., 2019; Spradley, Stull, et al., 2016). Migration patterns show that people are coming from central and south America because of the economic and political crisis happening in México, Honduras, Guatemala, El Salvador and many other countries, which is forcing them to flee from their countries (Anderson, 2008; Birkby, Fenton, & Anderson, 2008; Edgar, 2013; Hughes et al., 2013; Hughes et al., 2017; Humphries et al., 2015; Little et al., 2006; Rubi-Castellanos et al., 2009; Spradley et al., 2016; Spradley, 2013; Spradley et al., 2008; Weisensee & Spradley, 2018).

Many of the migrants who try to reach the U.S die on the Mexican side of the border, and throughout México's territory. Many of these migrants are from México, and many others are from central American countries. Attempting identification has become a challenge due to the lack of proper methods aimed to look at the different Hispanic groups (Birkby et al., 2008; Spradley, 2013; Spradley et al., 2008). Currently, there still needs to be a better understanding of the skeletal variation between these populations. My thesis project will investigate whether geometric morphometrics can be utilized to look at size and shape differences between highly related groups, in this case the Mexican population. If this proves to be useful, future research could focus on looking at variation between all the different central American countries as well in order to create a more efficient method to estimate the region of origins and skeletal variation in the Hispanic population not as a whole, but as different entities that compose it. Being able to ascertain if differences exist between these groups could potentially aid in narrowing down the list of missing persons based on the region in which the unknown individual best fits the data.

Being able to understand population specific traits or data, can be used to create the best method to estimate ancestry and therefore narrow down the region of origin based on cranio-morphological data. Narrowing the region of origin could aid in the identification of individual forensic cases that are believed to be of Mexican descent that have been found along the U.S. – México border, and who have perished due to the harsh environments, and those who fell victims of the organize crime in México (Algee-Hewitt, 2017; Hughes et al., 2013; Spradley, Stull, & Hefner, 2016).

Geometric Morphometrics

Geometric morphometrics will allow me to asses any size and shape differences and by using the coordinate data in a Generalized Procrustes analysis (GPA), which will transform coordinate data into Procrustes distances that can be used in traditional multivariate statistical analysis (Adams et al., 2004). Further, geometric morphometrics will allow me to have a visual representation of the data (Adams et al., 2004), which will facilitate the assessment of shape variation between the different geographical regions of México. Analyzing a series of cranial samples from across México will address questions related to this study.

Due to México's vast territory and historical admixture, one finds marked variation in cranial morphology, or craniometric differences, between residents from northern México and those from central and southern México (Algee-Hewitt, 2017b; Humphries et al., 2015; Rubi-Castellanos et al., 2009). It has been noted by several researchers that craniometric, morphological, and dental differences in México are possibly the result of admixture events in the past (Algee-Hewitt, 2017; Edgar, 2013; Hughes, Tise, Trammell, & Anderson, 2013; Humphries et al., 2015; Kirkwood, 2000; Rubi-Castellanos et al., 2009; Vogelsberg, 2018). The admixture between Native Amerindians, Europeans, and Africans is what gave rise to the cranial variation in the Mexican population and throughout the Latin American countries today (Little et al., 1986; Ruiz-Linares et al., 2014). The genetic and metric proportions of European ancestry increase from Southern México to Northern México, and it increases in Native American ancestry from North to South (Hughes et al., 2013; Kirkwood, 2000; Rubi-Castellanos et al., 2009).

Previous studies showed that metric, and geometry information can aid in further evaluating the biological profile, as well as providing more information on population specific identification methods (Birkby, Fenton and Anderson, 2008; Edgar, 2013; Humphries et al., 2015; Little, Buschang, Peña Reyes, Tan, & Malina, 2006; Neus et al., 2009; Spradley, 2013). Size and shape analysis could prove beneficial to the study on the Mexican population crania because it will permit to look at subtle variation through the regions. Mitteroecker defines size, shape, and form in his 2009 article on the advances in Geometric Morphometrics. Understanding what each one of the components that geometric morphometric analyses is important to understand what is being studied. Size is defined by Mitteroecker as the scale or dimensions of the space an object occupies on a plane, this is dependent on the dimension of the item being analyzed. Shape is used to define the geometry of an item, this description is independent of the item's dimensions, location, and the position of it on a plane. On the other hand, the form of an item uses both the shape and the size at the same time (Mitteroecker and Gunz, 2009).

Cranial Morphology

Cranial morphologies have been used to estimate ancestry, biodistance, and regional variances between and within populations, and employed to assess the accuracy of the biological profile (André Strauss and Mark Hubbe, 2010; Ann H Ross, Ubelaker and Falsetti, 2003; Hughes et al., 2013; Humphries et al., 2015; Little, Buschang, Pena Reyes, Swee Kheng Tan, & Malina, 2007; Neus et al., 2009; Ousley et al., 2009; Relethford, 2001; Spradley et al., 2016; Stull, Kenyhercz, & L'Abbé, 2014; Weisensee & Jantz, 2011). Cranial morphology can help identify any size and shape differences that could help to identify region differences between and within populations, distinguishing variation within a population, these morphology changes could also help narrow down certain characteristics that are unique to a geographical location; these variations allow us to understand how human populations diverge from the parental groups (André Strauss and Mark Hubbe, 2010; Sardi et al., 2005; Spradley et al., 2016; Spradley et al., 2008; Stull et al., 2014; Weisensee and Jantz, 2011).

Craniometric comparison between the regions in México has previously been done by Humphries (2015), and by Hughes (2013). Both researchers looked at cranial morphological variation in México while comparing the variation to the parental groups (European and Native American) and to geographical regions (Hughes et al., 2013;

Humphries et al., 2015). Hughes completed a study where she analyzed the genetic data and compared it to the cranial morphologies of the modern Mexican population (Hughes et al., 2013). Humphries, on the other hand, analyzed the regional geometric morphometrics in México and compared it to the parental populations of Spain and Africa (Humphries et al., 2015). Hughes and Humphries focused on comparing craniometric data to the parental samples from both Spain and Africa (Hughes et al., 2013; Humphries et al., 2015). Both Humphries and Hughes concluded that bigger samples and further exploration of these craniometric and cranio-morphological differences are needed in order to fully understand these metric variations between the groups studied (Hughes et al., 2013; Humphries et al., 2015). Hughes also concludes that a deeper analysis could further aid in the identification of the unidentified border crossers along the US-México border (Humphries et al., 2015).

Hughes utilized a sample size that was composed of 82 identified migrant males from the Pima County Office of the Medical Examiner (PCOME) forensic cases (Hughes et al., 2013). In her study, Hughes acknowledges that her sample size could be improved in order to expand the research on the biological variation, with a more in depth study on cranial morphologies rather than with an emphasis on genetics (Hughes et al., 2013). Humphries used a sample composed of 318 individuals, out of the total, 88 are crania of Mexican individuals. Humphries discusses that all the groups compared and analyzed on her study are significantly distinct from one another (Humphries et al., 2015). Both authors focused on the genetic and cranial variation between the three different regions in México compared to the parental groups from México past populations.

My research would fill the gap in the literature by exploring the classification of Mexican individuals based on their cranial shape and size differences, in comparison to their region of origin. A previous study argued that geometric morphometric analysis are best used when comparing samples that are from the same population rather than from different ones (Tarkhnishvili et al., 2018). Tarkhnishvili et al. (2018) tested whether geometric morphometric data could be used to identify closely related species. He tested the head shape of three closely related rock lizards, and found that geometric morphometrics is a better tool for identification (or discrimination) than other traditional methods (Tarkhnishvili et al., 2018). Digitizing data collection methods are the best for this study because it analyzes and preserves the geometry while removing the size any better than traditional methods. Geometric Morphometric methods evaluate shape while disregarding any size differences due to either sexual dimorphism or any biological factors that could be affecting cranial size (Spradley, & Jantz R. L., 2016; Mitteroecker & Gunz, 2009; Spradley, 2013).

Multivariate statistics will be conducted in order to check differences between all the cranial samples. Geometric morphometric landmark data will help to determine if size is a defining factor in the further classification of the Mexican population. For the shape analysis, removing size will allow to analyze if shape plays a determining role in the further classification of individuals based on their region of origin. For this study, crania from individuals that belong to the same country but different geographical regions will be analyzed and therefore geometric morphometrics will provide a tools to detect subtle differences in the shape variations in the Mexican population, as well as it gives a visual representation of the variation which makes data easier to understand (Tarkhnishvili et al,. 2018).

If this study proves to be valid, and shape and size variation can be distinguished based on region then this could further aid in identifying unidentified individuals at the border. This could also aid the Mexican medicolegal authorities to narrow down the region of origin for unknown murder victims of organized crime or unidentified migrants across the country.

This study aims to answer the following questions:

- Are there any differences in the cranial morphology between three regional groups in México? If so, are these differences size or shaped related?
- Is geometric morphometrics a good method for differentiating closely related groups?

Cranial size and shape differences between the Northern, Central and the Southern regions are expected. Previous research has shown that in the Mexican population various morphological changes seen in the skeletal elements could potentially be due to admixture events in the past (Algee-Hewitt, 2017; Edgar, 2013; Hughes, Tise, Trammell, & Anderson, 2013; Humphries et al., 2015; Kirkwood, 2000; Rubi-Castellanos et al., 2009; Vogelsberg, 2018). Due to the findings of previous research, it is possible that the results of this current study will be supporting those findings as well.

II. MATERIALS AND METHODS

Materials

To analyze cranio-morphological data for the Mexican population, data was collected from four different samples, including the Autonomous University of Yucatan (UADY) in Merida (n=55), the Pima County Office of the Medical Examiner (PCOME) in Tucson Arizona (n=68), Zimapan, Hidalgo at the National Autonomous University of México (UNAM) in México City (n=15) (Spradley, 2013), and Operation Identification (OpID) at Texas State University (n=1) (Spradley et al., 2019); for a total of 139 Mexican individuals from the three different regions in México (Table 1; Figure 1). These samples provide valuable information for this study as they are individuals that belong to a contemporary sample, which is why they will be analyzed for this study. Individuals who form part of modern samples are crucial for this study because they allow to see the current craniomorphology in the Mexican population. Since this study will be looking at the craniomorphological variation in the Mexican population as aims to narrow down region of origin to facilitate the identification of migrants who perish along the México and U.S. border; and throughout the Mexican territory, the analysis of modern samples best fits the population and the demographics of those individuals in need for identifications due to the migrant crisis.

UNAM

The collection is composed of 45 individuals from the town of Zimapan, Hidalgo which are now housed by the UNAM (Spradley, 2013). This collection is composed of contemporary Mexican individuals who were born around the middle of the 20^{th} century and belonged to a low socioeconomic status. Individuals who form part of this collection had to be relocated from their original resting place, and those families who could not afford the re-burying of the remains of their family members opted for donating the remains to the UNAM (Figueroa-Soto, 2012 ; Spradley, 2013). This collection provides vital osseous information about contemporary individuals from México. Since this study will be looking at the cranial differences based on the three regions in México, this collection proves to be essential for the analysis of individuals of the central region of México.

XOCLAN - UADY

The collection from Xoclan cemetery is located in UADY, it is composed of 158 skeletons out of which 84 are identified individuals which demographic information is well documented (Chi-Keb et al., 2013). The individuals from this collection were born from the years 1900 and 1990 and passed away between the years 1994 and 2004. Out of the 84 identified individuals, 56 are males and 28 are females, whose ages range from 8 years to 104 (Chi-Keb et al., 2013). The remains that form part of this collection are individuals that formed part of the town of Xoclan, Merida and are of individuals who formed part of the lower socioeconomic status (Chi-Keb et al., 2013). These individuals are a more representative sample of the southern population, which is mostly composed of the direct descendants of the Maya in México, Maya individuals and also individuals that held a Mexican citizenships as well as others who still formed part of the urban regions of Merida (Chi-Keb et al., 2013; Medina, 2013). Due to the low admixture events between the Maya and the Europeans, the Yucatan peninsula continues to house the most homogeneous and intact indigenous groups in all México (Medina, 2013).

PCOME

The PCOME in Tucson, Arizona is tasked with handling most of the deaths that occur in the county; Pima County also serves three additional counties around the Pima County (Spradley, Reineke, Doretti, & Anderson, 2016). These deaths also include the individuals that perish while attempting to cross the United States-México border through harsh terrains like the Sonoran Desert, in order to find a better life (Birkby et al., 2008). The majority of the border crossers that perish in the Tucson sector and are examined by the PCOME are males of Mexican origin, between the ages of 21 to 38 years of age (Anderson, 2008; Pima County Office of the Medical Examiner, 2017). Even though the individuals at the PCOME are not part of a collection, data is collected in order to estimate the biological profile to better aid the authorities in identifying the remains (Anderson, 2008; Birkby et al., 2008; Edgar, 2013; Humphries et al., 2015; Spradley et al., 2016; Spradley, 2013; Vogelsberg, 2018) . Between the years 2000 and 2017, 1520 individuals that are Mexican nationals have been identified, these individuals compose 83% of all undocumented border crosser identifications made (Pima County Office of the Medical Examiner, 2017). Due to the high number of identified individuals that are from México, this sample proves to be beneficial to this study. When the individuals are identified, information such as the nationality, and their region of origin may sometimes be documented (Anderson, 2008; Birkby et al., 2008; Pima County Office of the Medical Examiner, 2017; Vogelsberg, 2018), which is information that this study will be looking into.

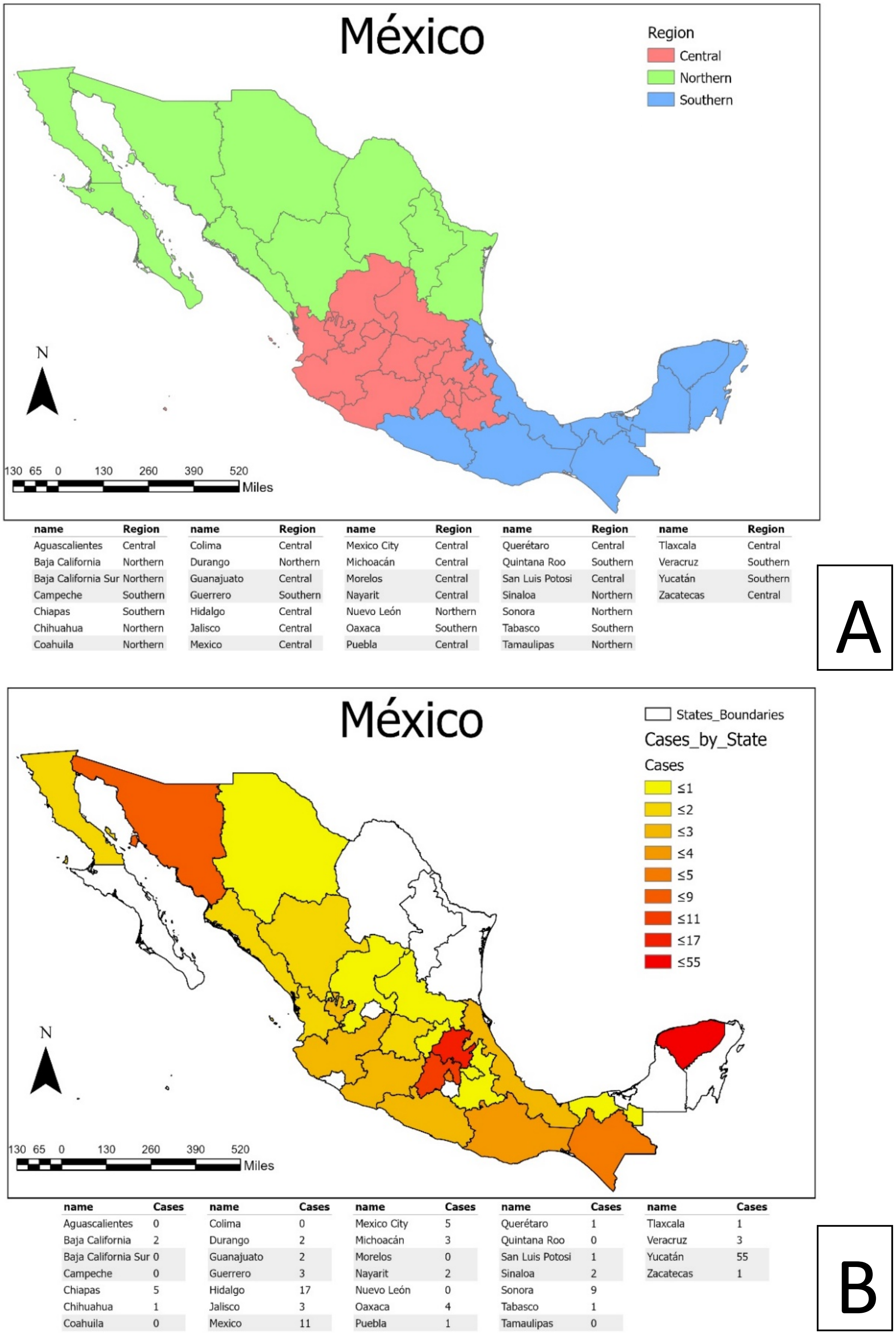
OpID

Operation Identification (OpID) at Texas State University in San Marcos Texas, is a project that was created in 2013 with the aim of helping the identification of the migrants who crossed the US-México border through the south Texas region (Spradley, 2018). Even though OpID is not a collection, but rather an aid in the identification process, it has helped to identify individuals of Mexican origin in which the region and state of origin were previously unknown. OpID curates pending forensic cases that are primarily from Brooks County, TX. OpID has a sample size of 233 individuals, 153 males and 80 females. The data that have been collected by OpID has assisted in the identification of 36 of these individuals (Spradley, 2018). Data collected from OpID could benefit by increasing my sample size as well as including more individuals of known Mexican origin to the study.

Table 1. Samples Arranged by Geographic Region in México

	Samples				Sex	
Regions*	**UNAM**	**UADY**	**PCOME**	**OpID**	**Male**	**Female**
Northern	0	0	16	0	16	0
Central	15	0	31	0	37	9
Southern	0	55	17	1	51	22
TOTAL	15	55	64	1	104	31
				Total Individuals		135

*Regions are grouped according to the Secetaria de Gobernacion de México website (*DOF - Diario Oficial de la Federación*, 2014).



name	Region	name	Region	name	Region	name	Region	name	Region
Aguascalientes	Central	Colima	Central	Mexico City	Central	Querétaro	Central	Tlaxcala	Central
Baja California	Northern	Durango	Northern	Michoacán	Central	Quintana Roo	Southern	Veracruz	Southern
Baja California Sur	Northern	Guanajuato	Central	Morelos	Central	San Luis Potosi	Central	Yucatán	Southern
Campeche	Southern	Guerrero	Southern	Nayarit	Central	Sinaloa	Northern	Zacatecas	Central
Chiapas	Southern	Hidalgo	Central	Nuevo León	Northern	Sonora	Northern		
Chihuahua	Northern	Jalisco	Central	Oaxaca	Southern	Tabasco	Southern		
Coahuila	Northern	Mexico	Central	Puebla	Central	Tamaulipas	Northern		

name	Cases	name	Cases	name	Cases	name	Cases	name	Cases
Aguascalientes	0	Colima	0	Mexico City	5	Querétaro	1	Tlaxcala	1
Baja California	2	Durango	2	Michoacán	3	Quintana Roo	0	Veracruz	3
Baja California Sur	0	Guanajuato	2	Morelos	0	San Luis Potosi	1	Yucatán	55
Campeche	0	Guerrero	3	Nayarit	2	Sinaloa	2	Zacatecas	1
Chiapas	5	Hidalgo	17	Nuevo León	0	Sonora	9		
Chihuahua	1	Jalisco	3	Oaxaca	4	Tabasco	1		
Coahuila	0	Mexico	11	Puebla	1	Tamaulipas	0		

Figure 1. A) Map of the three regions in México, B) Map of the number of cases per State

Methods

Data Collection

The program that was used for the 3-dimensional data collection is *3Skull* (Ousley, 2014). The *3Skull* program captures the cranial three-dimensional landmark coordinates using a Microscribe digitizer. Once all the available coordinates were collected, the *3skull* program calculated the Howells interlandmark distances and added these measures to an additional data table (one for interlandmark distances and one for the 3D coordinates). The use of these programs and the equipment facilitated the data collection process while at the same time increased the amount of data points collected, while diminishing the probability of any transposing errors from one data table to the other (Spradley, 2013).

A total of 35 craniometric landmarks are analyzed for this study (Table 2; Appendix 1). Geometric morphometric analysis requires that all the specimens being analyzed have the same landmark data collected. If a specimen was missing a landmark from table 2 (Appendix 1), then the specimen was not considered in the analysis.

Table 2. Craniometric Landmarks

Data Point	Landmark Name	Data Point	Landmark Name
1, 2	Alare	19, 20	Inferior Nasal Border
3 ,4	Asterion	21	Occipital Subtense Point
5	Basion	22	Opisthocranion
6	Bregma	23	Ophistion
7, 8	Dacryon	24	Parietal Subtense Point
9, 10	Ectoconchion	25, 26	Porion
11, 12	Euryon	27	Prosthion
13, 14	Frontomalare Anterior	28, 29	Frontotemporale
15	Glabella	30	Cheek Height Inferior
16	Lambda	31	Cheek Height Superior
17	Metopion	32, 33	Nasomaxillary Suture Pinch Point
18	Nasion	34, 35	Zygon

Shape

Landmark analysis was completed through the evaluation of a Generalized Procrustes Analysis (GPA). Transformation of the landmark data to Procrustes coordinates allows for the rotation, reflection, and the scaling of the landmark data in order to have an easier analysis. This removes the size component of the geometry of the crania and provides a picture of the shape of each cranium but also provides a consensus form for comparison (Rusk et al., 2016). Then, the Procrustes coordinates were transformed to principal components which allowed for maximization of variation, they were later used in the canonical variates' analysis (Rusk et al., 2016). The principal component analysis allows one to visualize the distance and the relationship between the groups based on cranial shape. The principal components were then analyzed through a canonical variate analysis, this allowed to see the representation of the relationship between the Procrustes coordinates and the region of origin. The canonical variate analysis also results on a Mahalanobis distance output. Mahalanobis distances were analyzed through a scale factor change through the program morphoJ, these distances permit to see how similar or dissimilar the groups shapes are in comparison to one another. A discriminant function analysis was also created in order to see how well the function classifies each group (North, Central, South) into their correct geographical region. These methods will aid in the analysis of the size variation between the regions.

The axes X, Y, Z were rotated in order to get a good description of the variation seen through all the different axes. The program morphoJ was used to process that data for it to be used in traditional multivariate analyses. Shape changes where addressed visually through the analysis of the wireframe graphs crated through the program

MorphoJ. The wireframe plots were examined in order to compare the variation of the mean values of all the landmark points collected throughout all the three geographical regions. Wireframes were created by creating connections between the landmarks, which allowed to visualize the canonical variates and the shape changes as three-dimensional plots that allowed for an easier comparison of the shape differences between the regions in México (Tarkhnishvili et al., 2018). The Wireframes exaggerate the morphological differences by allowing the user to change scale factors in which the data is being displayed.

Size

Through the data analysis in MorphoJ, the Procrustes analysis allowed for the creation of Procrustes coordinates. Through these coordinates, the size component was removed as a separate variable from the geometry component which then allowed for the separate analysis of the centroid size values. One-way ANOVA was performed to determine if significant differences exist in relation of centroid size to group. Centroid size was then compared to each one of the regions through an LSD post-hoc test, is a Fisher's test for least significant differences, this highlights how significant the size differences are between the northern and the central, the central and the southern and the northern and the southern regions. The LSD post-hoc test allows for a comparison of means between two groups.

III. RESULTS

Shape Analysis

The shape analysis results through the Canonical variate analysis show that the shape variation between the three geographical regions in México are dissimilar from one another (Figure 2). On this graph, canonical variate 1 (CV1) depicts the Northern and the Central regions within the negative axis and the Southern region within the positive axis. Canonical variate 2 (CV2) shows the Central and the Southern regions on the Negative axis, while the Northern region is on the positive axis. Axis 1 (CV1) represents 74.898% of the variation, while axis 2 (CV2) captures 25.102% of the variation.

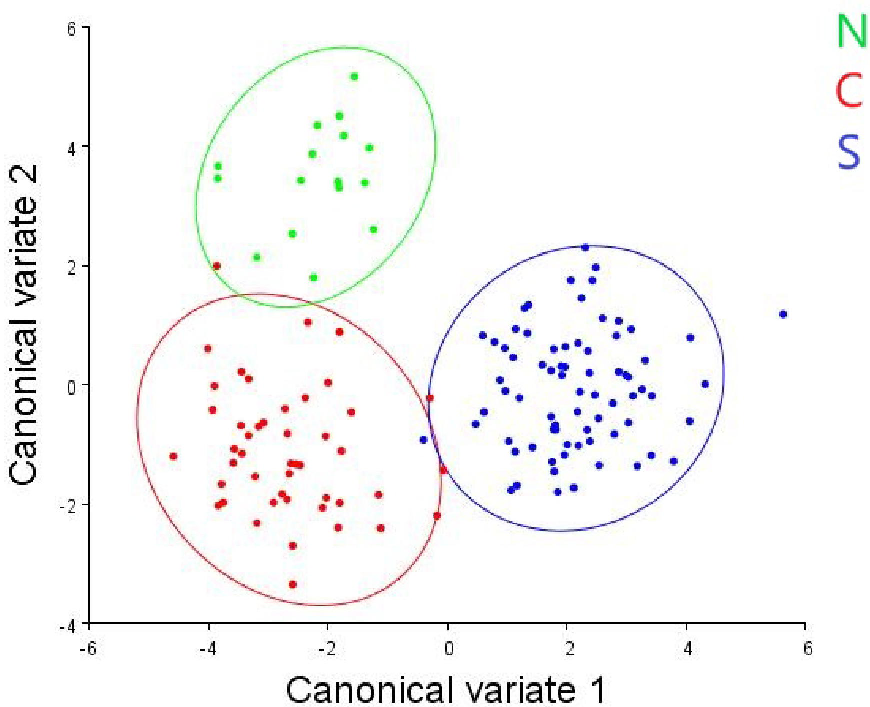


Figure 2. CVA representing 95% of the variation between the regions with confidence ellipses.

Discriminant Function Analysis

The classification of groups into their correct region of origin was completed through a linear discriminant function analysis with cross-validation. This analysis accurately classified 76.09% of the Central region correctly, 75.00% of the Northern region and 78.08% of the Southern region (Table 3). The overall accuracy of the discriminant function analysis was tested in order to see how well the function analysis separates every individual analyzed, with its corresponding regional group (Wilk's Lambda = .3057 ; $F(30) = 6.52$; $p < .0001$).

Table 3. Cross-validation summary per region using a Linear Discriminant Function

Cross-validation using Linear Discriminant Function				
Number of Observations and Percent Classified into Region				
From Region	**N**	**C**	**S**	**Total**
N	12 75%	4 25.00%	0 0.00%	16 100.00%
C	5 10.87%	35 76.09%	6 13.04%	46 100.00%
S	9 12.33%	7 9.59%	57 78.08%	73 100.00%
Total	26 19.26	46 34.07	63 46.67	135 100
Priors	0.33333	0.33333	0.33333	

Mahalanobis distances permit for an easier analysis of group similarities based on the cranial shape differences. The smaller the Mahalanobis distance the greater the similarity, while the larger the Mahalanobis distance the greater the difference between groups (McKeown & Schmidt, 2013). Mahalanobis distances showed that there are differences between the three regions. The Northern region and the Southern show the most dissimilarity in their Mahalanobis distances with one another, while the northern and the Central regions are more similar (Table 4).

Table 4. Mahalanobis distances expressed in significant values.

Mahalanobis Distances			
Generalized Squared Distance to Region			
From Region	**N**	**C**	**S**
N	0	4.63982	6.91994
C	4.63982	0	6.52741
S	6.91994	6.52741	0

Wireframe Analysis

Northern

Wireframe plots of the northern region were created in order to analyze the shape variation seen in this region as compared to the average shape of all three regions (Figure 3). A scale factor of 3.5 was utilized because this allowed for the best representation of the morphological differences between the northern region and the average of all three regions.

Axes 1 and 2 of the wireframe plots of the northern region show that the overall shape of the cranial vault is slightly shorter when compared to the average of the three regions combined. Axes 1 and 3 show that the northern region has a more elevated cranial vault around the sagittal suture region than the average, as well as an inferior straightening of the cranial vault in relation to the foramen magnum aperture and prosthion. This axis also shows that the glabella region is much more pronounced as compared to the average. Axis 2 and 3 show longer height, while at the same time showing small width of the cranium. In this axis it is also noticeable that the nasal aperture of the northern region sample is narrower and longer. The eye orbits also appear to be smaller and closer to one another. Overall, the northern region sample appears to have reduced width and greater cranial height than the average.

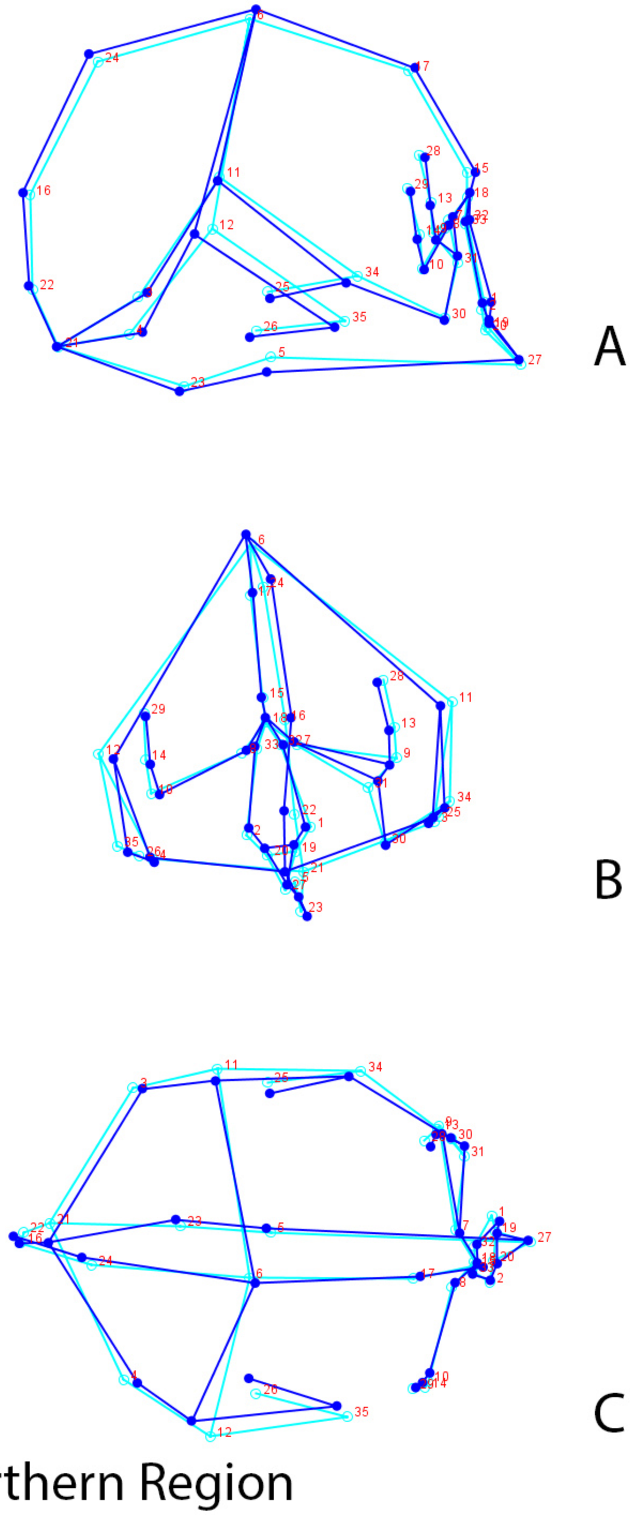


Figure 3. Wireframe from the Northern region.
A. Right lateral view of cranium, B. Frontal view of cranium, C. Superior view of cranium. Light blue represents the mean shape, while the dark blue represents the northern region mean shape.

Central

Wireframe plots of the central region were created in order to analyze the shape variation seen in this region as compared to the average shape of all three regions (Figure 4). A scale factor of 10 was utilized because this allowed for the best representation of the morphological differences between the northern region and the average of all three regions.

Axes 1 and 2 of the wireframe plots of the central region show that both the lateral portions of the cranium are flatter with higher pronunciation of flattening on the left side. The facial-cranial aspect of the crania is more concave than the average. Axes 1 and 3 show that in the central region the wider points of the cranium are located higher up than any of the other two regions, and it shows to be higher even than the average. The area of the calotte slightly higher than the average, and the overall shape of the cranium is much more circular. A more pronounced flatter occipital region can also be seen from this axis. Axis 2 and 3 show a slightly higher cranial height at bregma, while at the same time it shows a near average cranial width. In this axis it is also noticeable that the nasal aperture of the central region sample is more teardrop shaped. The eye orbits also appear to be slightly wider and farther apart. Overall, the central region sample appears to be much closer associated with the average between all three regions.

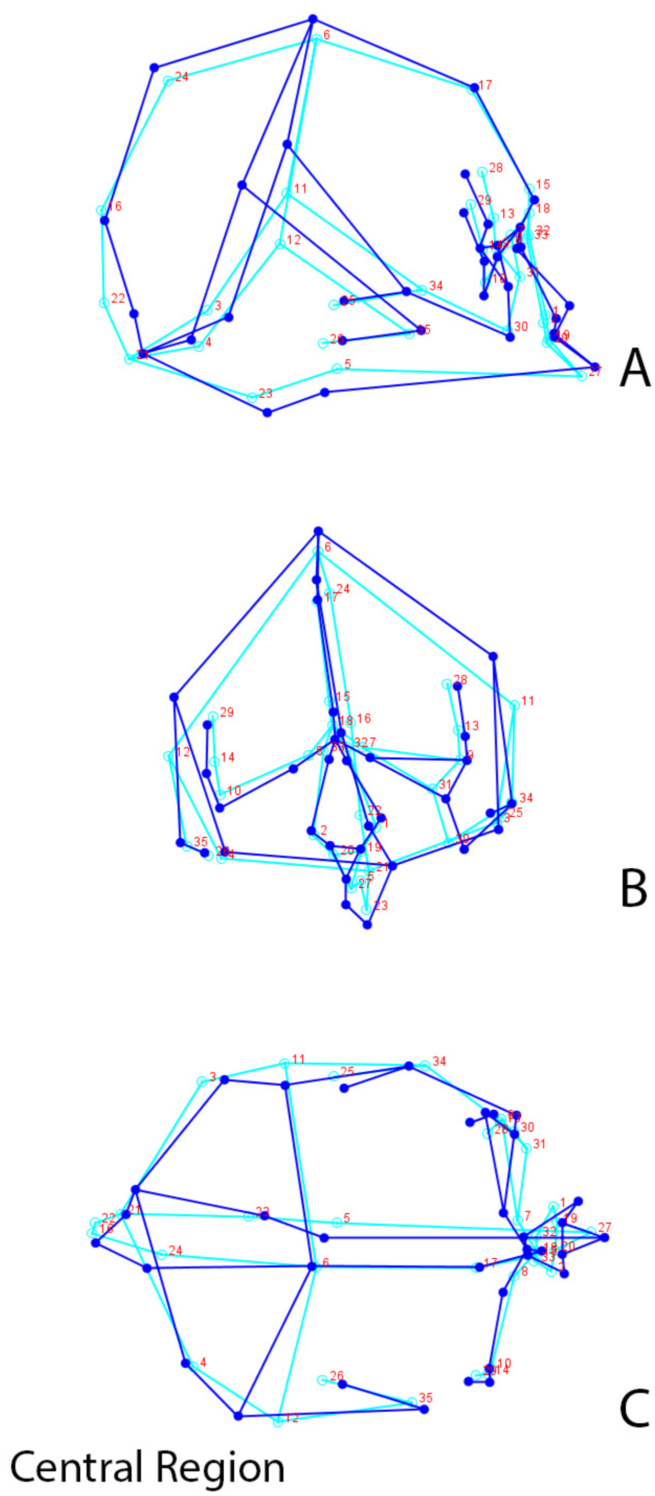


Figure 4. Wireframe from the Central region.
A. Right lateral view of cranium, B. Frontal view of cranium, C. Superior view of cranium. Light blue represents the mean shape, while the dark blue represents the central region mean shape.

Southern

Wireframe plots of the southern region were created in order to analyze the shape variation seen in this region as compared to the average shape of all three regions (Figure 5). A scale factor of 3.5 was utilized because this allowed for the best representation of the morphological differences between the northern region and the average of all three regions.

Axes 1 and 2 of the wireframe plots of the southern region show that the overall shape of the cranial vault is much wider and slightly longer than that of the average. Axes 1 and 3 show that the southern region has a much shorter cranial height and the glabellar projection is not evident. The inferior portion of the crania also showed higher angulation between the foramen magnum angle and a much more pronounced incline from basion to prosthion than the average. This axis also shows that the craniofacial aspect of the southern sample is much flatter than the average. Axis 2 and 3 show a smaller cranial height and a broader cranial width. The eye orbits also appear to be wider and more angular than the average. The nasal aperture also appears to be wider and bigger towards the base of the nasal aperture. Overall, the southern region sample appears to have a broader cranial width and a much shorter or flat cranial height.

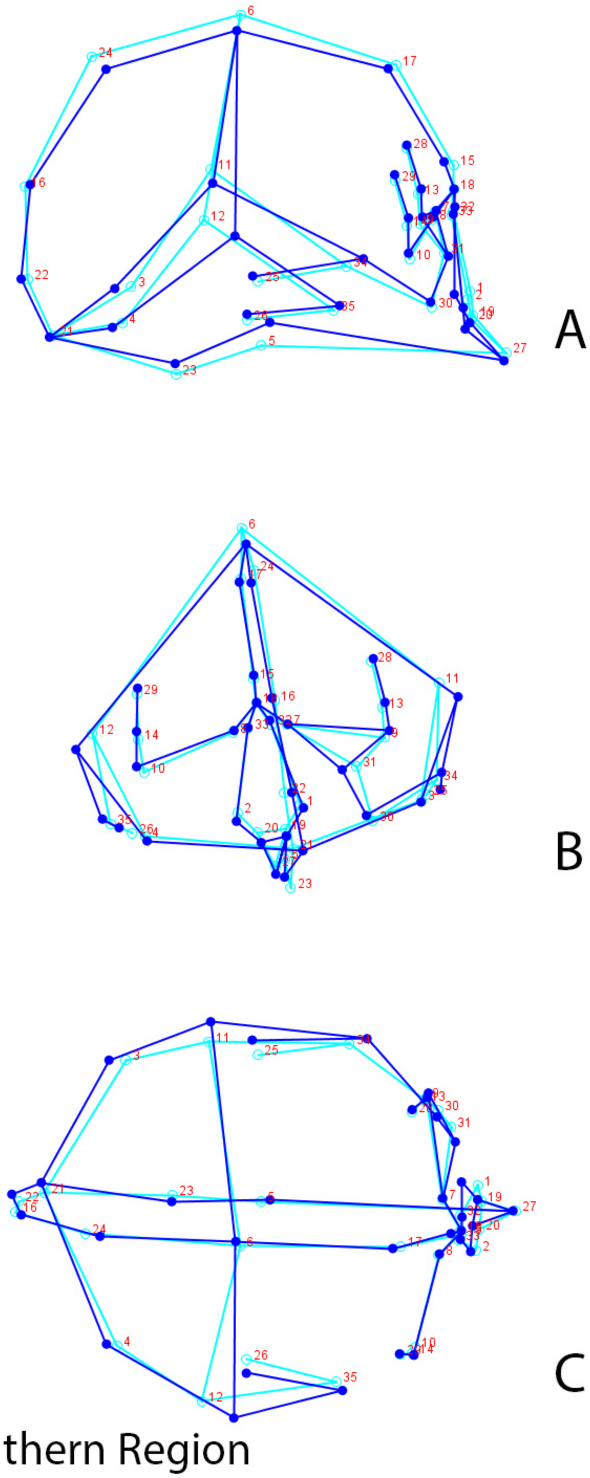


Figure 5. Wireframe from the Southern region.
A. Right lateral view of cranium, B. Frontal view of cranium, C. Superior view of cranium. Light blue represents the mean shape, while the dark blue represents the southern region mean shape.

Size Analysis

Visual inspection of the histograms and the Q-Q plots showed no violations to the assumption of normality, the northern sample is slightly positively skewed, though this is most likely due to the small sample size for the northern region. A test of homogeneity of variances was conducted and the test showed violations on the assumption of equal variances (p=0.013). The test between-groups showed significance between centroid sizes in the three geographic regions (Table 5).

Table 5. Centroid size means per geographic origin

Centroid Size		
Region	*Mean*	*Sample Size*
Northern	459.641	16
Central	451.829	47
Southern	443.603	76

ANOVA: **All Regions**				
n	*F-Value*	*Total Mean*	*Sd. Deviation*	*Significance*
135	7.657	448.057	17.1769	0.001

ANOVA: **Males**				
n	*F-Value*	*Total Mean*	*Sd. Deviation*	*Significance*
104	5.501	452.919	13.8159	0.005

ANOVA: **Females**				
n	*F-Value*	*Total Mean*	*Sd. Deviation*	*Significance*
31	0.107	431.2123	16.2769	0.746

For measuring the size differences between the regions, first the mean values for the centroid size were analyzed and compared. The centroid size mean values showed that the northern population has a larger cranial size when compared to the central and southern regions (Northern, mean= 459.641; n=16), the central region had an intermediate centroid size as compared to the northern and southern regions (Central, mean= 451.829; n=47), and the southern region had the smallest centroid size as compared to the other two geographical regions in México (Southern, mean= 443.603; n=76).

Centroid size comparisons were then separated into both sexes in order to define whether males and females had different centroid sizes throughout the three different geographical regions. The ANOVA indicated that females showed no statistically significant differences in their cranial size though the regions (p= 0.746). The ANOVA indicated that males do show statistically significant differences in their cranial size though the regions (p= 0.005).

A Profile Plot of the centroid sizes was created in order to have a visual representation of the data, significant variation between the three regions male centroid sizes and female centroid sizes are visible through the plots (Figure 7, Figure 8).

A post Hoc test for multiple comparisons was completed in order to analyze the differences between the males of each two groups, comparisons were as followed: North-Central, Central-South, South-North. A comparison between the northern region and the central region males yielded a p-value of 0.335. The central region and the southern region males yielded a p-value of 0.015. The northern and the southern region males yielded a p-value of 0.005. These results show that there are statistically significant differences between the males of the central and southern regions, as well as between the males of the northern and the southern region, however, the northern region males show no high significant differences from the central region males (Table 6). Summarized findings for each region are represented on table 7.

Table 6. LSD Post Hoc test for Male Centroid size comparisons between groups.

Multiple Comparisons				
Dependent Variable: Centroid Size; Grouped by: **Males**				
Region		Mean Difference	Std. Error	Significance
North	Center	3.8369	3.9641	0.335
	South	10.9261*	3.7964	**0.005**
Center	North	-3.8369	3.9642	0.335
	South	7.0867*	2.8611	**0.015**
South	North	-10.9236*	3.7964	**0.005**
	Center	-7.0867*	2.8611	**0.015**
*Mean Difference is significant at the 0.05 level.				

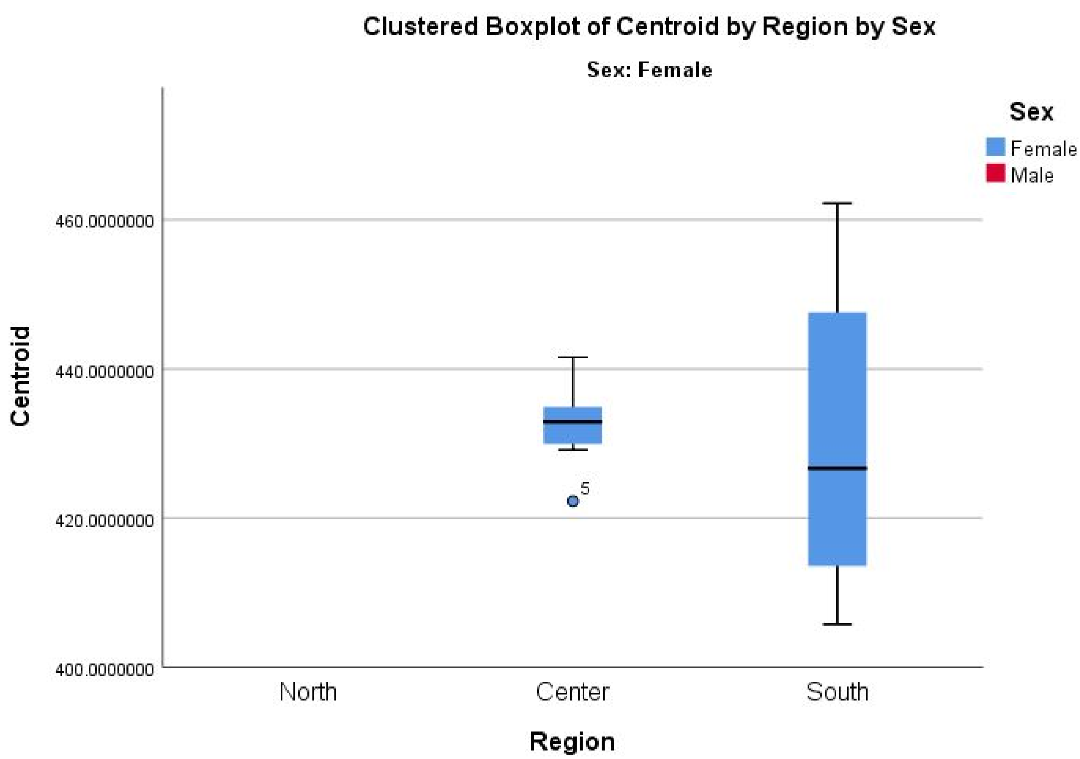


Figure 6. Boxplot of Female centroid sized grouped by region.

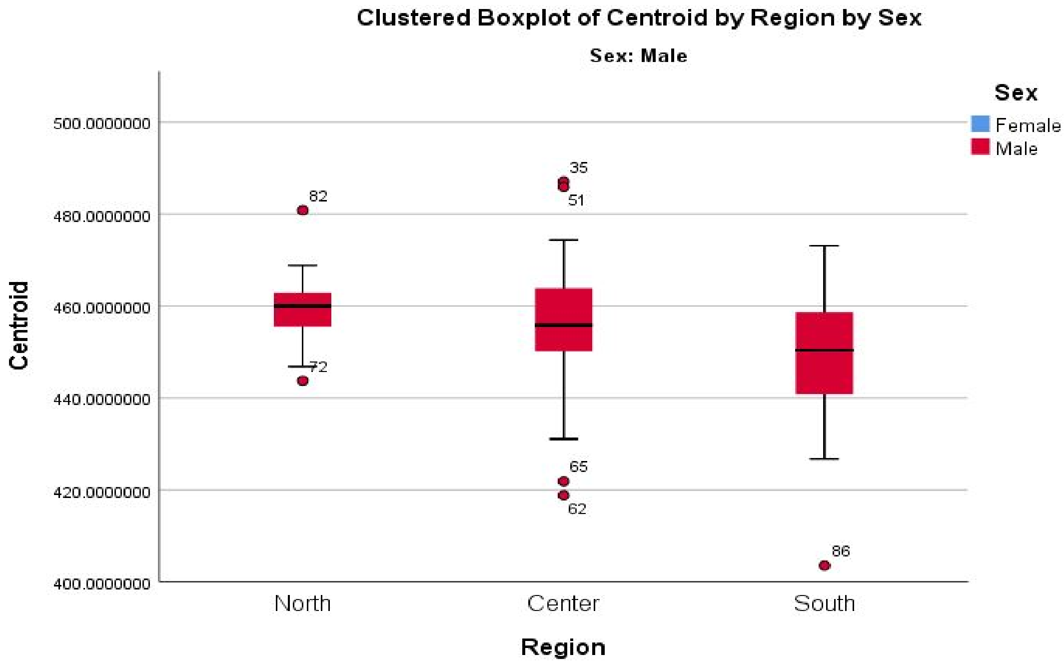


Figure 7. Boxplot of Male centroid sizes grouped by region.

Table 7. Summarized findings for each region

Region	Sample Size	Centroid Size Mean	Unique Shape Characteristics	Unique Size Characteristics
Northern	16	459.641	Less cranial width an increased cranial height, with a much narrower nasal aperture.	Largest cranial size
Central	46	451.829	Cranial height higher at glabella, a flatter posterior-cranial shape. The nasal aperture was bell-shaped	Intermediate cranial size
Southern	73	443.603	Wider crania, a shorter cranial height and slightly longer cranial depth.	Smallest cranial size

IV. DISCUSSION

The present study showed that there are differences in the cranial morphology between the three regional groups in México. The differences recorded were both size and shape related throughout all regions. When comparing the geometric differences in the Mexican population, cranial shapes appeared to be most different between the northern and the southern regions, while the central region was closer to the shape of the average. Size differences were statistical different between all regions.

Shape

The northern region sample has an overall higher cranial height and smaller cranial width, which a narrower nasal aperture, and a higher glabella projection. The shape differences seen in the northern sample could be due to the northern region having a much higher percentage of genetic admixture with European groups than any other region in México or central America (Cahua-Pablo et al., 2017). The northern states present a 62% genetic admixture with European groups, a 37% admixture with Native American groups and a 1% of African admixture (Cahua-Pablo et al., 2017). Even though they still have high admixture with Native groups from the northern region, the characteristics of their cranial morphology mostly resembles that of an European individual (Cahua-Pablo et al., 2017). This present study showed that the shape of the northern region has less cranial width and increased cranial height, with a much narrower nasal aperture. These features could be due to the potentially high European admixture in the northern population (Green et al., 2000a; Hughes, Algee-Hewitt, et al., 2017; Hughes et al., 2013; Humphries et al., 2015; Ruiz-Linares et al., 2014).

The central region was very closely related to the average, but they showed a higher recording point for maximum cranial width (Euryon), as well as more circular crania with a bell-shaped nasal aperture and a slightly more pronounced prosthion by Howells landmark. The shape differences seen in the Central region sample could be because the Spaniards, French, Africans and other Native American groups mixed, creating a major admixture event in the central region (Cahua-Pablo et al., 2017; Ruiz-Linares et al., 2014). DNA analysis for the admixture percentages in the central region of México have shown that in average 63% of the genetic proportion of the individuals is composed of Native American admixture, followed by a 31% European admixture and a 6% of African admixture (Cahua-Pablo et al., 2017). Though the genetic admixture with Native American groups, the European admixture is higher than that compared to the southern geographical region of México (Cahua-Pablo et al., 2017; Martínez-Cortés et al., 2013). The present study showed that the cranial shape of the central region appeared to be much closer to the average between all three groups. These features could be due to the potentially moderate European admixture, and the moderate native American admixture in the central population (Green et al., 2000a; Hughes, Algee-Hewitt, et al., 2017; Hughes et al., 2013; Humphries et al., 2015; Ruiz-Linares et al., 2014).

The shape of the Southern region could be because this region of México is mostly composed of individuals who isolated themselves when the Europeans arrived, through time they refused to mix with the foreigners that did not look like them due to lack of trust (Cahua-Pablo et al., 2017; Ruiz-Linares et al., 2014). It is important to note that throughout the years, the indigenous populations throughout Mexico have been isolated and controlled mostly by white individuals, who in turn oppressed and discriminated the indigenous groups throughout the southern region (Medina, 2013). The genetic structure of groups in the southern region showed that 70% of the genetic makeup was composed a Native American lineage, 27% of European and a 3% of African admixture (Cahua-Pablo et al., 2017). The genetic admixture of the southern region is highly composed of Native American admixture, thanks to the community unity that they have been able to sustain for more than 500 years (Medina, 2013). This research showed that the cranial shape for the southern region appears to be have a wider crania, smaller cranial height, and slightly longer crania. These characteristics might be caused to the possibly high native American admixture in the southern population (Johnson et al., 2011; Little & Malina, 1986; Martínez-Cortés et al., 2012b, 2013).

The results of the present study add more to the previous existing evidence that geometric morphometrics can be successfully utilized to look at differences between highly related groups. In this case, the present study tested geometric morphometric changes in the crania of the Mexican population and the results showed high differences that could be visually distinguishable between the Northern, Central and Southern regions.

The results of the present research could potentially be applied to try and identify the region of origin on individuals who are believed to be Mexican nationals and remain unidentified; or it could potentially be explored more further with an increased sample size for all three regions; specifically the northern region in order to define more clearly the variation in both shape and size for this region. These results support previous studies which have found variation in morphology and with admixture in the Mexican population (Hughes et al., 2013; Hughes et al., 2017; Humphries et al., 2015; Little & Malina, 1986;

Martínez-Abadías et al., 2006; Spradley et al., 2016). Anthropometric distinctions of groups such as this, could aid in better understanding skeletal differences among different groups, which in turn could aid in the identification of remains of unknown individuals.

Size

Size differences were significant and showed that the northern region has an overall higher centroid size than the central and the southern regions, while the southern region showed a much smaller cranial size when compared to any of the other two regions. The findings of this study have also shown that geometric morphometrics is a good tool for the identification of subtle differences between closely related groups. The present research results showed high statistical significances and visual differences between the three regions in México in both the shape and the size factors of the crania.

Size differences were highly variable between the three regions, and the results were expected based on the different admixture events that occurred historically through each one of the regions in México. The present study showed that the cranial sizes between the three regions are highly dissimilar from one another. The northern region showed to have a larger cranial size than the central and southern regions, whereas the southern region showed a much smaller cranial size than the other two regions. The higher the centroid size in the Northern region is likely due to previous admixture events with Europeans, or due to higher socioeconomic status. The average centroid size in the Central region is likely due to the other two regions being so highly dissimilar with one another. The smaller the centroid size in the Southern region is likely due to the lower numbers of admixture events and higher Native American ancestry, as well as the lower socioeconomic status that is seen throughout this region.

Previous studies have shown that throughout the southern regions in México, the admixture with Europeans is lowered and there is predominantly a higher Native Amerindian blood lineage, and therefore the skeletal features that could potentially be present due to European admixture are lowered further south in México (Green et al., 2000b; Martínez-Cortés et al., 2013).

Future studies could focus on expanding the sample size for the northern individuals, which could aid in further analyzing any major differences between the northern and the central regions, (Birkby et al., 2008; Spradley, 2013; Spradley et al., 2008), which showed to have higher similarities than when compared to the southern region. The northern sample size for this study was composed of 16 individuals, even though this was a small sample size compared to the other two regions the significance of the variation between the northern region and the central and southern regions was high enough to see variation in size and shape. The multiple comparison test for centroid size comparison between the northern and the central sample was p-0.335, this value could potentially be statistically significant if the sample for the northern region could be increased and re-analyzed.

V. CONCLUSION

In the regional samples used in the present analyses, shape and size differences can be recognized for all three regions. Even though the northern sample was relatively small compared to the other two regions, it yielded significant differences the shape and size factors of the cranial morphology of individuals in this region. Geometric morphometric analysis of the crania in highly similar groups shows to be a good method for the identification of shape and size differences that could potentially aid in the narrowing down of classification systems for ethnicities or regions of origin.

Some limitations of the current study lie on the small sample sizes that were analyzed. A further expansion on sample sizes could potentially yield better results and higher differentiation between the regions. A deeper analysis of regional genetic variation could also aid in differentiating craniomorphological aspects that are unique to each region, as well as understanding were the skeletal variation comes from. Further analysis on the Native American group category could aid in making a more in-depth analysis of variation within the southern population. A distinction of the groups that form part of the Native American category could yield better differentiation between Mayan heritage, Mesoamerican and other classifications that could be linked to other groups.

Findings could potentially aid in the identification of individuals who are believed to be Mexican national because by collecting their cranial landmarks and through a geometric morphometric analysis, they could then be compared to the three regions and see to which group they are most closely related. The findings of this study could be potentially used as a model to test data of individuals who have been identified and compare their cranial data to the one on this project in order to test its usefulness.

Through the examination, the biological profile estimates could then potentially be compared to the missing persons list from the region which best relates to the unknown individual and therefore narrow down the list of possible matches. If the individual does not fit to any of the regions, it could be then compared to other countries groups specific biological estimation methods. A closer analysis of the different groups that compose the Hispanic ancestry category, we could potentially generate biological profile specific methods for each one of the groups that make up this classification.

Further research could aid in narrowing down the factors that affect the shape and size differences throughout the regions. Through DNA analysis or secular change studies we could potentially see whether the changes are truly caused through admixture between populations, isolation events or if it could potentially be due to nutritional levels, stress factors, or economic strength in each one of the regions (Green et al., 2000a; Hughes, Algee-Hewitt, et al., 2017; Hughes et al., 2013; Humphries et al., 2015; Ruiz-Linares et al., 2014).

Additional exploration of the morphological differences between the three geographical regions in México could aid in the identification of region of origin of unidentified remains who are believed to be of Mexican nationals. Comparison of geometric morphometric data of other countries such as Honduras, Guatemala and El Salvador could possibly aid in the identification of region of origin of migrants who have died trying to cross the United States and México border. Further studies could potentially focus on increasing the sample size for the Mexican population, while at the same time analyzing other Central American countries in order to see if there’s any further differentiation between these countries. Through these analyses we could potentially narrow down the list of missing persons by looking at specific regions of origin or countries, instead of continuing to use the single category of Hispanic.

APPENDIX SECTION

1. Landmark abbreviations, number and definitions (Fleischman and Crowder, 2019)

Data Point	Number	Definition
alarl	1	The most lateral point on the margin of the nasal aperture taken on the anterior surface. Left side
alarr	2	The most lateral point on the margin of the nasal aperture taken on the anterior surface. Right side
astl	3	The point where the lambdoidal, parieto-mastoid, and occipitomastoid sutures meet. Left side
astr	4	The point where the lambdoidal, parieto-mastoid, and occipitomastoid sutures meet. Right side
bas	5	Midline point at the anterior margin of the foramen magnum.
brg	6	Point where the coronal and sagittal sutures intersect.
dacl	7	Anterior border of the junction of the lacrimal and frontal. Apex of lacrimal fossa on the frontal bone. Left side
dacr	8	Anterior border of the junction of the lacrimal and frontal. Apex of lacrimal fossa on the frontal bone. Right side
ectl	9	The intersection of the most anterior surface of the lateral border of the orbit and a line bisecting the orbit along its long axis. Left side
ectr	10	The intersection of the most anterior surface of the lateral border of the orbit and a line bisecting the orbit along its long axis. Left side
eurl	11	Instrumentally determined, ectocranial point of greatest cranial breadth. Left side
eurr	12	Instrumentally determined, ectocranial point of greatest cranial breadth. Right side
fmal	13	Point where the frontozygomatic suture intersects with the orbit. Point is taken anterior. Left side
fmar	14	Point where the frontozygomatic suture intersects with the orbit. Point is taken anterior. Right side
glb	15	The most forwardly projection point in the mid-sagittal plane at the lower margin of the frontal bone.
lam	16	Point where the sagittal and lambdoidal sutures meet.
met	17	Instrumentally determined, point where the frontal's elevation above the chord from nasion to bregma is greatest.
nas	18	Point of intersection of the nasofrontal suture and the mid-sagittal plane, on the frontal bone.
nlhil	19	Actual floor of the nasal cavity, taken inside the nasal aperture if there is guttering or the stylus will fit into the nasal aperture.

Data Point	Number	Definition
nlhir	20	If there is a nasal sill, place stylus on the anterior surface of the maxilla to approximate the location of nasal floor. Left and Right side
ocspt	21	The maximum subtense, at the highest point on the convexity along the lambda-opisthion chord, in midline.
opg	22	Instrumentally determined, the furthest point from glabella in midline.
ops	23	Midline point at the posterior margin of the foramen magnum.
paspt	24	The maximum subtense, at the highest point on the convexity of the parietal bones, within the bregma-lambda chord, in midline.
porl	25	Point at the most superior aspect of the EAM. Left side
porr	26	Point at the most superior aspect of the EAM. Right side
proH	27	Midline point at the most anterior point on the alveolar process of the maxillae.
wfbl	28	Point generally anterior and medial along the temporal line (minimum frontal breadth). Left side
wfbr	29	Point generally anterior and medial along the temporal line (minimum frontal breadth). Right side
wmhi	30	The minimum distance, in any direction, from the lower border of the orbit to the lower margin of the maxilla, medial to the masseter attachment.
wmhs	31	
wnbl	32	The minimum transverse breadth across the two nasal bones. Left side
wnbr	33	The minimum transverse breadth across the two nasal bones. Left side
zygl	34	Maximum lateral extent of the zygomatic arch. Left side
zygr	35	Maximum lateral extent of the zygomatic arch. Right side

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