



## Size and shape analysis of human molars: Comparing traditional and geometric morphometric techniques

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### Abstract

Dental size and shape have been widely used to study biological relationships among human populations. Although several techniques have been proposed to quantify dental form, few attempts have been made to compare results obtained by application of different techniques. This work aims at comparing the information about size and shape of molar contours obtained from linear measurements, landmarks and semi-landmarks as well as evaluating the variation patterns among populations obtained by each method. The crowns of 35 permanent upper second molars belonging to archaeological samples from three regions of Argentina were analyzed. Buccolingual and mesiodistal crown diameters were measured and centroid size and crown index were used as size and shape descriptors, respectively. Likewise, four landmarks and 79 semi-landmarks were collected from the molar outline and relative warps (RW) analysis was performed on partial warps and uniform vectors; centroid size was estimated to summarize tooth size. The results indicate that size was consistently estimated by the three variable types. On the contrary, noticeable differences were found when the results of molar shape described by linear measurements were compared with those obtained using either landmarks or semi-landmarks. This study shows that considerable information about molar contour is added by using landmarks instead of crown diameters and that some morphological features, such as degree of cusp development, can only be captured by means of

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semi-landmark analysis. Finally, the interpretation of similarities among samples differs according to the selected description system.

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## Resumen

El tamaño y forma dental han sido variables tradicionalmente empleadas en el estudio de relaciones biológicas entre poblaciones humanas. Varias técnicas de medición han sido propuestas con el fin de cuantificar la forma dental, sin embargo, son escasos los trabajos tendientes a evaluar los resultados obtenidos mediante el empleo de diferentes técnicas. El objetivo de este trabajo es comparar la información acerca del tamaño y la forma del contorno de los molares obtenida a través de medidas lineares, *landmarks* y *semi-landmarks*, así como evaluar el patrón de variación entre poblaciones obtenido mediante cada técnica. Se analizaron 35 segundos molares superiores permanentes provenientes de muestras arqueológicas de tres regiones de Argentina. Se midieron los diámetros bucolingual y mesiodistal, a partir de los cuales se obtuvieron el tamaño centroide y el índice de la corona que fueron empleados como descriptores de tamaño y forma, respectivamente. Asimismo, se digitalizaron cuatro *landmarks*, y setenta y nueve *semi-landmarks* sobre el contorno de los molares. Para analizar la variación en tamaño y forma a partir de ambos tipos de coordenadas se estimó el tamaño centroide y se realizaron análisis de *relative warp* sobre el componente no uniforme (*partial warps*) y los vectores uniformes de variación. Los resultados indican que el tamaño dental fue estimado consistentemente por las medidas lineares, los *landmarks* y los *semi-landmarks*. Sin embargo, se encontraron diferencias notables al comparar los resultados de la forma del contorno molar descriptos por estas variables. Este trabajo muestra que el empleo de *landmarks* adiciona información sobre la forma en relación a los diámetros de la corona y que algunos rasgos morfológicos tales como el grado de desarrollo de las cúspides solo pueden ser capturados por los análisis de *semi-landmarks*. Finalmente, el patrón de relaciones biológicas entre las muestras se modificó de acuerdo con las variables empleadas.

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## Introduction

Variation in shape and size of complex structures such as molar teeth arises during ontogeny as a result of heritable and non-heritable factors that control development, growth and morphogenesis (Atchley and Hall, 1991; Hall, 2003). However, it is well known that dental development has a high genetic control and is also a highly conserved evolutionary process (Scott and Turner II, 1997; Thesleff, 2000). Additionally, due to the protected environment in which they develop, teeth represent patterns of genetic variation much more precisely than other body tissues (Dempsey and Townsend, 2001; Dempsey et al., 1995; Sperber, 2004; Townsend and Brown, 1979). As a consequence, studies of tooth form within anthropological research have mainly focused on interspecific variation and biological relationships among human populations (Bailey, 2002; Coppa et al., 1998; Mahler, 1980), although other issues such as the degree of fluctuating asymmetry (Fields et al., 1995; Kieser and Groeneveld, 1988; Livshits and Kobylansky, 1991; Perzigian, 1977) or

sexual dimorphism (Bermúdez de Castro et al., 1993; De Vito and Saunders, 1990; Garn et al., 1964) have been investigated as well.

Traditionally, studies of evolutionary relationships have been based on non-metric crown and root traits recorded using a graded scoring system (Hrdlička, 1920; Turner II et al., 1991), as well as on distance measurements and indices constructed from them (e.g. crown index) that are used to describe tooth form.

During the last two decades, geometric morphometrics began to be applied to the analysis of dental morphology (Bailey, 2004; Ferrario, 1999; Robinson et al., 2002). To date, only geometric morphometric methods based on sets of two-dimensional coordinates of biological landmarks (i.e. homologous reference points), and elliptic Fourier analysis for the description of closed contours, have been used in dental analysis. However, newer methods for the description and analysis of outlines, based on techniques developed for landmark-based analysis with the addition of points called semi-landmarks (points distributed along a homologous contour) (Bookstein, 1997; Sampson et al., 1996), have not yet been applied to the quantification of dental morphology. This approach permits the combination of landmarks and semi-landmarks in the same statistical analysis and is a very powerful tool for the analysis of shape variation in biological structures with few or no landmarks (Bookstein, 1997).

There are several theoretical reasons for favoring geometric morphometric methods over traditional ones. An important feature of these techniques is that they allow the capture of the geometry of the morphological structures of interest, and preserve this information throughout the analyses (Adams et al., 2004). In addition, geometric methods are able to remove size from the landmark coordinates more efficiently than the methods available for traditional data (Monteiro et al., 2002). Nevertheless, empirical evidence is required in order to assess possible significant differences in the results obtained for a particular problem (Monteiro et al., 2002). This is because there are no a priori more efficient methods; the intrinsic quality of a given method varies according to the objects analyzed and problems addressed. In consequence, due to the multiplicity of available approaches for quantification of the morphological variation of biological structures (e.g. linear measurements, landmark-based methods, analysis of outlines), there is increased interest in comparing results obtained by application of different techniques and establishing which is more adequate to address a particular problem (Loy et al., 2000; Lynch et al., 1996; McKeown and Jantz, 2002; Navarro et al., 2004; Palmqvist et al., 1996; Perez, 2003). Although tooth contour has been described using traditional and geometric morphometric techniques, few attempts have been made to evaluate and compare the amount of information extracted by these methods.

Hence, this work aims to compare the information about size and shape of molar contour obtained from linear measurements, landmarks and semi-landmarks, and to evaluate the pattern of variation among populations obtained by each method. In order to evaluate the differences between results obtained from different data: (a) buccolingual and mesiodistal crown diameters were measured, and centroid size and crown index were used as size and shape descriptors, respectively; (b) four landmarks and seventy nine semi-landmarks were collected from the molar outline,

and relative warps (RW) analysis was performed on partial warps and uniform vectors; centroid size was estimated to summarize tooth size. The ordinations resulting from each set of data were compared for similarity.

## Materials and methods

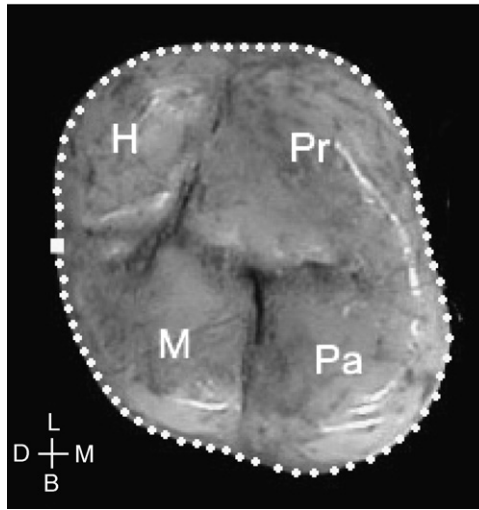
### Sample

The crowns of 35 permanent upper second molars ( $M^2$ ) of male and female individuals were analyzed. These teeth belong to archaeological samples from three regions of Argentina: North Patagonia (Chubut,  $n = 13$ ), West-Central or Cuyo (San Juan,  $n = 10$ ) and Northeast Argentina (Delta,  $n = 12$ ). The samples were obtained from the osteological collection at División Antropología, Facultad de Ciencias Naturales y Museo in La Plata (Argentina). Teeth that were not completely erupted, obscured by crowding, presented carious lesions or exhibited marked wear were excluded from the analysis. The scoring system proposed by Scott (1979) was used to quantify the degree of occlusal wear; only teeth with wear facets or small dentine patches corresponding to levels 1, 2 and 3 of Scott's system were included in this study. Likewise, 40 upper second molars were randomly selected from other archaeological samples from Pampean and Chacoan regions to assess intra-observer error. This study focuses on maxillary molars due to their lower size and shape variability with respect to mandibular molars (i.e. mandibular molars generally present a variable pattern and number of cusps, ranging between 4 and 7; Scott and Turner II, 1997; Turner II et al., 1991). Although  $M^2$  is not the most stable member within its morphogenetic field (Butler, 1939; Kieser, 1986) it was chosen based on the fact that it is morphologically less variable than  $M^3$  and better represented in archaeological samples than  $M^1$ , and is thus observable in a greater number of individuals. The low availability of  $M^1$  for this study is due to the higher levels of dental wear that occur in this molar.

### Data acquisition

Mesiodistal and buccolingual crown diameters of the molars were measured. Mesiodistal diameter was measured between the mesial and distal contact points of each crown (Goose, 1963). Buccolingual diameter was measured as the greatest distance between the buccal and lingual surfaces of the crown, taken at right angles to the plane in which the mesiodistal diameter was taken (Goose, 1963). All crown diameters were measured using a digital caliper (Tesa, Digit-Cal capa system) with 0.01 mm accuracy.

To perform geometric morphometric analyses each studied skull or individual tooth, if not *in situ*, was placed on a platform. Each tooth was photographed separately in occlusal view, using a C3030 Olympus digital camera with 3.3 megapixels resolution that was mounted on a special platform situated 100 mm from the occlusal surface of molars. Each tooth was oriented so that the occlusal surface was parallel to the camera lens. The scale was provided by means of a 10 mm ruler



**Fig. 1.** Allocated landmark (■), and semi-landmarks (●), on second upper molars. H: hypocone; Pr: protocone; Pa: paracone; M: metacone. The diagram on the bottom left indicates the orientation of lingual (L), mesial (M), buccal (B) and distal (D) surfaces.

placed level with the occlusal plane. Digital images were subsequently edited so that each tooth was aligned horizontally along its mesiodistal axis and detached from the background.

Next, four landmarks were digitized at the same points as used to measure inter-landmark distances (i.e. crown diameters) using tpsDig 1.40 (Rohlf, 2004). According to Bookstein (1991) these are type III landmarks because they are placed at extreme points of molar contour. Likewise, the equatorial outline (i.e. maximum breadth) of each molar was automatically recorded as coordinates of 80 equidistant points (Fig. 1) using tpsDig 1.40 (Rohlf, 2004). Each tooth was oriented along its mesiodistal axis, and digitization started at the mesial-most point. This point is actually a landmark; thus the outline was described by 1 landmark and 79 semi-landmarks (i.e. points distributed along a homologous contour). The configurations of point coordinates are hereafter referred to as specimens.

### Measurement error

Three sources of error may arise when identifying and recording landmark locations from specimens or their digital images: method-related – e.g. from specimen presentation, instrumental – e.g. from optical or digital distortion and personal – e.g. from subjective decisions (Arnqvist and Martensson, 1998). As the instrumental effects on the accuracy and precision of recorded data are expected to be negligible, subjective orientation of specimens and/or subjective location of landmarks by the operators will represent the primary sources of error (Robinson

et al., 2002). In consequence, intra-observer error in both measurements and tooth orientation was evaluated in this study.

Mesiodistal and buccolingual diameters were measured twice on each tooth. The degree of intra-observer error was evaluated by means of intraclass correlation coefficient (ICC; Shrout and Fleiss, 1979; Zar, 1999) and paired *t* test (Zar, 1999). Distributional normality of the two variables was tested by means of Shapiro–Wilk test before the computation of ICC and paired *t* test (Weber and Skillings, 2000).

An experimental design was devised in order to evaluate intra-observer error in landmark location and specimen orientation using the random sample of 40 upper molars. Operator inconsistency in landmark location was evaluated by taking a series of linear measurements (i.e. mesiodistal and buccolingual diameters) from the same set of images twice with a 1-week interval. A second set of images was obtained in order to evaluate error due to different orientation during photographing, and mesiodistal and buccolingual diameters were measured on each set of images. The degree of intra-observer error was assessed using the intraclass correlation coefficient (ICC; Shrout and Fleiss, 1979; Zar, 1999) and paired *t* test (Zar, 1999).

### Analysis of dental size and shape

For linear measurements size was measured using centroid size rather than crude diameters; the former is defined as the square root of the sum of squares of distances of all the points from their center of gravity (Bookstein, 1989). There are several size measures and not one is especially more valid. In this paper centroid size was used because it is mathematically independent of shape and also the most commonly used size measure in geometric morphometrics (Zelditch et al., 2004). Thus, the results obtained for linear measurements can be compared with geometric morphometric data. Dental shape was described by means of the crown index, calculated as buccolingual diameter expressed as a percentage of the mesiodistal diameter ( $BL/MD \times 100$ ).

In landmark-based analysis, shape can be defined as the information remaining in a configuration of landmark points after the differences due to location, scale and orientation have been removed (Bookstein, 1991, 1996a). A generalized Procrustes analysis (Gower, 1975; Rohlf, 1990; Rohlf and Slice, 1990) was performed on the landmark configuration in order to optimally translate, rotate and adjust the landmarks for size (computed as centroid size). The set of *x*- and *y*-coordinates of any single specimen's digitized two-dimensional landmark points are first centered at the origin (0,0) by subtracting the centroid or mean location of all landmarks from each (*x*, *y*) pair. After the specimen has been centered, the centroid size of the configuration is set to one through division of the coordinates by the initial centroid size of the specimen. An iterative procedure is used to determine the mean form onto which all specimens are aligned. During this iterative procedure, all specimens are first aligned as a single specimen, and the mean shape of all specimens is calculated. All specimens are then rotated to minimize the added squared differences of landmark coordinates between each specimen and the estimated mean shape or

reference form. This procedure is repeated until the mean shape does not change substantially after iteration of the orientation procedure (Rohlf, 1999).

Outline analysis was performed following the approach proposed by Bookstein (1997) and Green (1996). This approach incorporates points, called semi-landmarks, along a curve and permits the analysis of outlines using techniques developed for landmark-based analysis. This operation extends the standard Procrustes superposition procedure: in addition to optimally translating, scaling, and rotating landmarks, the semi-landmark points are slid along the outline curve until they match as well as possible the positions of corresponding points along an outline in a reference specimen (Adams et al., 2004). This is done because the curves or contours should be homologous, whereas the individual points in these are not claimed to be homologous from subject to subject (Bookstein, 1997). Consequently, the variability along tangent directions is not informative, and only the coordinate normal to the outline carries information about differences between specimens or groups (Bookstein, 1997; Bookstein et al., 2002). Various criteria exist for sliding points along an outline. In this study the perpendicular projection criteria or minimum Procrustes distances (Bookstein et al., 2002; Sampson et al., 1996; Sheets et al., 2004) were used; this method slides the points to minimize the distances between the curve of the reference and each individual in the sample. The perpendicular projection criterion removes the difference along the curve in semi-landmark positions between the reference form and each specimen by estimating the tangent direction to the curve and removing the component of the difference that lies along the tangent to the curve (Sheets et al., 2004). The semi-landmarks were aligned along their respective curves using the Semiland6 software (Sheets, 2003).

Principal components analysis (PCA) was carried out on the partial warp scores plus uniform components derived from the partial Procrustes aligned landmark and semi-landmark coordinates by means of thin-plate spline decomposition based on the bending energy matrix (Bookstein, 1996b; Dryden and Mardia, 1998). The partial warp scores are components along the orthogonal eigenvectors of the bending energy matrix, and describe non-affine patterns of shape difference, whereas the uniform components (Bookstein, 1996b) describe affine differences in shape. The thin-plate spline decomposition has become a standard technique in geometric morphometrics, as it yields a convenient set of variables to carry out multivariate statistical analyses since the partial warp plus uniform component scores express shape changes in the same number of variables as there are independent measurements. Additionally, the thin-plate spline method allows for use of the deformation grid diagram to depict shape changes. A PCA carried out on the partial warp scores is called a RW analysis and was performed using tpsRelw 1.40 (Rohlf, 2004). Centroid size was used as size measurement. The PCA on partial warps plus centroid size was performed in order to describe the major trends in molar form. This PCA was obtained from the correlation matrix to allow for the scale differences between the centroid size and the partial warps.

Pearson's correlation was used to compare the three estimations of centroid size based on linear measurements, landmarks and semi-landmarks. In addition, the following correlations were calculated in order to assess the degree of association

among the three types of data on dental shape: (a) correlation between crown index and the specimens' scores along the first RW as well as along all RW, both for landmark and semi-landmark data; and (b) correlation between the ordinations obtained from RW analysis for landmark and semi-landmark data. The following parameters were calculated in order to contrast the information about form extracted by the different types of variables: (a) correlation between the ordination obtained from buccolingual and mesiodistal diameters and PC performed on partial warps plus centroid size, both for landmarks and semi-landmarks; and (b) correlation between the ordinations along the first two PC, as well as along all PC, for landmark and semi-landmark data. A Procrustean superimposition approach (Gower, 1971) was used to assess the overall degree of association between these matrices. In this analysis the matrices were scaled and rotated in order to find an optimal superimposition to maximize their fit. The sum of the squared residuals between configurations in their optimal superimposition can then be used as a metric of association (Gower, 1971). A permutation procedure (Procrustes test) implemented by Jackson (1995) was then used to assess the statistical significance of the Procrustean fit (Peres-Neto and Jackson, 2001). The comparisons among matrices were performed using both the first two PCs and all PCs, because the discussion of the PCA results takes into account their two most common interpretations: (1) as the linear combinations of the shape variables that have the greatest variance in the original data; and (2) as the dimensions that best reproduce the observed distances between all the forms of the data set using linear combinations of the original variables (interpretation as principal coordinates, see Reyment and Jöreskog, 1993).

Pearson's correlation, PCA and Procrustes test were performed using R 1.9.1 (Ihaka and Gentleman, 1996).

## Results

### Intra-observer error

Normality cannot be rejected for any variable according to the Shapiro–Wilk test ( $p > 0.05$ ).

The results of intraclass correlation for the linear measurements taken with digital calipers indicate significant correlations both for mesiodistal (CCI = 0.98,  $p < 0.01$ ) and buccolingual diameters (CCI = 0.98,  $p < 0.01$ ). Moreover, the paired  $t$  values show no statistically significant differences between repeated measurements of both variables ( $t = 1.57$ ,  $p > 0.05$  for mesiodistal;  $t = 1.09$ ,  $p > 0.05$  for buccolingual diameter).

The results of intraclass correlation indicate very good agreement in the location of landmarks to measure both mesiodistal (CCI = 0.95,  $p < 0.01$ ) and buccolingual diameters (CCI = 0.98,  $p < 0.01$ ). Moreover, the paired  $t$  values show no statistically significant differences between repeated measurements of both variables ( $t = 0.56$ ,  $p > 0.05$  for mesiodistal;  $t = 0.90$ ,  $p > 0.05$  for buccolingual diameter). The

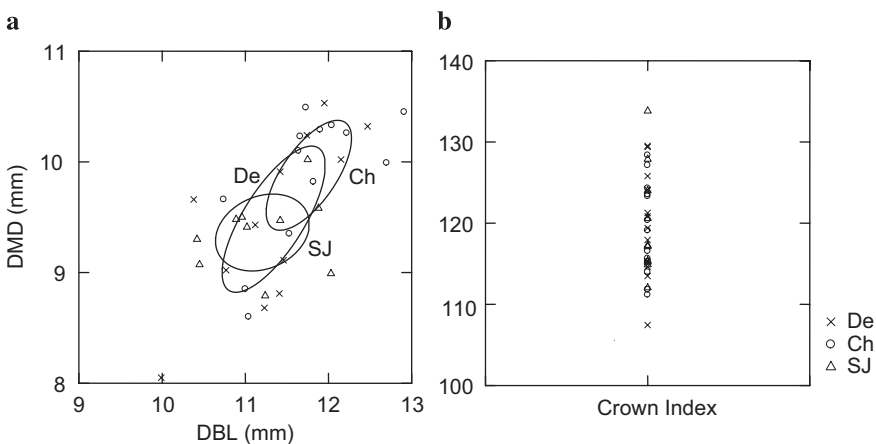
measurements taken on two different images of the same tooth agree very well, both for mesiodistal ( $CCI = 0.95$ ,  $p < 0.01$ ) and buccolingual ( $CCI = 0.92$ ,  $p < 0.01$ ) diameters. Likewise, there are no significant differences between measurements taken on different images ( $t = 0.72$ ,  $p > 0.05$  for mesiodistal;  $t = 1.14$ ,  $p > 0.05$  for buccolingual diameter). These results indicate that differences due to specimen orientation are negligible.

### Size and shape analyses

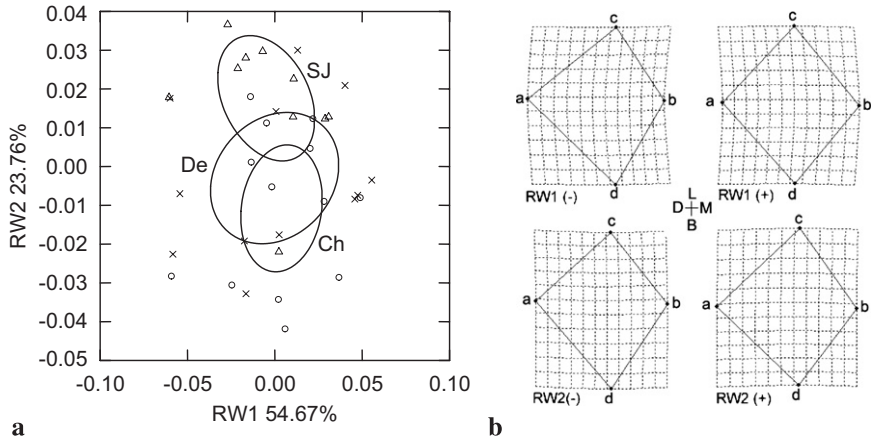
When buccolingual diameter is plotted against mesiodistal diameter, the three samples show extensive superposition (Fig. 2(a)). Likewise, no shape differences between the samples are seen when crown index is used (Fig. 2(b)).

Fig. 3(a) is a plot of the first two RW calculated from the four landmarks, which account for 78.37% of the explained variance. The first RW explains 54.61% of the variance, and reflects mainly the position of landmarks 2 and 4 (Fig. 3(b)). These landmarks are located at the maximum buccolingual diameter. The second component describes differences in the position of the landmarks corresponding to the mesiodistal diameter (i.e. landmarks 1 and 3). When among-group variation is considered, the sample from West-Central Argentina is set apart from the other two samples along the second RW axis. Similar results are seen in the PCA performed on partial warps plus centroid size (Fig. 4).

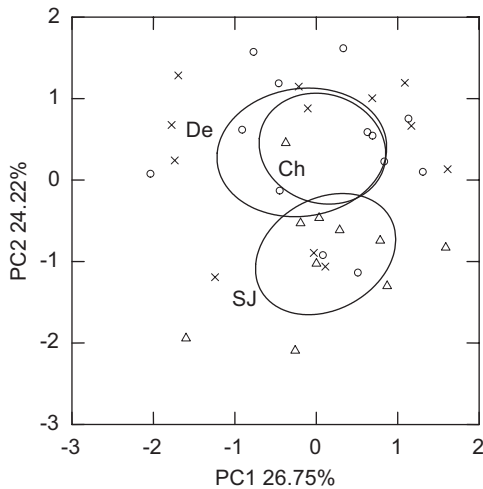
Fig. 5(a) shows the first two RWs calculated from the landmarks and semi-landmarks of dental contour, which account for 55.26% of the explained variance. The first RW explains 32.13% of the variance, and the molars at its most negative values are characterized by a projecting paracone cusp and well-developed protocone and hypocone cusps (Fig. 5(b)). On the contrary, those molars located at the most positive values have a reduced hypocone and are buccolingually shorter



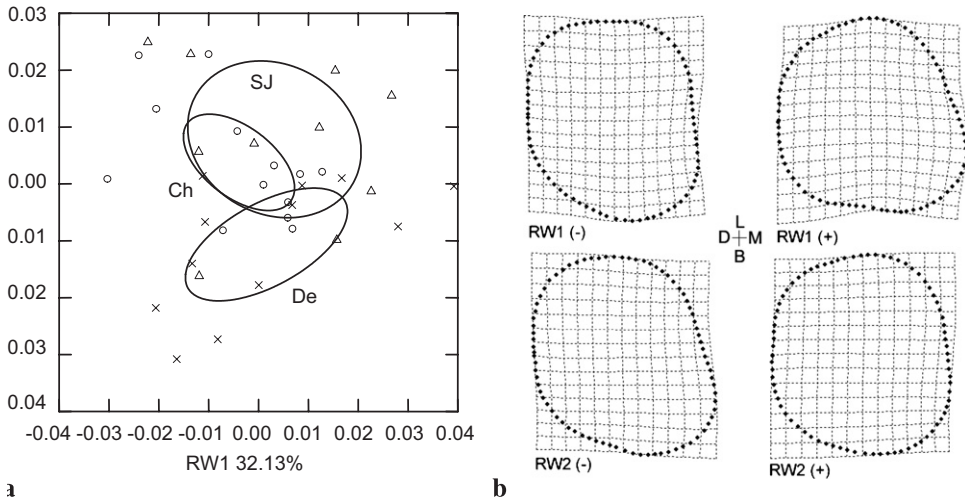
**Fig. 2.** (a) Buccolingual diameter plotted against mesiodistal diameter; (b). Plot of crown index by sample. Ch: Chubut; SJ: San Juan; and De: Delta.



**Fig. 3.** (a) First two relative warps obtained from landmark analysis of second upper molar Ch: Chubut; SJ: San Juan; and De: Delta; **Fig. 3(b).** The deformation grids represent shape changes with respect to the molar consensus configuration at the negative and positive extremes of the first and second relative warps axes. The differences among molars along the first axis are mainly due to variation in the distal landmark. The shape differences along the second axis, which separates the San Juan sample from the others, are more subtle and located in distal and buccal landmarks. The letters represent landmarks digitized at the same points used to measure mesiodistal (a–b) and buccolingual (c–d) diameters.



**Fig. 4.** First two principal components derived from shape variables plus size obtained from landmarks analysis. The samples are separated along the second axis. Ch: Chubut; SJ: San Juan; and De: Delta.

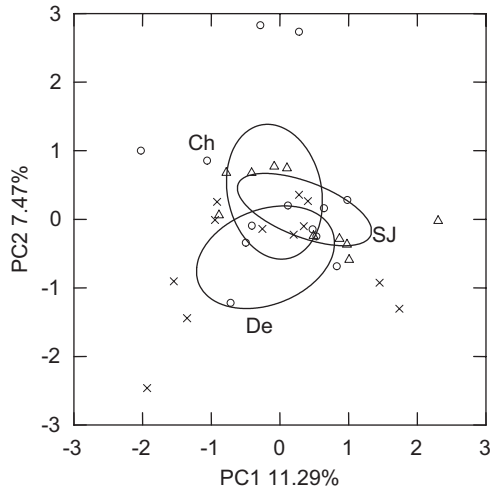


**Fig. 5.** (a) First two relative warps obtained from semi-landmark analysis of second upper molar contour Ch: Chubut; SJ: San Juan; and De: Delta; **Fig. 5(b).** Deformation grids represent shape changes with respect to the molar consensus configuration at the negative and positive extremes of the first and second relative warps axes. The molars located at the negative extreme of RW1 have relatively longer buccolingual axis and shorter mesiodistal axis than those located at the positive extreme. The main variation along the second RW axis is located in the paracone cusp; development of this cusp is greater in the molars at the negative extreme than in those at the positive extreme.

than the molars with negative values. The second component describes mainly differences in paracone development (**Fig. 5(b)**). This cusp projects buccally in those molars at most negative values. When among-group variation is considered, the Northeast sample is differentiated from the other two samples along the second RW axis. This sample is characterized by a greater projection of the paracone cusp than either the West-Central or North Patagonian samples. The PCA results change slightly when centroid size is incorporated into the geometric morphometric analysis (**Fig. 6**), but even so the Northeast sample is still separated from the other two samples.

The correlation between size measurements used in traditional and geometric morphometric analyses was high and significant. The centroid size calculated from crown diameters is highly correlated with the centroid size estimated from four landmarks ( $r = 0.91$ ,  $p < 0.01$ ), and also with the centroid size from the semi-landmarks analysis ( $r = 0.89$ ,  $p < 0.01$ ). The correlation between centroid sizes from landmark and semi-landmark-based analyses is high and significant ( $r = 0.97$ ,  $p < 0.01$ ).

The results of Pearson's correlation analysis show low non-significant association between index values and scores on the first RW, both for landmark-based ( $r = 0.14$ ) and semi-landmark-based ( $r = 0.26$ ) analyses. The Procrustes test indicates low



**Fig. 6.** First two principal components derived from shape and size variables obtained from semi-landmarks analysis. Ch: Chubut; SJ: San Juan; and De: Delta.

association between crown index values and all RW performed on 2D coordinates, both for landmarks ( $r = 0.32$ ,  $p < 0.05$ ) and semi-landmarks ( $r = 0.28$ ,  $p < 0.05$ ). The association between specimen ordinations along all RWs in landmark and semi-landmark-based analysis is significant but relatively low ( $r = 0.47$ ;  $p < 0.05$ ). Similar results are achieved when only the axes with greater variation are considered. The results of the Procrustes test indicate very low association between specimen ordinations along the first two RW in landmark and semi-landmark-based analysis ( $r = 0.29$ ,  $p > 0.05$ ).

The ordination of specimens based on the buccolingual and mesiodistal diameters is fairly well correlated with the PC scores from landmark data, which describe the variation in shape plus size ( $r = 0.51$ ,  $p < 0.01$ ). In contrast, when linear measurements are compared with PCA calculated from semi-landmarks, the correlation is low and non significant ( $r = 0.22$ ,  $p = 0.46$ ). Similar results were achieved when comparing ordinations along all PCs axes for landmark and semi-landmark data ( $r = 0.38$ ,  $p = 0.24$ ). The correlation between the first two PCs of the PCA of centroid size plus partial warp for landmark based analyses and the PCs obtained from semi-landmarks was low but significant ( $r = 0.40$ ,  $p < 0.05$ ).

## Discussion

The results indicate that size is consistently estimated by the three variable types (i.e. linear measurements, landmarks and semi-landmarks). The obtained centroid size values are highly correlated among the three types of data; therefore the former supply similar information.

The contour of human molars can be seen as a structure simpler than the molars of other mammals. Thus, it would seem that a small set of linear measurements could capture the main variations concerning the crown contour. However, noticeable differences are found when the results of molar shape described by linear measurements are compared with those obtained using four landmarks. When index values, which contain all the shape variation captured by linear measurements, were compared with all the RW calculated from landmarks, the association between ordinations was very low. This means that relevant information about molar shape variation was gained by using geometric morphometric techniques. Even though the points digitized in landmarks-based geometric morphometric analyses are the same ones used to take linear measurements, the results were different. This implies that the location of maximum length is an important source of variation among molars that is not captured by traditional techniques. According to Bailey (2004), although both mesiodistal and buccolingual diameters, as well as the index constructed from these measurements, are useful ways of quantifying the shape of rectangular teeth, they are insufficient to accurately describe irregularly shaped teeth. This happens because one serious limitation of traditional morphometrics is that interlandmark measurements convey no information about geometric structure; this implies that the relative position of the homologous points or landmarks is lost in the analyses (Adams et al., 2004; Rohlf and Marcus, 1993). On the contrary, geometric morphometrics can extract and preserve the information about the spatial location of morphological variation (Adams et al., 2004). The differences found between index values and RW analysis may also arise from the fact that traditional methods measure size rather than shape, whereas landmark-based methods are able to remove size very efficiently. This does not mean that traditional data include no information about shape but rather that it is extremely difficult to extract shape information from interlandmark distance as well as to separate information about shape from size (Monteiro et al., 2002; Zelditch et al., 2004). Even though the procedures needed for data acquisition and analysis in geometric morphometrics require more time and observer training than linear measurements, the abovementioned advantages justify their application to study dental variation.

The analyses performed show that semi-landmark analysis yields results that differ from those based either on crown diameters or landmark data. Semi-landmarks permit the capture of variations in hypocone shape and degree of development that cannot be shown either by linear or landmark-based techniques. When this cusp is reduced the lingual surface of the molars tends to be rounded, whereas good development of the hypocone and protocone results in square-shaped molars. The differences between the results obtained by these two methods might be partly due to the fact that in the case of semi-landmark analysis, the information on shape variability is extracted from the entire outline and may include axes of variation that are not revealed by the four analyzed landmarks. This shows that additional information was gained from the analysis of outlines. Among the techniques that have been proposed for the analysis of shape variation patterns in biological structures with few or no landmarks, semi-landmark-based analysis would be preferable due to the fact that this approach allows for the combination of

landmarks and semi-landmarks in the same statistical analysis tool (Bookstein, 1997). Likewise, the use of semi-landmarks is preferable to other outline methods such as Elliptic Fourier Analysis (Ferson et al., 1985; Kuhl and Giardina, 1982; Rohlf and Archie, 1984) because its relationship with Kendall shape space is known (Bookstein and Green, 1993). In the analysis of human molars, this approach makes it possible to combine contours, described by semi-landmarks, with landmarks located on features of the occlusal surface, such as cusp tips. Accordingly, additional information can be gained by combining landmark and semi-landmark based analyses due to the fact that the analysis of crown components, such as intercuspal measurements, might provide data that are biologically more meaningful than conventional measures of whole tooth crowns (Peretz et al., 1998; Sekikawa et al., 1988; Townsend, 1985).

The results of the Procrustes test show that the three description systems (i.e. linear measurements, landmarks and semi-landmarks) result in different ordinations of specimens when molar form (i.e. shape plus size) is compared. As the correlations were low for all comparisons, the various morphological mappings constructed express different patterns of relationships among specimens. In addition, the interpretation of similarities among samples varies according to the selected description system. It was not possible to differentiate among the three archaeological samples analyzed by using crown diameters, whereas landmark and semi-landmark based analysis allowed the separation of the samples along the first two PCs axes. As PCA is used to compare patterns of morphological variation among samples, only the first two components, which account for the greater percentages of explained variance, are considered in almost all cases. Thus, the results show that the relative position of the samples with respect to these first two PC axes changes according to the variable employed (e.g. landmarks or semi-landmarks). When landmarks are used, the Chubut and Delta samples are close to each other, whereas the San Juan sample is separated from the others along the second PC. Conversely, the results based on semi-landmarks show a close relationship between Chubut and San Juan samples. Such discrepancy among ordinations might be partly due to the fact that each type of variable extracts different amounts of information about molar shape, and also that morphological variation among human populations is low. In this way, several authors have shown that the differences between results achieved by diverse techniques seem to be related to the morphological variation in the analyzed samples (Adams and Funk, 1997; Baylac et al., 2003; Navarro et al., 2004). Navarro et al. (2004) found that linear-based descriptors of *Microtus* molars appear to be very effective at capturing major features of shape and performing species discrimination. These authors showed that interspecific differentiation is well recovered from morphospaces based either on traditional or geometric morphometric techniques, while intraspecific differentiation is not quantified in the same way and is markedly different. Geometric morphometric techniques are also more powerful than traditional ones for separating biological species that are extremely similar in morphology, termed cryptic or sibling species (Adams and Funk, 1997; Baylac et al., 2003). Therefore, when analyzing biological relationships among modern human populations that

display low levels of morphological variation (Barbujani et al., 1997; Lewontin, 1972; Relethford, 1994), it is desirable to employ techniques that allow capturing subtle shape differences. Accordingly, Falk and Corruccini (1982) found that traditional dental measurements sort individuals into human populations worse than cranial measurements do. However, this may be attributable to the technique used to extract morphological information, rather than to the fact that teeth are less indicative of the processes differentiating human populations. According to our results, landmarks and semi-landmarks allowed molar shape differences among samples to be explored in greater detail than linear measurements did and were more appropriate for finding similarities and differences between human populations. The results obtained by the semi-landmark based analysis agree with those from other anthropometric studies of these populations. A recent study aimed at assessing degree of variation of craniofacial morphology of South American Amerindians using linear variables, found that Delta sample was not closely related to Chubut sample (Sardi et al., 2005). Likewise, Perez (2006), in a geometric morphometric analysis of facial morphology, also found that San Juan and Chubut samples were close to each other in the RW analysis, whereas Delta sample was quite distant from the others. In summary, these studies support the hypothesis that the Delta populations are not biologically related to those from San Juan and Chubut.

## Conclusions

This study shows that considerable information about molar contour is added by using landmarks instead of crown diameters. Additionally, some morphological features can only be captured by means of semi-landmark analysis. Therefore, such analyses appear to be more powerful for revealing variations in the relative position and degree of development of dental cusps. An important consequence of the differences in the amount and type of shape information captured by these methods is the difference in results concerning biological relationships among the three archaeological samples analyzed according to the method employed. Differences among the samples were only found by means of geometric morphometric analyses. Due to the relatively low levels of morphological variation occurring among modern human populations, the use of such techniques, which allow complete description of shape, becomes more appropriate.

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