CHAPTER 5

MATERIALS AND METHODS

5.1 Materials

In order to be able to identify and side individual hand bones, an initial sample of 20 sets of hands from each sex-race group (blacks, whites, males and females) were collected. These hands were obtained from cadavers, aged between 21 and 86 years that had been completely dissected by second year medical and dental students.

To estimate stature and sex of an individual, an additional sample of 30 sets of hand bones from each of the four groups were used. These hands were obtained from skeletons currently housed in the Department of Anatomy at the University of Pretoria. These skeletons are from individuals aged between 21 and 81 years. The entire study thus comprised 50 sets of hands from each sex-race group giving a total sample of 200 individuals.

5.1.1 Pretoria Bone Collection

The Anatomy Department receives on an annual basis approximately 50 to 100 bodies which are either unclaimed or donated. The unclaimed bodies are obtained from local state hospitals in the Tshwane Metropolitan Area (L'Abbé *et al*. 2005). Under the Human Tissues Act, No 65 of 1983, anyone may donate his or her body to science for the purpose of tissue transplants, medical training and research. This act also covers destitute people who die in public hospitals. If these individuals are not claimed within a twenty-four hour period, the body is transferred to the nearest medical school where they are embalmed and stored for about a year. All bodies that are received by the Department of Anatomy at the University of Pretoria, are of known age, sex and population affinity. Additional information regarding the height, weight and cause of death is also known.

These bodies are then dissected by students registered in the Department of Anatomy. After being dissected the bodies undergo a cleaning process which includes maceration and defatting. Complete skeletons are then reserved for the Pretoria Bone Collection and used for

research purposes. The incomplete skeletons are allocated to the Student Collection and used for teaching purposes. The Pretoria Bone Collection currently has approximately 2000 skeletons. The currently appointed director for the Pretoria Bone Collection is EN L'Abbé who can be contacted for any additional information including access to this collection (L'Abbé *et al*. 2005).

5.2 Methods

5.2.1 Preparation of the dissected hand bones for identification and siding (Figures 5.1a-f)

The method for preparation of the hand bones as described by Scheuer and Elkington (1993), were used as a guide for the present study. The right and left hands necessary for purposes of identification were disarticulated from each cadaver after the students had completed dissecting them, removing as much of the soft tissue as possible. These hands were placed into a single calico bag which was divided into two pockets to separate the right and left hand belonging to the same cadaver (Figure 5.1). An aluminium identification label which would not be destroyed by the boiling process was attached to each hand. The calico bags were then placed into a larger linen bag and transferred to a huge drum filled with water. The drum was then positioned over an open fire and the hands were boiled for approximately four days in order to soften the tissue. It should be stressed that if the hands are boiled for a longer period than stipulated, it may soften the tissues to a point where individual hand bones may mix and thus make it difficult to assign them to a digit.

Once the hands were boiled for the specified time period, they were cleaned manually by N Navsa. The cleaning process involved disarticulating the individual hand bones and removing the surrounding soft tissue. Once disarticulated and cleaned, all five metacarpals and 14 phalanges from each hand were placed into specially designed calico bags. Each calico bag was divided into 38 small pockets which were carefully sewn to accommodate and isolate the 19 hand bones from each hand of one cadaver. Each pocket was labelled exactly

the same way as for each finger bone. The metacarpals were each assigned a single digit number, namely, 1 for thumb, 2 for index finger, 3 for middle finger, 4 for ring finger and 5 for little finger. Each phalangeal bone was assigned two numbers. The first number indicated the bone associated with a specific finger, namely, the number 1 (thumb), 2 (index finger), 3 (middle finger), 4 (ring finger) and 5 (little finger). The second number would indicate the row that the bone belongs to, namely, 1 for proximal phalanx, 2 for middle phalanx and 3 for distal phalanx. As an example, the phalanges belonging to the index finger are numbered as 21, 22 and 23 for proximal, middle and distal phalanges respectively, those for the middle finger as 31, 32 and 33, etc.

In the final stages of preparation, the labelled calico bags containing the hand bones were boiled in Trichloroethylene solution for 24 hrs in order to remove all traces of fat. The hands were then removed from the individual pockets, air-dried on racks and labelled as mentioned above. All 80 sets of hand bones from each of the four groups, including both right and left hands, were prepared by N Navsa. In this way, each bone was correctly assigned to the appropriate digit.

The individual bones were carefully examined and characteristic features recorded for identification purposes. Various anatomical textbooks were used to assist in compiling detailed descriptions of each hand bone. Unique features of individual hand bones were used to assign them to either the right or left hand. These unique features were listed at the end of the descriptions of each hand bone. Right and left hands were cleaned and employed in the identification and siding process, however, only the bones from the left hand were used for the rest of this study. For purposes of orientation, the following terms were used with the hand in the anatomical position, namely, dorsal, palmar, medial, lateral, articular end of the head and articular end of the base. To make the descriptions easy to follow, numbers are placed next to each bony landmark identified. These numbers coincide with the numbers on the corresponding figure.

5.2.2 Problems which arose throughout the preparation of the dissected hand bones for identification and siding

Once the maceration process was complete, the hands were laid out on tables and hand diagrams were then made to record morphological features. In the process of noting landmarks, asymmetries were observed, not only in a series of bones, but also between corresponding bones of the right and left hand. The characteristic features noted for identification purposes were also used for siding the hand bones. Once a list of identifying and siding features had been put together, the question arose as to the presentation of this information. Line diagrams were attempted but these did not bring out detail that was required. Photographs of each hand bone were taken and these were redone on a few occasions so that the morphology of each bone could be clearly seen. Each photograph was then re-looked and it was decided to highlight certain features with a thick broken line to emphasize the bony landmark. During the entire process, terminologies had to be standardized which meant that labelling on photographs of each hand had to be checked to maintain consistency throughout.

Photographs of the individual hand bones were re-done on many occasions in order to enhance key morphological traits that were described in the text. While terminologies of the metacarpals in most anatomy textbooks proved to be limited for descriptive purposes, the phalanges, especially the distal row in this series of hand bones, lacked an adequate description in most anatomy textbooks. In these cases, new terminologies were introduced which best represented characteristic features of each bone. A shortfall in the use of new terminologies is that different persons may view bony landmarks differently. For example, a tubercle to one researcher may be considered to be a tuberosity to another.

5.2.3 Measurements of the hand bones (Figures 5.2 to 5.17)

Only left hands from a total sample of 200 (50 white males, 50 white females, 50 black males and 50 black females) individuals were measured. A total of seven measurements, as opposed to six used by Scheuer and Elkington (1993), were taken on each hand bone, namely, length, dorsal palmar and medial lateral width of the base, dorsal palmar and medial

lateral width of the head and dorsal palmar and medial lateral width of the midshaft region. All measurements were recorded to the nearest 0.01 mm, using a digital caliper.

Length measurement:

Maximum length was measured as opposed interarticular length used by Scheuer and Elkington (1993). The reason for taking the maximum length is that proximal and distal articular ends of each bone varied in shape and this may affect accurate length measurements. Maximum length was recorded by placing the digital caliper on the lateral aspect of the bone. All length measurements were taken along the longitudinal axis of the bone from the proximal to the distal end (illustrated in Figures 5.2, 5.4, 5.6, 5.8, 5.10, 5.12, 5.14 and 5.16).

Base measurements:

The maximum medial-lateral measurements were always taken from the most medial to the most lateral point on the base of each hand bone (Figures 5.2, 5.4, 5.6, 5.8, 5.10, 5.12, 5.14, and 5.16). Maximum diameters were also recorded from the most dorsal to the most palmar point (Figures 5.3, 5.5, 5.7, 5.9, 5.11, 5.13, 5.15, and 5.17). This was done to overcome the morphological variation which exists at the proximal end of each of these bones. This variation was more marked in the metacarpals than in the phalanges. All measurements were carried out using a caliper which was always positioned in the medial lateral and dorsal palmar plane.

Head measurements:

Maximum medial-lateral (Figures 5.2, 5.4, 5.6, 5.8, 5.10, 5.12, 5.14, and 5.16) and dorsal-palmar (Figures 5.3, 5.5, 5.7, 5.9, 5.11, 5.13, 5.15, and 5.17) measurements of the head were also recorded. Unlike the base, the morphology of the distal ends of each hand bone displayed fewer variations which made it easy to record the maximum dimensions. All measurements of the head were also recorded with a calliper positioned in the medial lateral and dorsal palmar plane.

Midshaft measurements:

Scheuer and Elkington (1993) used a single measurement for the midshaft, namely, the maximum dimension. In the present study, the midshaft was found to have a maximum medial-lateral (Figures 5.2, 5.4, 5.6, 5.8, 5.10, 5.12, 5.14, and 5.16) as well as a maximum dorsal-palmar (Figures 5.3, 5.5, 5.7, 5.9, 5.11, 5.13, 5.15, and 5.17) dimension. The midshaft of each hand bone was established by noting the halfway mark of the maximum length recorded. The midshaft region for all hand bones was found to be the narrowest part of the shaft. Both diameters of the midshaft region were recorded with a caliper which was positioned in the medial-lateral and dorsal-palmar plane.

5.2.4 Measurements of the humerus, radius, ulna, femur and tibia

The length of the bones most commonly used for stature estimation, namely, humerus, radius, ulna, femur, and tibia, had to be measured. This dimension was needed as the length of each hand bone had to be regressed to that of a long bone. Only long bones belonging to the left side of the skeleton were used and these long limb bones came from the same cadavers and skeletons as the hand bones. The maximum length of each of the five long bones was recorded using an osteometric board (Figures 5.18 to 5.22).

5.3 Statistical analysis

A data file was created using Statistical Product and Service Solutions (SPSS®, version 11.5). All variables used in this study were defined according to the measurements recorded on each hand bone. All data collected were subsequently entered separately for black males, black females, white males and white females into the SPSS spreadsheet. Once entered and before any analyses was done, screening and cleaning of the data was done. In this way any errors in the data was checked and corrected. In other words, any value/s that fell outside the range of possible values for a variable was checked and corrected. All minimum and maximum values were looked at to see if they were within the range of possible scores for

that variable. Valid and missing cases were also screened for any errors that may have occurred when entering the data. A paired *t*-test for inter- and intraobserver test was carried out to test for repeatability of the measurements.

To test for intra-observer repeatability in the metric analysis, hand bones from 36 individuals were randomly selected from a total sample of 200. These bones were the metacarpal, proximal, middle, and distal phalanges of the thumb and little finger. All seven hand bone dimensions recorded on the initial sample of 200, were re-measured on this random sample after all the initial data was collected. The repeated metric data was statistically compared to the original data set using a one-way analysis of variance (ANOVA).

To test for inter-observer repeatability in the metric analysis, a PhD student in Anthropology was asked to re-measure the randomly select left hand bones from the 36 individuals. The student was not involved in this study at all. The metric data collected by this student was statistically compared to the original set of data also using a one-way ANOVA.

Once the data were screened for errors and the repeatability test was carried out, a basic statistical analysis was done to establish whether the 7 hand bone dimensions were significantly different firstly between whites and blacks, and secondly, between males and females. To do this an independent sample T-test was carried out and the descriptive statistical analysis which was calculated included the means, standard deviations, and ranges. The independent samples T-test will indicate whether there is a statistically significant difference in the mean scores for the 7 dimensions carried out on the hand bones (metacarpals and phalanges) and the length dimensions of long bones (humerus, radius, ulna, femur and tibia). That is, it will establish whether the hand and long limb bone dimensions differ firstly, between whites and blacks and secondly, between males and females of the South African population.

5.3.1 Stature determination

The mean values of hand and long limb bone measurements in the descriptive statistics indicated few statistically significant differences between whites and blacks, the data was thus pooled. In order to reconstruct the length of a long bone, a correlation was established between the metacarpals and phalanges to each of the five long limb bones, namely, the humerus, radius, ulna, femur, and tibia. A correlation analysis is done to indicate the strength and direction of the linear relationship between the hand bones to each of the long limb bones, otherwise it will not be possible to use them for linear regressions. The Pearsons correlation test was chosen for this analysis.

As most of the skeletons lacked documented cadaver lengths and the reliability of documented cadaver lengths when present, was questionable, it was decided to regress the length of a hand bone to that of a long limb bone. The length of a long bone is frequently used when determining stature of an unknown individual.

Generally, for stature estimation both univariate and multivariate analyses in a direct and stepwise manner is carried out. In the present study, a univariate analysis was done where the hand bones were regressed against each of the long bones of the upper (humerus, radius, and ulna) and lower (femur and tibia) limbs. Multivariate analyses of these variables were not carried out because of the numerous combinations possible, which would have been beyond the scope of this thesis. It was thus decided to carry out only a single variable analysis. From the regression analyses, the correlation coefficient (r) standard error of estimate (SEE), slope and intercept were obtained.

The value obtained from this analysis could then be inserted into a second formula such as that devised by Lundy and Feldesman (1987) and Dayal *et al*. (2008) in order to estimate final stature. The statistical analysis is discussed further under the chapter of stature estimation.

5.3.2 Sex determination

As the intention of this analysis was to provide sex discriminant functions, it was necessary to determine whether or not levels of sexual dimorphism varied between the white and black groups. An analysis of variance (ANOVA) was then carried whereby a detailed description of data for males, females, whites, and blacks was generated. From the ANOVA analysis, data for whites and blacks were pooled and a discriminant function was run on the pooled data.

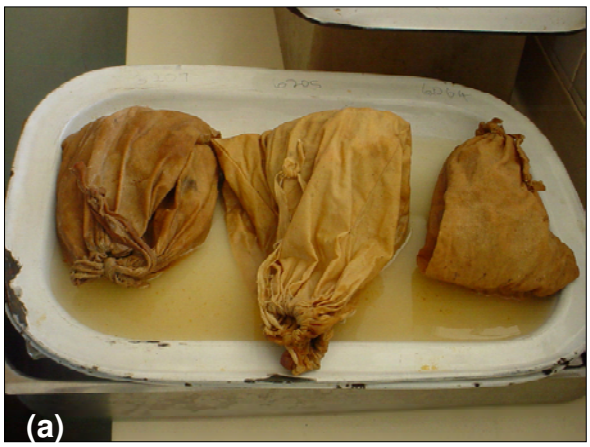
Discriminant function analysis is primarily used to sort binomial characteristics (e.g. sexual dimorphism) between two groups (e.g. males and females). This method identifies those variables that are competent at separating groups, selects variables which perform equally well and it also selects variables with similarities and differences. Discriminant analysis provides a predictive model for group membership (e.g. males or females) based on the observed characteristics (e.g. measurements) of each case. The statistical output generates a discriminant function for the two groups or a set of discrimant functions if there are more than two groups. These functions are generated from a sample of cases for which group membership is known and then applied to new cases with the available measurements where group membership is unknown (Pallant 2001, Tabachnick & Fidell 2007).

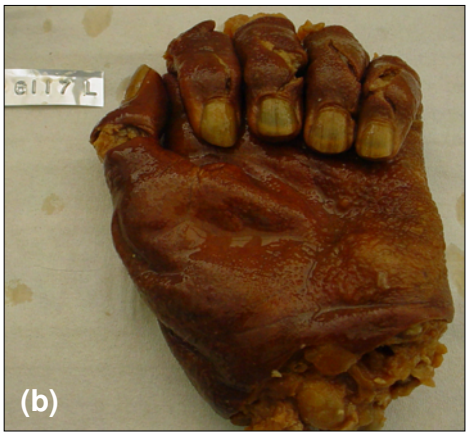
The accuracy of the discriminant function is expressed by an F-ratio and Wilks Lambda value. The F-ratio is used to assess whether the differences between the groups are statistically significant, with higher F-scores indicating a stronger significance. Wilk's Lambda identifies the contribution each variable had in distinguishing between the sexes and defines the order in which dimension will appear in the discriminant function formula. If a score=1 it means that the groups are equal. Smaller Lambda values indicate increased variability between the groups (Pallant 2001, Tabachnick & Fidell 2007). After the stepwise analysis was calculated, the variable that appeared to best distinguish between the sexes, was selected and subjected alone to direct discriminant analysis so as to develop a formula for determing sex from fragmented remains.

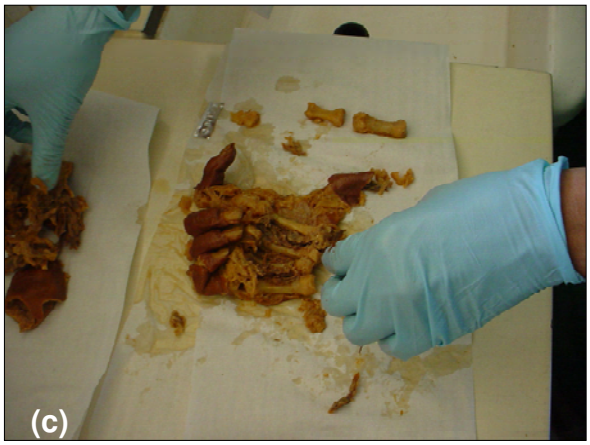
Classification accuracies were then tested. A “leave one out classification” procedure was employed to measure the effectiveness of the discriminant function. This method of analysis classified each specimen from the functions derived from all the other cases. The results were then cross-validated to determine whether or not specimens were correctly assigned to either the male or female group.

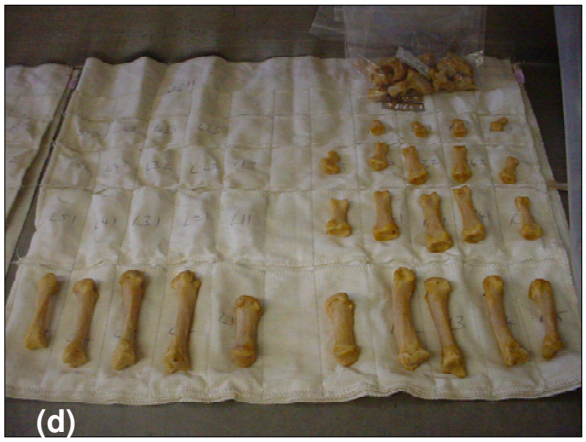
Discriminant function statistics explore the predictive ability of a set of independent variables on a single categorical dependent measure. In other words, it takes the measured seven dimensions of each hand bone and uses them to predict the sex (male or female) of an individual with a certain degree of accuracy. The use of linear discriminant analysis for deriving classification functions is dependent on the assumption of multivariate normality of the data and equality of variance-covariance matrices (Tabachnick & Fidell 2007). In other words, the distribution of each variable within each of the classes must be normal. Studies have shown that the level of sexual dimorphism varies within the skeleton by population (Garn *et al*. 1973, Meadows & Jantz 1957).

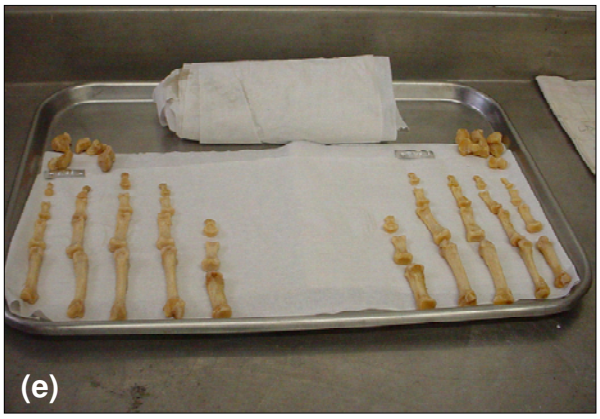
Figure 5.1: Paired hands in calico bags for boiling (a), hand after boiling (b), disarticulation and cleaning of individual bones (c), calico bag with 30 pockets for individual bones ready for defatting process (d), hand bones being air-dried (e), labelling of individual bones (f).











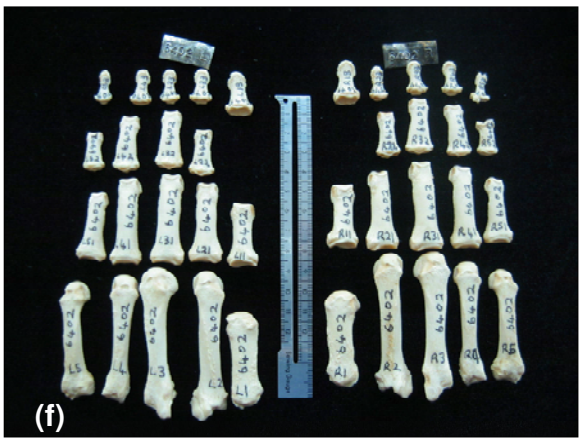


Figure 5.2: Palmar view of metacarpal I - thumb (m-l = medial lateral measurement)

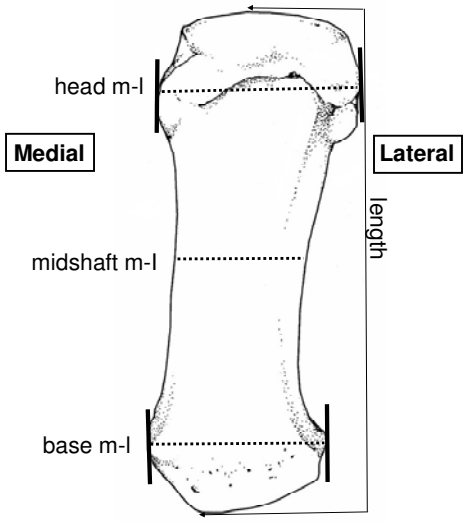


Figure 5.3: Lateral view of metacarpal I - thumb (d-p = dorsal palmar measurement)

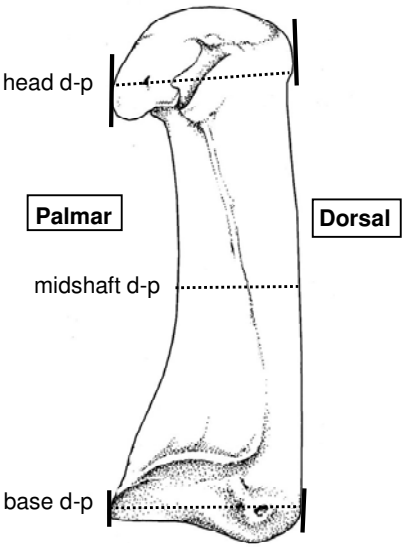


Figure 5.4: Palmar view of metacarpal II - index finger (m-l = medial lateral measurement)

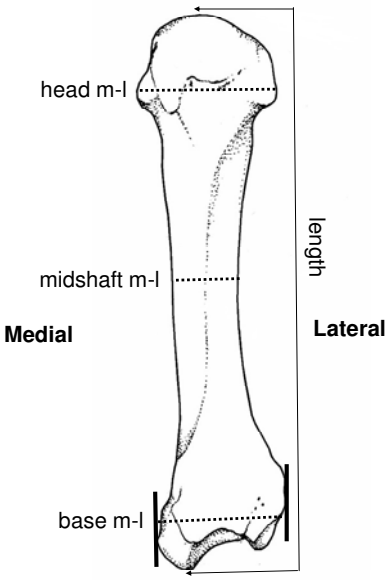


Figure 5.5: Lateral view of metacarpal II - index finger (d-p = dorsal palmar measurement)

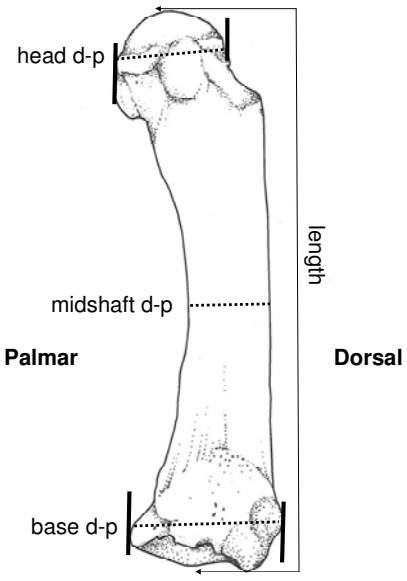


Figure 5.6: Palmar view of metacarpal III - middle finger (m-l = medial lateral measurement)

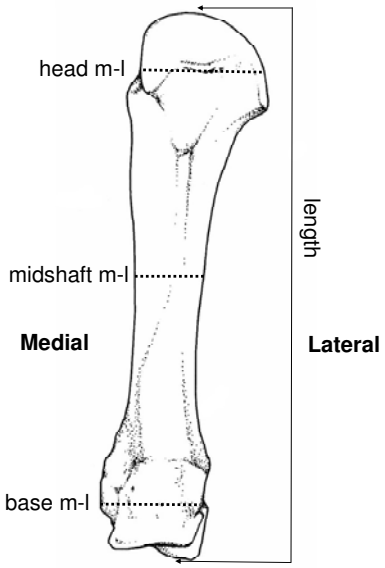


Figure 5.7: Lateral view of metacarpal III - middle finger (d-p = dorsal palmar measurement)

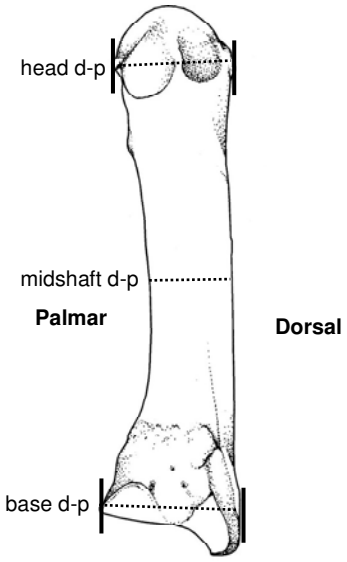


Figure 5.8:Palmar view of metacarpal IV - ring finger (m-l = medial lateral measurement)

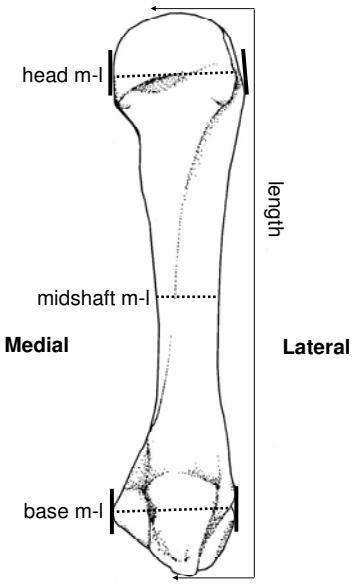


Figure 5.9: Lateral view of metacarpal IV - ring finger (d-p = dorsal palmar measurement)

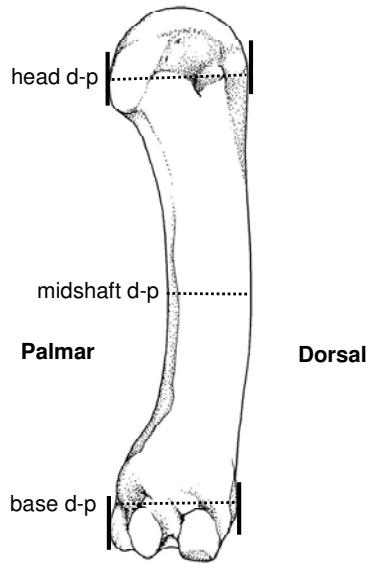


Figure 5.10: Palmar view of metacarpal V - little finger (m-l = medial lateral measurement)

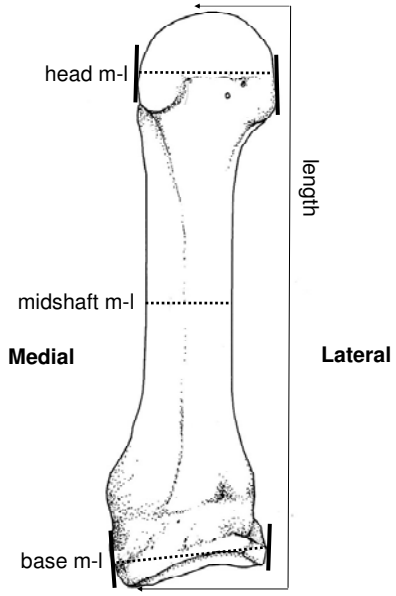


Figure 5.11: Lateral view of metacarpal V - little finger (d-p = distal palmar measurement)

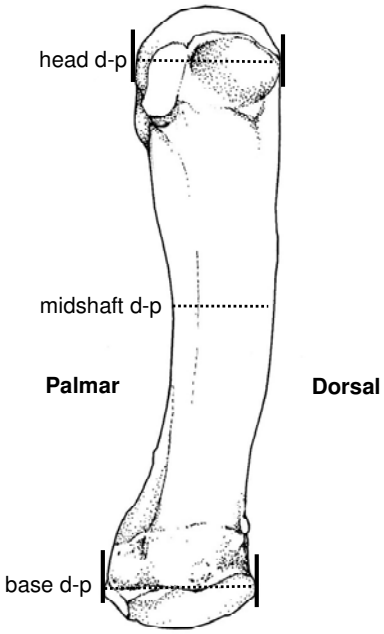


Figure 5.12: Palmar view of proximal phalanx - index finger (m-l = medial lateral measurement)

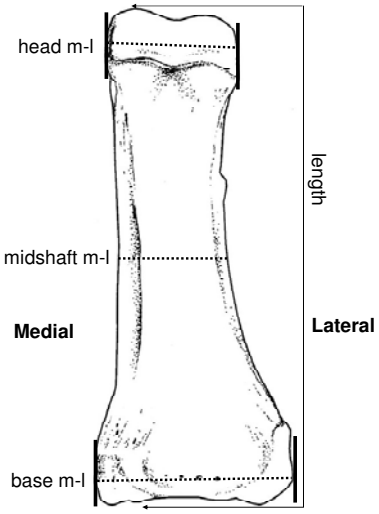


Figure 5.13: Lateral view of proximal phalanx - index finger (d-p = distal palmar measurement)

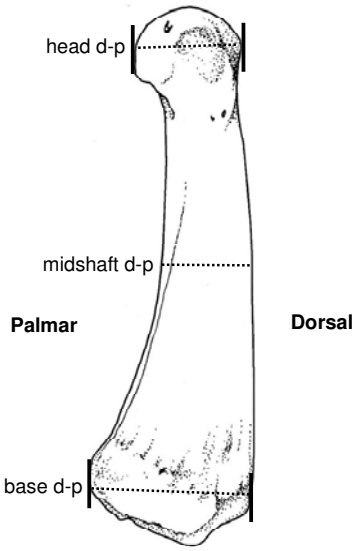


Figure 5.14: Palmar view of middle phalanx - index finger (m-l = medial lateral measurement)

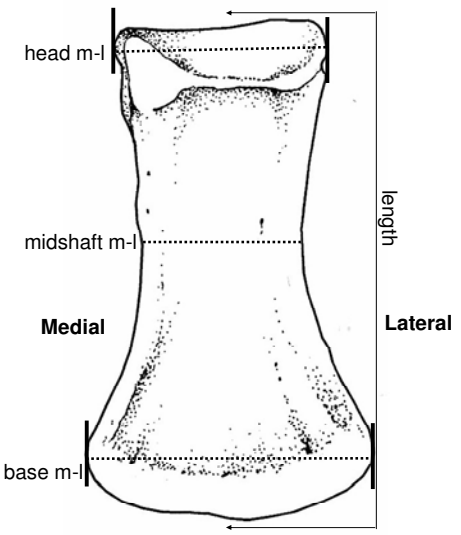


Figure 5.15: Lateral view of middle phalanx - index finger (d-p = dorsal palmar measurement)

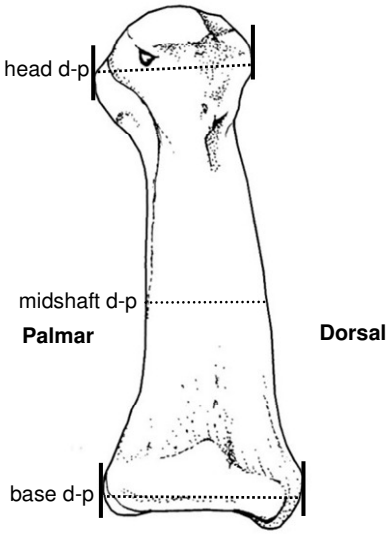


Figure 5.16: Palmar view of distal phalanx - index finger (m-l = medial lateral measurement)

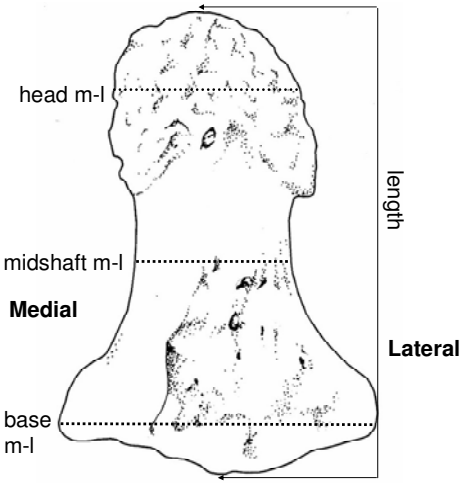


Figure 5.17: Lateral view of distal phalanx - index finger (d-p = dorsal palmar measurement)

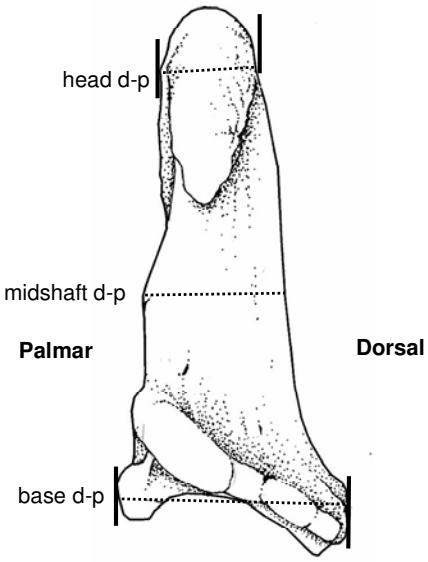


Figure 5.18: Maximum length measurement of the left humerus

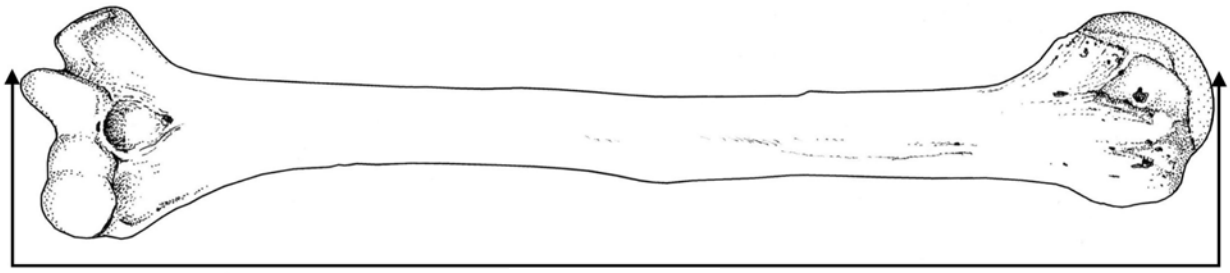


Figure 5.19: Maximum length measurement of the left radius

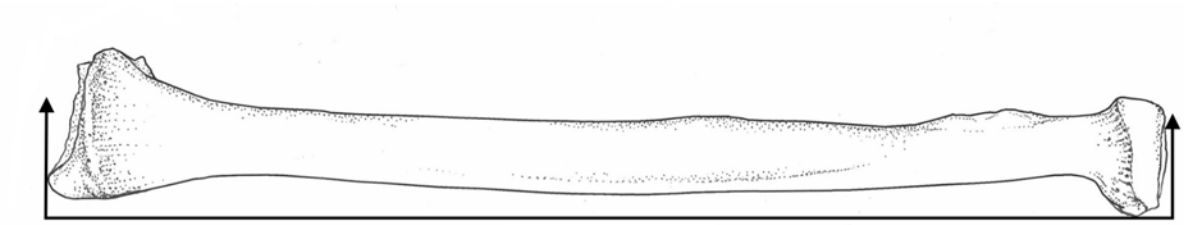


Figure 5.20: Maximum length measurement of the left ulna

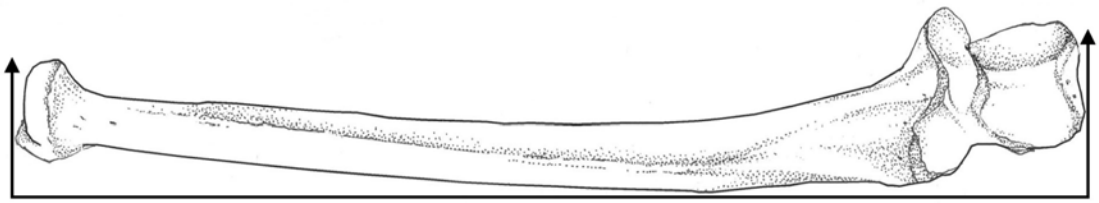


Figure 5.21: Maximum length measurement of the left femur

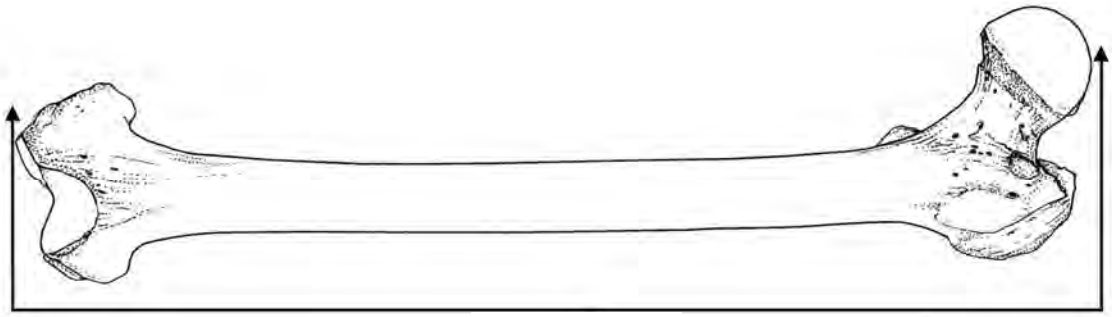


Figure 5.22: Maximum length measurement of the left tibia