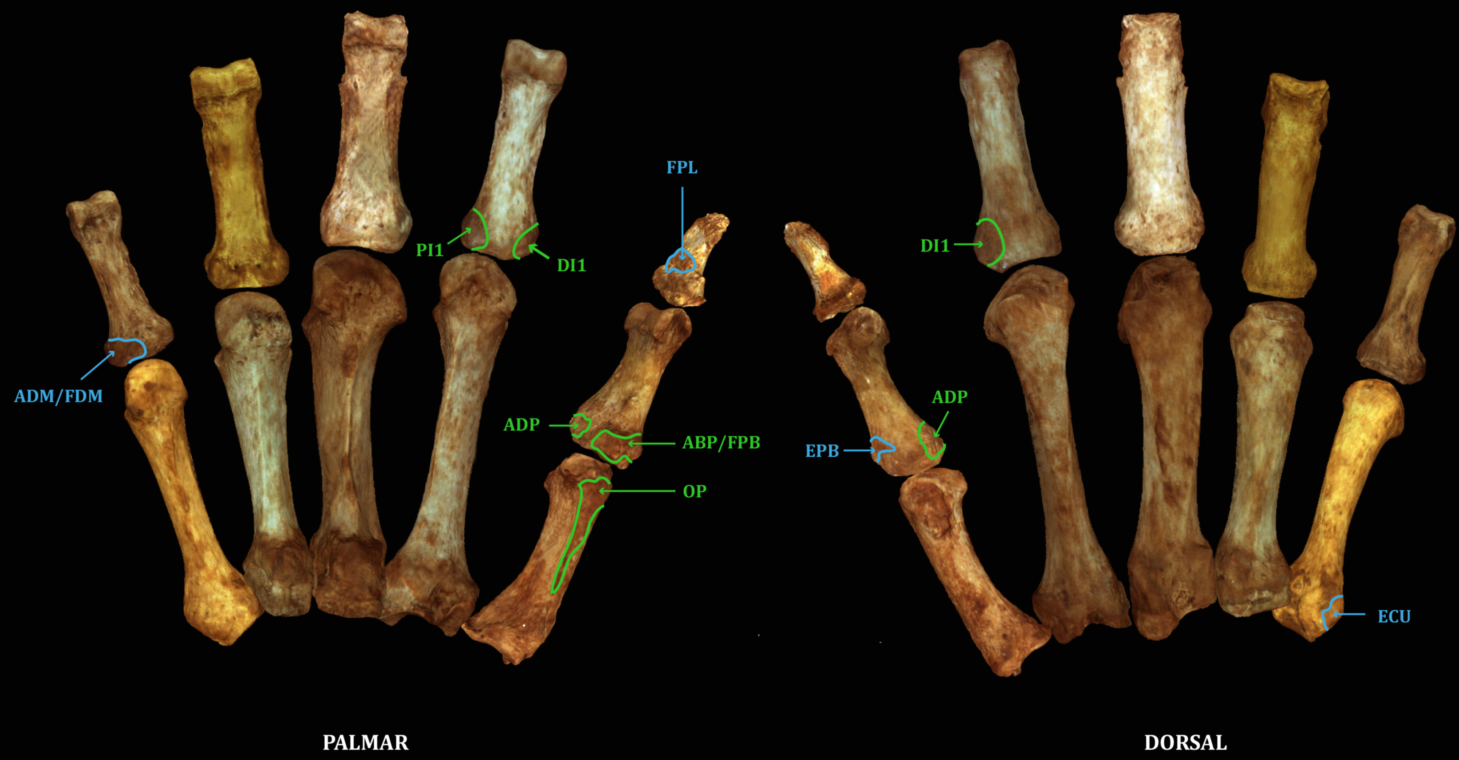
Investigating the effect of manual physical activity on the form of human hand entheses



Fotios Alexandros KARAKOSTIS

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Senckenberg Center for Human Evolution and Palaeoenvironment

INVESTIGATING THE EFFECT OF MANUAL PHYSICAL ACTIVITY ON THE FORM OF HUMAN HAND ENTHESES

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Fotios Alexandros KARAKOSTIS

aus Marousi / Griechenland

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Dekan:	Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatter:	Prof. Dr. Katerina Harvati
2. Berichterstatter:	Prof. Dr. Joachim Wahl

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ABSTRACT

Reconstructing physical activity based on human skeletal remains comprises a fundamental objective of anthropological sciences. Entheses, the areas of muscle attachment on the bone surfaces, have been widely utilized as occupational stress markers. However, most previous methods for analyzing entheseal morphology (size and/or shape) are characterized by substantially low precision, lack of three-dimensional (3D) multivariate statistical analysis, and absence of individuals documented for their long-term physical activities. Furthermore, no previous study used histological methods for assessing the effect of physical activity on the interindividual variability of entheseal surfaces. My past research put forth a precise methodology for measuring the 3D size of hand entheseal areas, identifying two main multivariate patterns among entheses. On this basis, this PhD thesis performed a multi-disciplinary approach to the analysis of hand entheseal form (a term encompassing both size and shape) and its potential relationship with habitual physical activities.

In Paper I, the hand entheses of a thoroughly documented sample were analyzed using a highly precise 3D method of quantification, followed by multivariate statistical analysis. The utilized material is part of an anthropological collection which is gradually becoming a unique universal reference for the most detailed documentation of the specimens' lifelong physical activities. The results revealed a close statistical association between multivariate patterns of hand entheses and the nature of individuals' long-term occupational profiles.

In the framework of an ongoing multidisciplinary research project, the developed method of entheseal analysis was applied for reconstructing the occupational profile of an unidentified individual from Basel (Paper II). The results indicated that this specimen was involved in precise manual activities relying on thumb-index finger interactions. This outcome came in agreement with other research on the physical activities of this individual.

Paper III introduced a new and precise geometric morphometric approach for investigating hand entheseal 3D shape, identifying a statistically significant interaction between 3D size and shape variation in three hand entheses. In this way, it set a novel basis for future research on both aspects of entheseal form (3D size and shape), bridging the gap between quantitative and qualitative methods of entheseal analysis. Furthermore, the results

showed that larger phalangeal entheses present a proportionally more projecting surface, which is linked to greater moment arm and biomechanical efficiency for the attaching muscle.

Finally, paper IV presented a microscopic histological analysis of hand entheses, which reported an interaction between entheseal morphology and the levels of applied biomechanical forces. Particularly, the elevated bone areas of hand entheses were associated with greater concentrations of calcified fibrocartilage, which is widely considered as a direct indicator of biomechanical stress. As a consequence, individual entheses with elevated marginal areas comprised evidence of greater biomechanical stress.

Overall, this PhD thesis identified a clear interaction between physical activity and human hand entheses based on three methodological approaches and four separate analyses. The multivariate 3D analysis showed that the patterns among different hand entheses reflect the nature of an individual's lifelong occupational activities. At the same time, the shape of larger entheseal areas is proportionally projecting, providing greater biomechanical efficiency for the attaching muscle. Finally, higher entheseal bone elevation seems to be related with greater biomechanical stress (i.e., higher concentrations of calcified fibrocartilage).

ZUSAMMENFASSUNG

Die Rekonstruktion körperlicher Aktivitäten basierend auf spezifischen Merkmalen des menschlichen Skelettes stellt ein grundlegendes Ziel der anthropologischen Wissenschaften dar. Muskelansatzstellen auf der Knochenöberfläche, auch Enthesen genannt, sind in der Vergangenheit häufig zur Interpretation beruflich bedingter, wiederkehrender Belastungen verwendet worden. Ein Großteil der bisherigen Studien zur Enthesen-Morphologie (Größe und / oder Gestalt) ist durch eine sehr geringe Präzision, dem Fehlen von multivariaten statistischen Analysen in drei Dimensionen (3D) und dem Fehlen von longitudinalen Daten zur körperlichen Aktivität der untersuchten Individuen, gekennzeichnet. Die Existenz histologischer Studien zur Beurteilung der möglichen Auswirkungen körperlicher Aktivität auf die interindividuelle Variabilität enthesealer Oberflächen an vergleichbaren Stichproben ist nicht bekannt. Anhand meiner bisherigen Forschung konnte eine präzise Methode zur 3D-Größenmessung der Handenthesen entwickelt werden. Mit Hilfe dieser Methode ist es möglich zwei Hauptgruppen an Individuen in der multivariaten Analyse der Enthesen zu unterscheiden. Basierend auf dieser Grundlage wurde in der hier vorliegenden Dissertation eine multidisziplinäre Formanalyse der Handenthesen (ein Begriff, der sowohl Größe als auch Gestalt einschließt) und deren möglicher Beziehung zu gewohnheitsmäßigen körperlichen Aktivitäten durchgeführt.

In Paper I wurden die Handenthesen einer sorgfältig dokumentierten Stichprobe mittels einer hochpräzisen 3D-Quantifizierungsmethode analysiert, gefolgt von einer multivariaten statistischen Analyse. Das verwendete Material ist Teil einer anthropologischen Sammlung, welche eine einzigartige universelle Referenz für die sehr detaillierte Dokumentation lebenslanger körperlicher Aktivität multipler Individuen darstellt. Die Ergebnisse deckten eine enge statistische Assoziation zwischen multivariaten Mustern von Handenthesen und der Art der langfristigen beruflichen Profile von Individuen auf.

Im Rahmen eines laufenden multidisziplinären Forschungsprojektes wurde die entwickelte Entheseanalyse zur Rekonstruktion des beruflichen Profils einer nicht identifizierten Person aus Basel (Paper II) angewendet. Die Ergebnisse wiesen darauf hin, dass das Individuum in präzise manuelle Aktivitäten involviert war, die hauptsächlich auf

Daumen-Zeigefinger-Interaktionen beruhten. Dieses Ergebnis stimmte mit anderen Untersuchungen über die körperlichen Aktivitäten dieses Individuums überein.

In Paper III wurde ein neuer und präziser geometrisch-morphometrischer Ansatz zur Untersuchung der dreidimensionalen Gestalt von Handenthesen vorgestellt, der eine statistisch signifikante Interaktion zwischen 3D-Größe und Gestaltvariation in drei Handenthesen identifiziert. Auf diese Weise wurde eine neue Grundlage für zukünftige Forschungen zu beiden Aspekten der Entheseform (3D-Größe und -Gestalt) geschaffen und die Lücke zwischen quantitativen und qualitativen Methoden der Entheseanalyse geschlossen. Darüber hinaus zeigten die Ergebnisse, dass größere Phalanx-Enthesen eine proportional mehr vorstehende Oberfläche aufweisen, die mit einem größeren Momentarm und biomechanischer Effizienz für den anhaftenden Muskel verbunden ist.

Darüber hinaus wurde in Papier IV eine mikroskopisch-histologische Analyse der Handenthesen vorgestellt, die eine Interaktion zwischen Enthese-Morphologie und dem Ausmaßder angewandten biomechanischen Kräfte aufzeigt. Insbesondere die erhöhten Knochenbereiche von Handenthesen waren mit höheren Konzentrationen von verkalkten Faserknorpel verbunden, was weithin als ein direkter Indikator für biomechanischen Stress angesehen wird. Dem zu Folge waren einzelne Enthesen mit erhöhten Randbereichen einen erhöhten biomechanischen Stress ausgesetzt.

Insgesamt wurde in dieser Doktorarbeit eine klare Interaktion zwischen wiederholter körperlicher Aktivität und menschlicher Handenthese anhand von drei methodischen Ansätzen und vier separaten Analysen nachgewiesen. Die multivariate 3D-Analysekonnte zeigen, dass die Muster verschiedener Handenthesen die Art der lebenslangen beruflichen Aktivitäten eines Individuums widerspiegeln. Zudem ragt die Gestalt der enthesealen Bereich proportional zur Enthesengröße über die umgebende Knochenoberfläche heraus, wodurch eine größere biomechanische Effizienz für den anhaftenden Muskel erreicht wird. Letztlich scheint eine höhere Knochenerhebung mit einer größeren biomechanischen Belastung (d.h. höheren Konzentrationen von verkalktem Faserknorpel) verbunden zu sein.

LIST OF PUBLICATIONS FOR CUMULATIVE DISSERTATION

Four publications submitted in fulfillment of the requirements of this cumulative dissertation. Numbers in parentheses represent percentage of own contribution to the articles or manuscripts (original idea/data analysis/writing/publication).

Paper I (100/100/90/90):

Karakostis, F. A., Hotz, G., Scherf, H., Wahl, J., Harvati, K. (2017). Occupational manual activity is reflected on the patterns among hand entheses. *American journal of Physical Anthropology*, 164, 30–40.

Paper II (20/30/20/20):

Hotz, G., Doppler, S., Gamma, M.-L., Gysin, D., Haas, P., Helmig, G., Huber, L., Kramis, S., **Karakostis, F. A.**, Meyer, L., Lopreno, G. P., Rauber, J., Roewer, L., Rothe, J., Spycher, A., Wittwer-Backofen, U., Zulauf-Semmler, M. (2017). Theo der Pfeifenraucher: Ein genealogisch-naturwissenschaftliches Identifizierungsprojekt. *Yearbook of the SSGS*, 44, 29-61.

Paper III (100/100/90/90):

Karakostis, F. A., Hotz, G., Scherf, H., Wahl, J., Harvati, K. (in revision). A repeatable geometric morphometric approach to the analysis of hand entheseal three-dimensional form. Accepted in the *American journal of Physical Anthropology.*

Paper IV (100/90/90/80):

Karakostis, F. A., Vlachodimitropoulos, D., Piagkou, M., Scherf, H., Harvati, K., Moraitis, K. (submitted). Is bone elevation in hand muscle attachments associated with biomechanical stress? A histological approach to an anthropological question. Manuscript submitted to the *Journal of Anatomy*.

INTRODUCTION

"Human skeletal research aiming at reconstructing past activities [...] *has been embraced as a kind of 'Holy Grail' by an entire subfield of human osteology."*

Jurmain et al., 2012.

Background of the research

The importance of skeletal markers of occupational stress

Identifying a consistent link between physical activity and human skeletal remains has long been regarded as a fundamental research objective of anthropological sciences (Foster et al., 2012; Henderson et al., 2017), having been characteristically referred to as the "Holy Grail" of bioarchaeology (Jurmain et al., 2012). This is because such an association could provide the long-expected basis for safely reconstructing the habitual behavior of past human populations from both bioarchaeological and paleoanthropological contexts. In this way, it would allow establishing a solid connection between the biological and cultural remains of humans, thus contributing to a holistic approach of the factors driving human history and evolution.

Particularly, accurately reconstructing the habitual activities of past human communities would provide a safer basis for testing some of the cornerstone hypotheses in paleoanthropology and bioarchaeology. In human evolution, these include the association between early hominins and the first lithic tool industries (Almecija et al., 2010; Kivell et al., 2011), the cultural factors underlying the extinction of the Neanderthals (Niewoehner, 2001), the complexity of *Homo floresiensis'* behavioral patterns (Tocheri et al., 2007), and the emergence of behavioral modernity in modern humans (involving standardized production of sophisticated items and distribution of labor) (Kunh & Stiner, 2009). In bioarchaeological contexts, assessing physical activities could comprise an essential tool which could re-evaluate our current understanding of past societies' manual activities, distribution of labor, and social structure or transformations (e.g., Hawkey and Merbs, 1995; Karakostis et al., 2015).

Based on standard anthropological practice, the skeletal markers typically utilized for reconstructing physical activity involve osteopathology, accessory articular facets, robusticity, cross-sectional morphology, and entheses (Galtes et al., 2007). The occurrence of osteoarthritis and/or accessory articular facets in various joints of the human skeleton is thought to result from excessive biomechanical strain (e.g., Lai & Lovell, 1992; Becker, 2016). Furthermore, the external or internal (i.e., cortical and trabecular) bone architecture is considered to undergo transformations throughout life, due to the bone's reaction to biomechanical stress inflicted by physical activity and the lifelong process of bone remodeling (e.g., Wolff, 1892; Beaulieu et al., 2015; Karakostis et al., 2015; Skinner et al., 2015). However, until present, no previous research has provided evidence for solid associations between any of these traits and the exact nature or intensity of physical activities (Jurmain et al., 2012). This is mainly because the degree in which long-term physical activity affects these bone aspects is still not determined, while previous research has not yet concluded on the influence of other important factors of variation, such as genetic variability, biological age, body size, hormone levels, nutrition, and pathology (Rauch, 2005; Foster et al., 2012; Karakostis & Lorenzo, 2016; Henderson et al., 2017). The main reason for this fundamental research gap is related to the absence of a human anthropological sample composed of individuals thoroughly documented for their biological, medical, socioeconomic, and occupational characteristics.

Entheses as occupational stress markers

Entheses are defined as the areas of the bones where muscles or tendons attach, comprising the only preserved human remains of the musculoskeletal mechanism responsible for muscle recruitment (Standring, 2008; Foster et al., 2012). Based on the concept that the morphology of entheseal areas undergoes lifelong changes inflicted by the bone's response to habitual biomechanical stress (Wolff, 1892; Rauch, 2005), experts postulate on the nature of long-term physical activity by analyzing the robusticity and the pathological manifestations of entheseal bone surfaces (i.e., enthesopathies) (Villotte et al., 2010; Foster et al., 2012). Enthesopathies, which may be osteophytic or osteolytic, are considered to be possible indicators of excessive stress (Mariotti et al., 2004; Villotte et al., 2010). However, these manifestations seem to be also strongly associated with other factors, including age-related degenerative changes, various musculoskeletal diseases, or traumata (Alves-Cardoso & Henderson, 2010).

Entheseal robusticity refers to variability in the form (a term encompassing both size and shape) of muscle attachment sites (Foster et al., 2012; Henderson et al., 2017). The traditional techniques for assessing the stage of entheseal development involve naked-eye observation of entheses followed by the application of various ordinal scoring systems (e.g., Hawkey & Merbs, 1995; Mariotti et al., 2007; Cashmore & Zakrzewski, 2013; Henderson et al., 2017). However, the precision of these traditional qualitative methods is proven to be significantly low, involving substantial inter-observer disagreement (Davis et al., 2013; Wilczak et al., 2016). Particularly, the reported repeatability error for these methods is typically 20% or above (Davis et al., 2013; Wilczak et al., 2016). Furthermore, the relationship between biomechanical stress and entheseal form remains highly controversial (Foster et al., 2012; Henderson et al., 2017), while there is low consistency among studies regarding the morphological criteria of each entheseal stage of development (Villotte et al., 2016). In fact, several studies have questioned the relationship between entheseal morphology and muscle contraction or physical activities (e.g., Zumwalt, 2006; Djukic et al., 2015; Rabey et al., 2015; Williams-Hatala et al., 2016; Wallace et al., 2017). These previous works attempted identifying bivariate correlations between each entheseal structure and corresponding muscle size or physical activity patterns. By contrast, a few more recent approaches focused on the multivariate relationship among different entheseal patterns, proposing that these multivariate patterns (rather than the entheseal forms themselves) are associated with lifelong physical activity (Milella et al., 2015; Karakostis & Lorenzo 2016).

Research on the entheses of the human hand

Throughout human evolution and history, the hand comprises a fundamental means of human interaction with the surrounding environment. For this reason, previous studies have relied on the analysis of hand bone morphology for assessing the evolution of manual dexterity and habitual manual behavior in past human species or populations (e.g., Almecija et al., 2010; Richmond et al., 2016). In this framework, the form of hand entheses has been utilized as an indicator of muscle hypertrophy and the frequent performance of particular hand movements, which are in turn linked to certain cultural contexts (Niewoehner, 2001; Almecija et al., 2010). However, these past assessments usually relied on macroscopic observations or linear bone measurements (e.g., Niewoehner, 2001; Almecija et al., 2010). In fact, until recently, the only method for assessing the development of hand entheses involved a binary scoring system based on the naked-eye visibility of each entheseal structure.

Recent research by Karakostis & Lorenzo (2016) has put forth a new and three-dimensional (3D) quantitative method for identifying, delineating, and computing the surface size of hand entheses. This approach was proven to be highly precise, showing high levels of intra-observer and inter-observer repeatability. Furthermore, that study performed a multivariate analysis of entheseal area measurements, reporting that the morphometric relationship among different entheses seems to directly reflect manual muscle synergies, which are fundamental for the performance of basic power and precision grips. The conclusion of that work was consistent with previous experimental research on bone mass and formation (Lohman et al., 1995; Heinonen et al., 1996; Bennell et al., 1997; Palombaro, 2005), which indicated that the distribution of bone mineral across different skeletal areas is regulated by the physical activities of each individual. This promising methodology, which combines high precision with multivariate analysis of high-resolution 3D data, established a novel basis for investigating whether entheseal form reflects habitual physical activity.

The high necessity of a thoroughly documented skeletal sample

Previous anthropological studies on occupational stress markers were based on occupation-at-death (e.g., Alves Cardoso & Henderson, 2010; Alves Cardoso & Henderson, 2012; Lopreno et al., 2013). However, this level of documentation is not reliable for predicting habitual activities (Alves Cardoso & Henderson, 2012). This is mainly because this information is usually inadequate, relying exclusively on a job mentioned in individuals' death certificates. Moreover, previous research has demonstrated that the process of bone remodeling leading to bone formation requires an average of four to eight months to be complete (Ganda, 2013), while experimental research based on biochemical markers did not detect bone formation in athletes over a 12-month period of systematic training (e.g., Bennell et al., 1997). Therefore, research on entheses should only rely on specimens whose documented occupational activities were consistent for years before death. Additionally, given that the mechanisms of entheseal change undergo substantial degenerative changes after approximately the age of 50 or 60 years (Niinimaki et al., 2011; Milella et al., 2012), entheseal research should not utilize specimens above this approximate age range.

Nevertheless, meeting these sampling requirements is impossible without access to a skeletal series with highly detailed documentation regarding the individuals' lifelong habitual activities. If such a collection were available, applying the aforementioned methodology (Karakostis & Lorenzo, 2016) could indicate whether the hand entheseal patterns of individuals vary according to the nature of their long-term habitual activities. Moreover, such an extensive documentation could be utilized to control for the various potential factors affecting entheseal form (Foster et al., 2012; Henderson et al., 2017), such as population group, sex, exact biological age, body size, manual pathologies (including handicaps), socioeconomic status, as well as direct relatedness among individuals. Until now, no previous anthropological research on entheses has relied on a sample with this high level of documentation. Thus, previous results concerning the effect of long-term physical activity on entheseal morphology could be regarded as questionable.

In search of a precise method for assessing entheseal shape variation

The above-mentioned multivariate methodology is based on the 3D size of entheseal surfaces (Karakostis & Lorenzo, 2016), without taking into account their 3D shape. Therefore, it remains unclear whether the observed 3D measurements of entheses also reflect shape variability across specimens. This is particularly important because the proportional extension of hand entheses is directly associated with greater biomechanical efficiency (Maki & Trinkaus, 2011; Richmond et al., 2016), while the size of entheses is correlated with muscle forces throughout the animal kingdom (Deymier-Black et al., 2015; Rossetti et al., 2017).

This research gap is mainly due to the fact that no previous study has introduced a highly repeatable geometric morphometric analysis of entheseal 3D form. This is in spite of the plethora of past entheseal studies relying on macroscopic observations of entheseal shape for postulating on the factors affecting entheseal morphology (see extensive literature in Foster et al., 2012). Due to substantial subjectivity in visually assessing the stage of entheseal change, the accuracy and precision of these traditional methods is reported to below (Davis et al., 2013; Wilczak et al., 2017). Developing an original coordinate-based geometric morphometric approach to the analysis of hand entheses would offer a new and semi-automated 3D technique of quantification for entheseal shapes, whose resulting shape variables could be statistically tested for intra-observer and inter-observer repeatability (Mitteroecker & Gunz, 2009).

Subsequently, this precise methodology could be used for bridging the existing gap between the quantitative (e.g., Nolder & Edgar, 2013) and qualitative (e.g., Hawkey & Merbs, 1995; Mariotti et al., 2007; Henderson et al., 2017) approaches for assessing entheseal variation. As it is not yet known whether these two aspects of entheseal form (3D size and shape) are correlated, it remains unclear whether there is ground for agreement between the two groups of methodological approaches. Utilizing the methods of geometric morphometrics could allow establishing an association between size and shape, by statistically evaluating the strength of allometry in hand entheseal surfaces. If an allometric relationship was identified, future works could rely on both aspects of entheseal form (3D size and shape), on the basis that these two vary together. It should be mentioned that the output of the same analytical process (involving the digitization of landmarks across the surfaces, followed by Procrustes superimposition of the raw landmark coordinates) includes both size and shape variables. These can be used for producing 3D visualizations of morphological variation, allowing for greater interpretability of the resulting patterns (Mitteroecker & Gunz, 2009). In addition,the methods of geometric morphometrics could be used for identifying statistically significant entheseal variation attributable to various factors, such as biological age or bone size (Foster et al., 2012; Henderson et al., 2017).

Utilizing histological evidence of biomechanical stress on entheses

Until now, anthropological studies have not utilized the scientific methods of histology for addressing the effect of biomechanical stress on the interindividual variation of entheses. Therefore, past anthropological research on entheses has not included the analysis of their distinct microscopic zones of calcified and uncalcified gradients. This is in spite of the rich histological literature on the microscopic structure of entheses and its reaction to biomechanical loading (e.g., Benjamin et al., 1986; Evans et al., 1991; Benjamin & Ralphs, 1998; Benjamin et al., 2006; Beaulieu et al., 2015). Several of these studies have established a direct association between the level of cumulative biomechanical stress subjected on fibrocartilaginous entheses and their concentration of calcified fibrocartilage (e.g., Benjamin & Ralphs, 1998; Beaulieu et al., 2015). However, no previous anthropological or histological work has investigated whether interindividual variation in entheseal bone morphology is linked to respective differences in the concentration of calcified fibrocartilage.

One of the main characteristics of entheseal morphological variation involves the proportional extent of bone elevation along the bone surface (Mariotti et al., 2007; Foster et al., 2012; Villotte et al., 2016; Henderson et al., 2017). In human evolution, increased bone extension in some hand entheses has been considered as an indicator of greater biomechanical efficiency (Maki & Trinkaus, 2011; Richmond et al., 2016), while entheseal size differences among various animal species is reported to reflect different levels of biomechanical stress (Deymier-Black et al., 2015; Rossetti et al., 2017). Interestingly, the proportion of bone projection in hand entheseal surfaces also appears to differ greatly among human individuals (**Figure 1**) (Cashmore & Zakrzewski, 2013; Karakostis & Lorenzo, 2016). On this basis, provided that calcified fibrocartilage reflects the level of accumulated biomechanical strain (e.g., Beaulieu et al., 2015), identifying a higher concentration in entheses with greater bone projection would suggest an association between entheseal form and the degree of accumulated biomechanical strain. Such a connection would provide a reasonable argument for future comparative studies on entheses (in the fields of bioarchaeology and paleoanthropology) to utilize the extent of relative elevation along the bone as an indicator of accumulated biomechanical forces.

This PhD project aims at exploring the effect of physical activity on hand entheses by addressing all the above-mentioned research gaps and questions. This will be attempted through the implementation of three distinct methodological approaches, involving morphometric, geometric morphometric, and histological analyses. The specimens used present a rarely extensive documentation with regard to their biological, medical, and occupational characteristics.

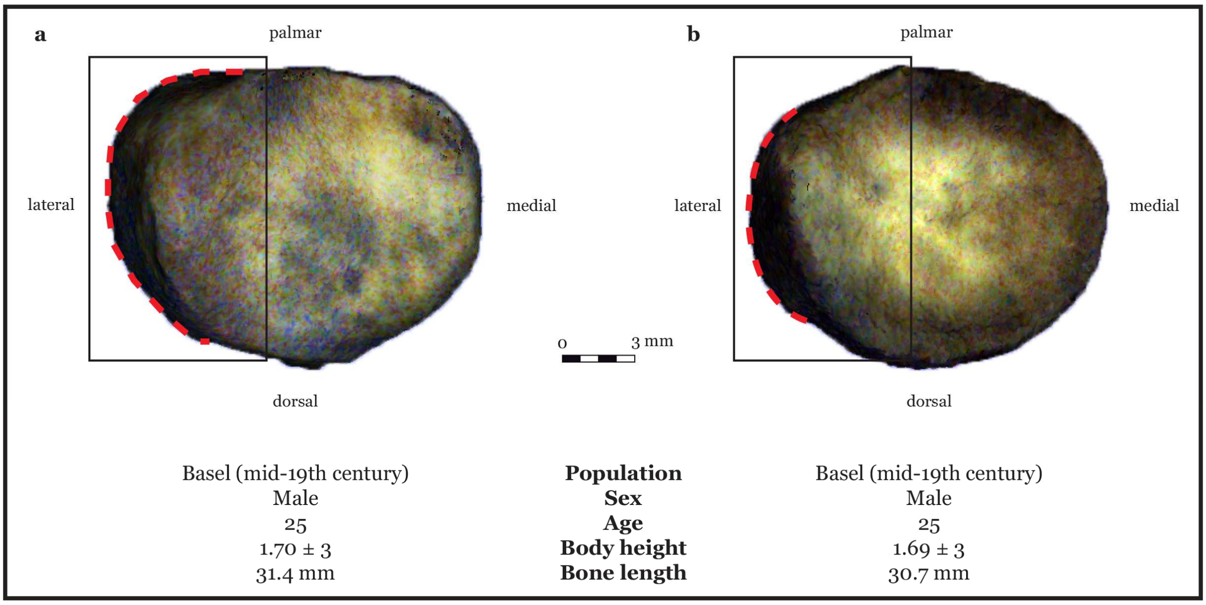

Figure 1. Proximal aspect of the phalangeal enthesis of the left *abductor pollicis brevis / flexor pollicis brevis*, in two individuals with very similar biological profiles (figure from Paper IV). The black rectangles indicate the area of attachment on the bones, while the lateral tubercles of the entheses are indicated with red dashed lines. The left specimen (**a**) presents elevation on its lateropalmar aspect, whereas the tubercle of the right specimen (**b**) is restricted to the lateral surface of the bone.

Objectives and expected output of the thesis / doctoral research

The primary objective of this PhD was to apply my previously developed, repeatable, 3D methodology (Karakostis & Lorenzo, 2016) on an anthropological sample documented in detail for the individuals' lifelong occupational activities, biological profile (population, sex, and age), medical record, as well as socioeconomic background. Its purpose is to identify whether individuals with the same or similar lifelong occupational activities demonstrate similar multivariate patterns of hand entheses. Additionally, given its promising results, this methodology was further tested on a well-known case-study from the same historical context, in the framework of an ongoing and multidisciplinary identification project (Hotz et al., 2017a; 2017b). The exact steps of the applied method are described in detail elsewhere (Karakostis & Lorenzo, 2016). Briefly, the borders of the entheseal areas on the bone 3D surfaces were delineated according to elevation, coloration, and surface complexity. Then, the delimited entheseal surfaces were measured in square millimeters, to be analyzed using multivariate statistical analyses.

Secondly, provided that the 3D surfaces of entheses can be now precisely delineated and digitally extracted from the surrounding bone surface (Karakostis & Lorenzo, 2016), this PhD research intended to design and evaluate an original landmark-based and semi-automated analysis of hand entheseal shape. This precise methodology was used for calculating the degree of allometry in the expression of entheses, in order to establish a link between the qualitative (shape) and the quantitative (size) methods of assessing entheseal variation. Furthermore, this study evaluated the influence of two important factors affecting entheses, including biological age and bone length (Rauch, 2005; Karakostis and Lorenzo, 2016). The latter is mainly regulated by systemic factors (e.g., genes, body size, hormones, and nutrition) during development and it is thus not considerably affected by lifelong bone remodeling (Rauch, 2005).

Finally, this PhD project aimed at putting forward a histological pilot study on the cadaveric thumb entheses of fully-documented body donors. Its purpose was to identify an association between hand entheseal morphology and biomechanical stress, by testing the hypothesis that individual entheses with greater bone elevation along the bone present more calcified fibrocartilage (i.e., an indicator of the accumulated amount of biomechanical stress).

Materials

As the holistic approach of this PhD project is multi-disciplinary, it required different types of samples and/or datasets for each analysis performed. In order for the materials to fulfill the research purposes, they were compiled based on certain sampling strategies, determined *a priori*. These strategies –and corresponding samples– are outlined below. More detailed information is provided in the respective manuscripts.

The sample used in the morphometric and geometric morphometric analyses

Human variation in entheseal form is considered to be affected by multiple biological and secular factors, including population, sex, age-at-death, body size, genetic relatedness, interindividual genetic variability, pathologies, as well as long-term physical activities (Rauch, 2005; Foster et al., 2012; Karakostis et al., 2017). However, the degree of each factor's effect on entheses is not yet understood, and therefore the conclusions of previous research on hand entheses and physical activity remain questionable (Foster et al., 2012; Villotte et al., 2016; Henderson et al., 2017). The main reason for this fundamental research gap is the fact that previously analyzed samples do not present a full documentation for individuals (Alves Cardoso & Henderson, 2012). For this purpose, the fundamental requirement of this research objective was to locate an anthropological collection which met full documentation criteria. The sample used here is part of the "Spitalfriedhof Saint Johann collection, housed at the Natural History Museum of Basel (Hotz & Steinke 2012). This collection is gradually becoming a unique universal reference for the most detailed documentation of the individuals' lifelong physical activities (Hotz & Steinke, 2012; see also Karakostis et al., 2017; Mani-Caplazi et al., 2017). The archived documentation includes the duration of the individuals' activities, their different occupations in life, as well as their exact status at work (e.g., director or employee). Moreover, the origin (population), medical records, cause of death (including the relevant circumstances), genealogy, and socioeconomic characteristics of specimens are included in their accessible documentation (Hotz & Steinke, 2012).

Among all individuals of this collection, a total of 45 were used for this research. These specimens were selected on the basis of ten criteria:

1. Excellent state of preservation of the individuals' hand bones;
2. Complete hand bone sets of the right anatomical side, for controlling for the potential effects of bilateral asymmetry;
3. No reported medical pathologies involving or affecting their hands;
4. No direct relatedness across specimens;
5. Same geographical origin and ethnicity;
6. A similar socioeconomic status (middle or low socioeconomic classes);
7. Male sex (for controlling for the effects of sexual dimorphism);
8. Highly detailed occupational documentation;
9. Adult biological age of less than 50 years, given that after the age of 50-60 years extensive degenerative changes affect human entheses (Niinimaki, 2011; Milella et al., 2012;).
10. Excellent preservation of the individuals' femoral head, which was used as proxy for calculation of body mass (Auerbach & Ruff, 2004; Arsuaga et al., 2012).

For each individual, a total of nine entheses of six hand bones were utilized based on the conclusions of previous research on hand entheses (Karakostis & Lorenzo, 2016), which put forth the precise multivariate method and reported the two morphometric patterns of entheses. The selected entheses are indicated in **Figure 2,** with respect to the morphometric pattern in which they belong based on previous research (Karakostis & Lorenzo, 2016). All entheses were 3D scanned using a high-resolution structured-light surface scanner, Breuckmann Smartscan scanner (Breuckmann Inc., Baden, Germany), which presents an accuracy of nine micrometers. The derived 3D models were used for all morphometric and subsequent geometric morphometric analyses.

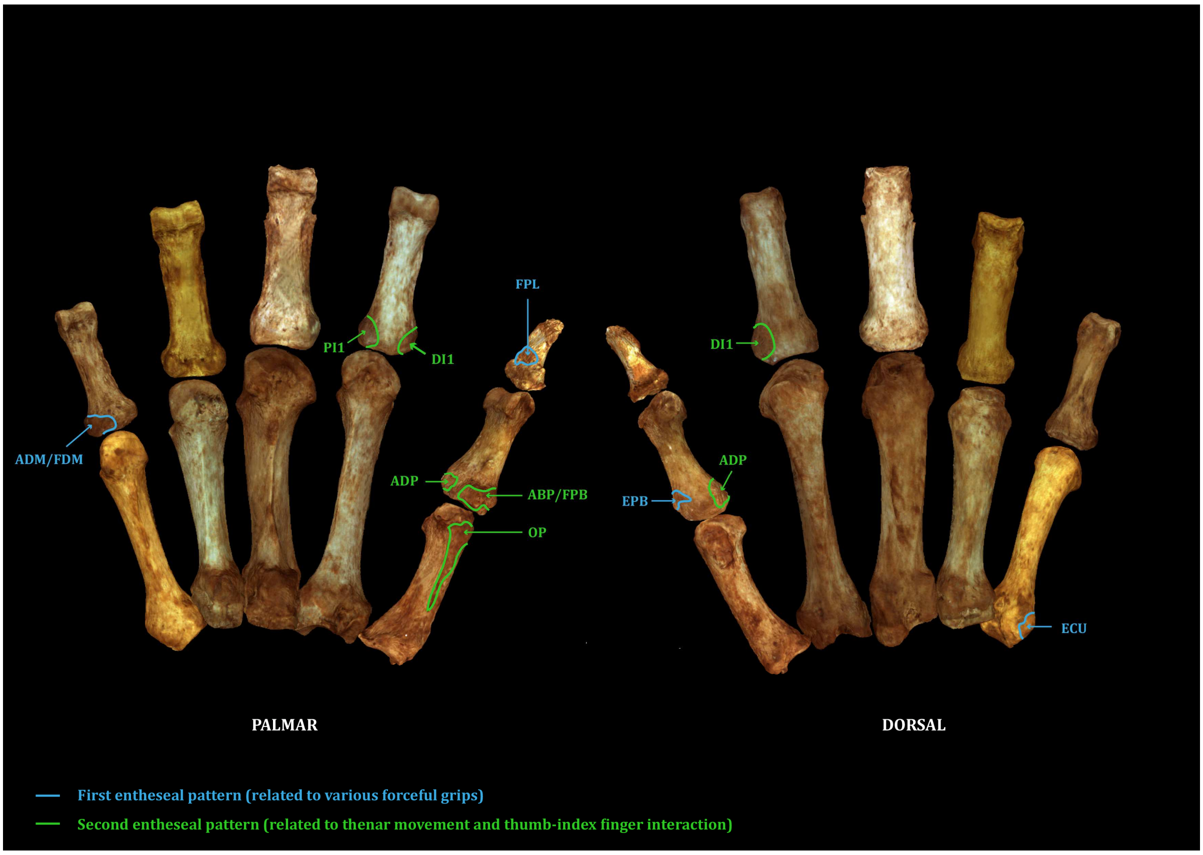

Figure 2. The nine entheseal surfaces of the two synergistic muscle groups described in previous research (Karakostis & Lorenzo, 2016). Each color (green or blue) represents one of the two observed entheseal patterns. For purposes of better demonstration, all five metacarpals and proximal phalanges are included in the figure (figure from Paper I). In two cases, the same enthesis corresponds to two different muscles ("ABP/FPB" and "ADM/FDM").
Abbreviations: ABP: *abductor pollicis*; ADM: *abductor digiti minimi;* ADP: *adductor pollicis*; DI1: first dorsal *interosseus*; ECU: *extensor carpi ulnaris*; EPB: *extensor pollicis brevis*; FDM: *flexor digiti minimi*; FPB: *flexor pollicis brevis*; FPL: *flexor pollicis longus*; OP: *opponens pollicis*; PI1: first palmar *interosseus* (Karakostis et al., 2017).

Histological analysis of hand entheses

Given that this research objective required the combined presence of bone and soft tissue at the tendon-bone junctions, the specimens used derived from the cadaveric hands of human body donors who provided written consent for the use of their bodies for purposes of basic research. These specimens, curated at the Medical School of Athens, consist of three males and one female of Greek origin, who lived in the city of Athens mainly in the second half of the 20th century. These individuals were selected based on four criteria:

1. Complete and accessible documentation of their biological (population origin, sex, age, body height, body weight, hand length, and complete medical record) and occupational profile;
2. No pathologies related to the hands or their ability for manual movement;

3. For the males a biological age of less than 60 years of age (ranging between 45 and 55), given that intense degenerative changes affect the entire human musculotendinous system after around the age of 60 (Deschenes, 2004; Maimoun& Sultan, 2011; Milella et al., 2012);
4. A relatively older (73 years old) and smaller female individual was included in the sample, in order to observe whether the resulting patterns would differ for an individual of distinct characteristics.

The three entheses analyzed were corresponded to thenar muscles of the thumb: *oponenns pollicis, abductor pollicis / flexor pollicis brevis,* and *adductor pollicis* (Figure 2). These particular entheses were selected because they play a fundamental role for human grasping performance (Napier, 1957; Clarkson, 2000). They also greatly contributed to the two morphometric patterns of entheses observed in the multivariate analysis of this PhD project (Karakostis et al., 2017). Following standard anatomical practice (Standring, 2008), the left hand bones were utilized for this research.

Methods

Given the multi-disciplinary approach of this PhD thesis, three different methodological approaches were utilized. These involved the multivariate analysis of hand entheseal surface areas, the geometric morphometric analysis of their form, and their histological analysis. These methods are described in detail in the three respective manuscripts, while a brief summary is presented below.

Multivariate analyses of entheseal surface areas

The primary research project aimed to identify multivariate patterns of hand entheses in extensively documented individuals (Karakostis et al., 2017). All statistical analyses were performed using the SPSS software package (IBM Inc., Armonk, NY; version 24 for Windows). Firstly, the entheseal areas were delineated on the 3D surface models and measured in square millimeters, following the repeatable methodology of Karakostis & Lorenzo (2016). All 3D measurements of the nine entheseal surface areas were utilized as independent variables in a principal component analysis using a correlation matrix (Field, 2013). This analysis allowed us to observe the underlying morphometric patterns among entheses which explain the maximum amount of sample variation, without assuming any *a priori* group categorization for the specimens (Jackson, 1991; Field, 2013). Subsequently, the variables were size-adjusted using the geometric mean and a second principal component analysis was performed (Almecija et al., 2010), for assessing the morphometric patterns among entheses after controlling for the effects of overall size. Finally, the strength of the relationship between the observed patterns and various factors of variation (biological age, body mass, and bone length) was estimated using Pearson's correlation coefficient (r) (Campbell, 2006). All statistical assumptions required for performing the above analyses (Field, 2013) were thoroughly tested before the analyses (Karakostis et al., 2017).

Subsequently, the developed method was also applied on the unidentified specimen "Theo", in the framework of an ongoing multidisciplinary research project, whose objective is to reconstruct this individual's identity (Hotz et al., 2017b). In that case, given that some hand bones of this individual were not preserved, the size-adjusted principal component analysis was based on fewer variables (i.e., entheses).

Geometric morphometric analysis

For the shape analysis of entheseal form, three entheses of the right thumb were used, which correspond to its four thenar muscles (**Figure 3**). Each of them was digitally delineated on the bone and extracted as a separate 3D model, following the precise method introduced in Karakostis & Lorenzo (2016). All further analyses were performed in the Geomorph package (version 3.0) of the R statistical software (Adams & Otarola-Castillo, 2013). For each of the three entheses, a set of fixed landmark points were digitized along the entheseal outline, which were geometrically corresponding across individuals. The precision of landmark placement was thoroughly tested for intra-observer and inter-observer error using the method introduced by Singleton (2002), while an original approach of error simulations was implemented for testing the applicability of this particular method on the sample analyzed.

The outline fixed points were used as a basis for digitizing a set of surface sliding semilandmarks across the entire entheseal surface, following the algorithm of Gunz et al. (2005). The coordinates of these landmarks were subjected to generalized Procrustes superimposition, which removes the effects of size. The resulting coordinates (shape variables) and centroid size (size measure) were used in allometric regression, which was in turn used to assess the correlation between size and shape in the sample (von Cramon-Taubadel et al., 2007; Drake & Klingenberg, 2008). Additionally, shape regression analyses were used for calculating the effect of biological age and bone length on entheseal form (Mitteroecker & Gunz, 2009).

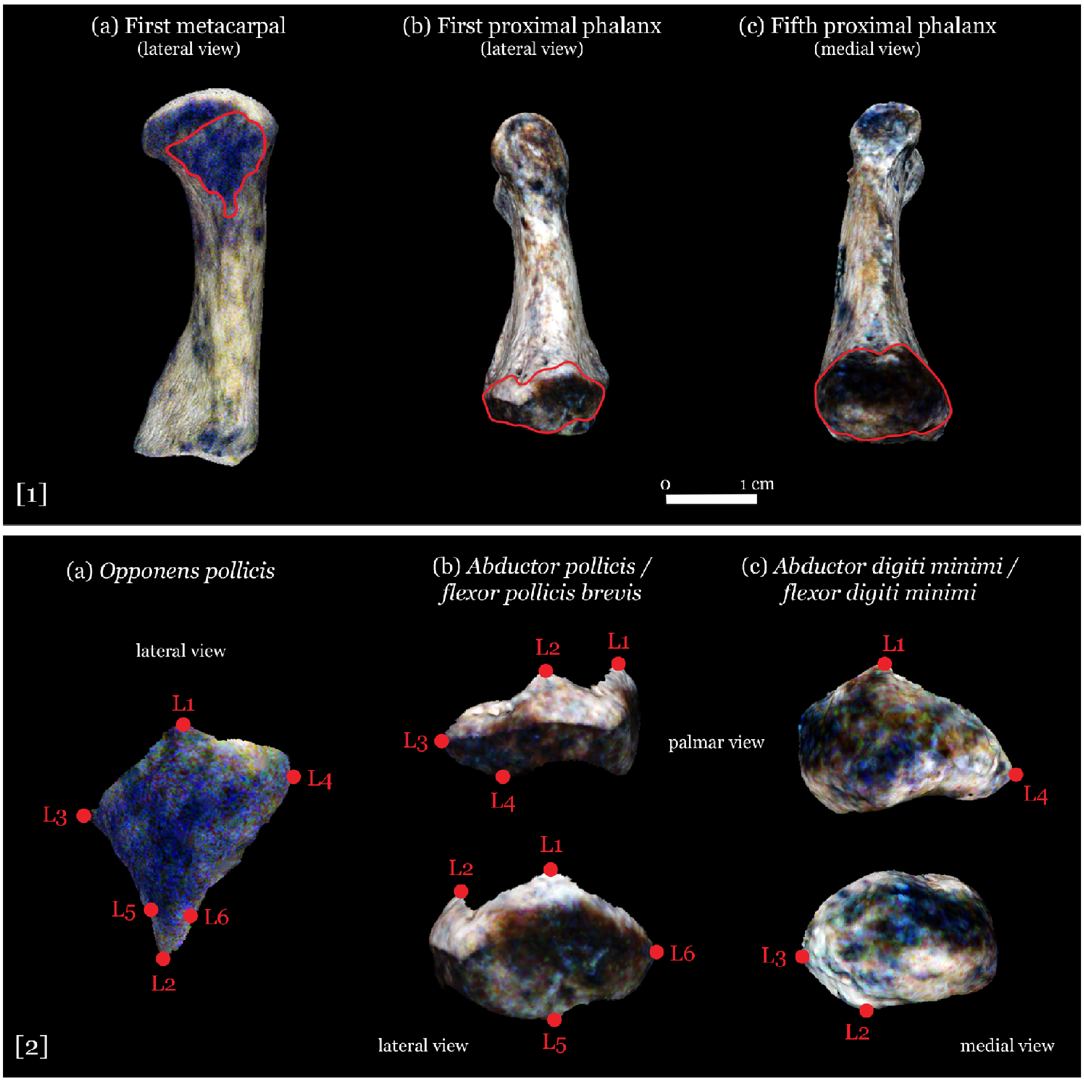

Figure 3. The location of the entheseal surfaces on the bone 3D models (1) and the position of the fixed pseudo-landmarks (L) on their outline (2) (figure from Paper III). These were used for the calculation of surface semilandmarks. In the depicted bone models, the color histogram is equalized and expanded to fit all possible colors, as described in Karakostis & Lorenzo (2016).

Histological analysis

For the final research project, the maximum width (millimeters) and surface area (square millimeters) of the calcified fibrocartilage zone in entheses were quantified using high-resolution photographs (at 20 and 40 magnifications), obtained using an optical microscope and the camera Leica DFC500 (Leica Microsystems, Wetzlar, Germany). These photographs were imported into the software package IC-Measure (The Imaging Source, Bremen, Germany), where all measurements were performed (**Figure 4**).

These measurements were represented directly in graphs and tables, for comparing the quantity of calcified fibrocartilage across the four individuals, the different entheses, and the nine entheseal regions. It should be mentioned that the enthesis related to *adductor pollicis* presented scarce evidence of fibrocartilage (i.e., suggesting a likely more "direct" nature of attachment) and could thus not be included in the analysis. Therefore the analysis was focused on two entheses, corresponding to the remaining three thenar muscles of the thumb (*opponens pollicis*, *abductor pollicis brevis*, and *flexor pollicis brevis*).

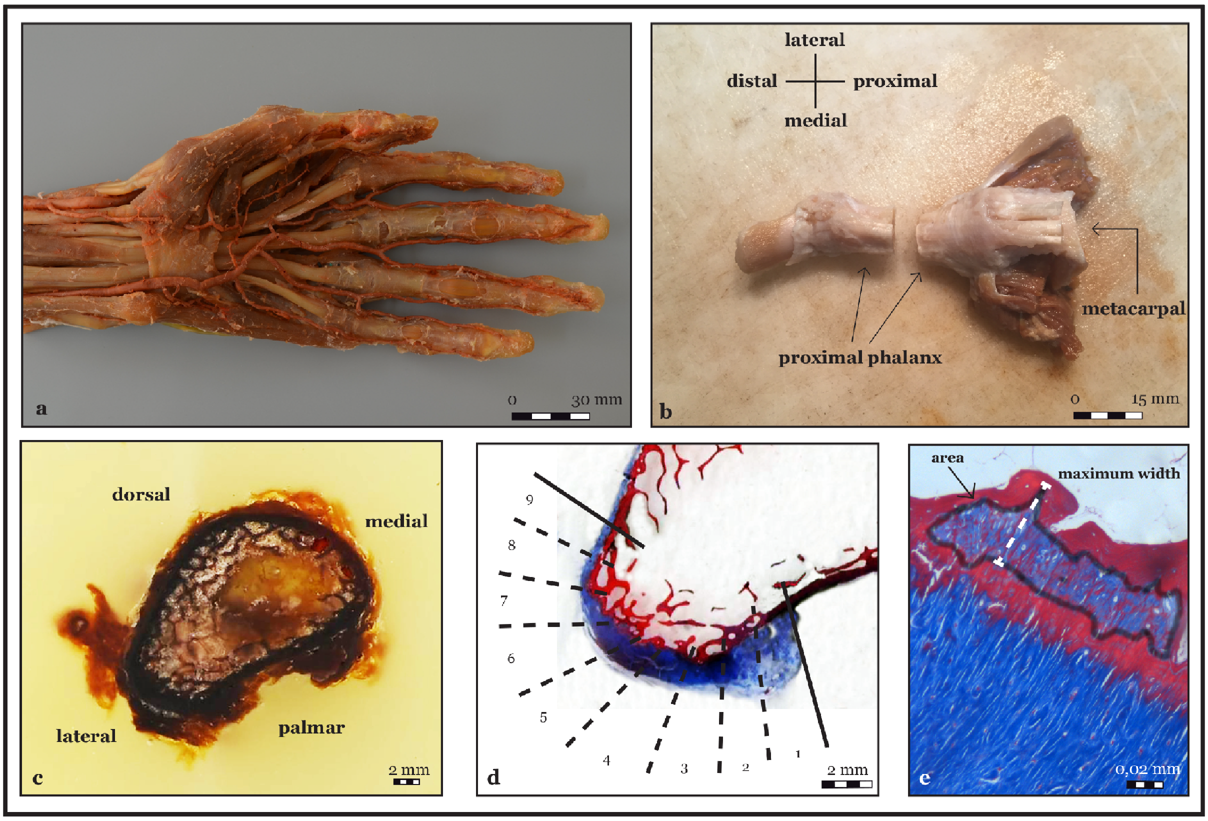

Figure 4. The steps of the analytical process: **a**. hand dissection; **b.** block cutting; **c.** obtaining of the sections; **d.** staining of the five sampled sections and digital separation of its bone area into nine equally-spaced regions; **e.** digital quantification of the calcified fibrocartilage's maximum width and area (figure from Paper IV).

Results

In this PhD thesis, each of the objectives was addressed in a separate research project, whose results were published in an independent article. Below, the results are summarized for each research project.

Morphometric analysis of a documented sample

The multivariate analysis of hand entheses from the extensively documented sample demonstrated that the two previously reported entheseal patterns (Karakostis & Lorenzo, 2016) can occur in distinct population groups. More importantly, variation related to these multivariate patterns provides a clear morphological discrimination between individuals involved in strenuous activities requiring sustained high grip force (mainly construction workers) and individuals with long-term occupations of lower intensity and/or highly mechanized nature (**Figures 5** and **6**). The latter individuals were associated with an entheseal pattern related to precision grasping through thumb-index finger interactions, while the former individuals were exhibited an entheseal pattern reflecting power grips using the thumb and the little finger (Marzke et al., 1998; Clarkson, 2000; Karakostis & Lorenzo, 2016). It should be highlighted that, based on the sampling strategy and the correlation analyses of this study, these two entheseal patterns were not correlated with any other factor associated with bone remodeling and entheses (Rauch, 2005; Foster et al., 2012; Henderson et al., 2017), including population, sex, age (below 50 years), body size, bone length, direct relatedness, or socioeconomic status.

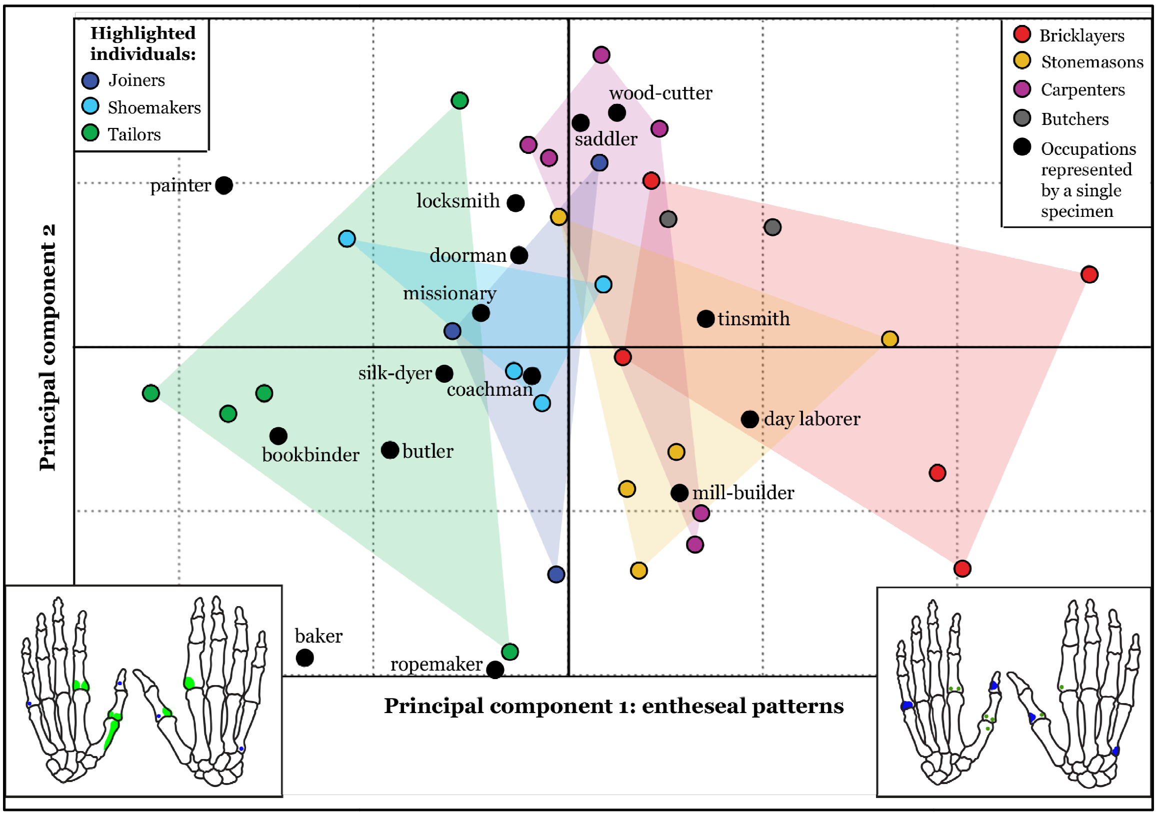

Figure 5. Scatter plot of the principal component analysis on size-adjusted 3D entheseal measurements without *a priori* group categorization (figure from Paper I). Individuals with the same occupation were highlighted (Karakostis et al., 2017).

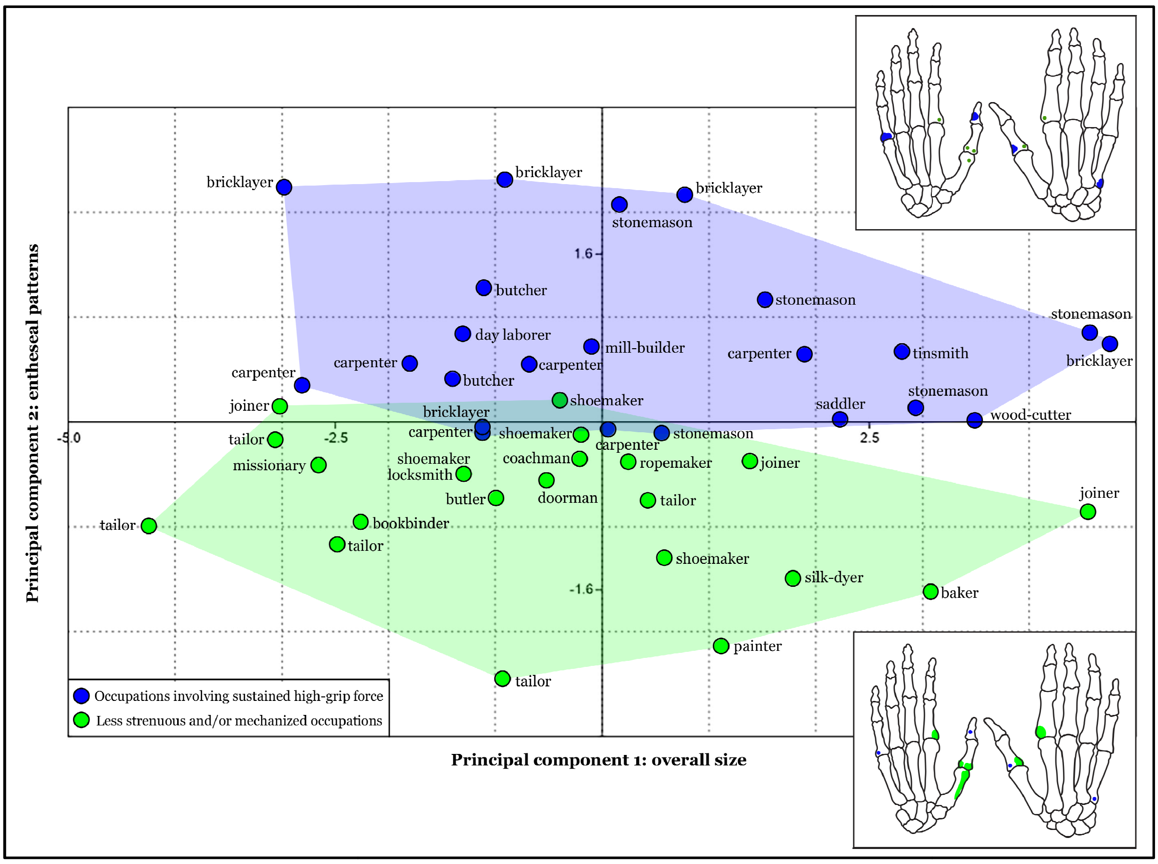

Figure 6. Scatter plot of the principal component analysis on raw entheseal 3D measurements without *a priori* group categorization (figure from Paper I). Occupations were classified (colored) based on the intensity of their lifelong manual activities (Karakostis et al., 2017).

Application of the method on a case-study

The application of the developed methodology on the unidentified specimen “Theo” provided a distinctive component score for this individual (**Figure 7**). This was indicative of a morphometric pattern which involves strong correlations among the thumb and index finger entheses, reflecting the systematic performance of intense precision grasping (Hotz et al., 2017a). This in spite of the fact that fewer entheses could be used in this case-study (due to relatively poor preservation of this individual’s hand bone set), which reduced the overall level of discrimination between the two occupational groups. The component score of “Theo” overlapped only with three tailors and a painter included in our comparative, documented, sample (**Figure 7**). On this basis, the results for “Theo” are consistent with long-term manual activities of rather low intensity, which involve manual activities relying on the thumb and the index finger (Hotz et al., 2017a). This prediction regarding the occupational behavior of “Theo” is in agreement with the conclusions of previous, as well as ongoing, research on the identification of this individual and his lifelong occupational activities (Hotz et al., 2017a; 2017b).

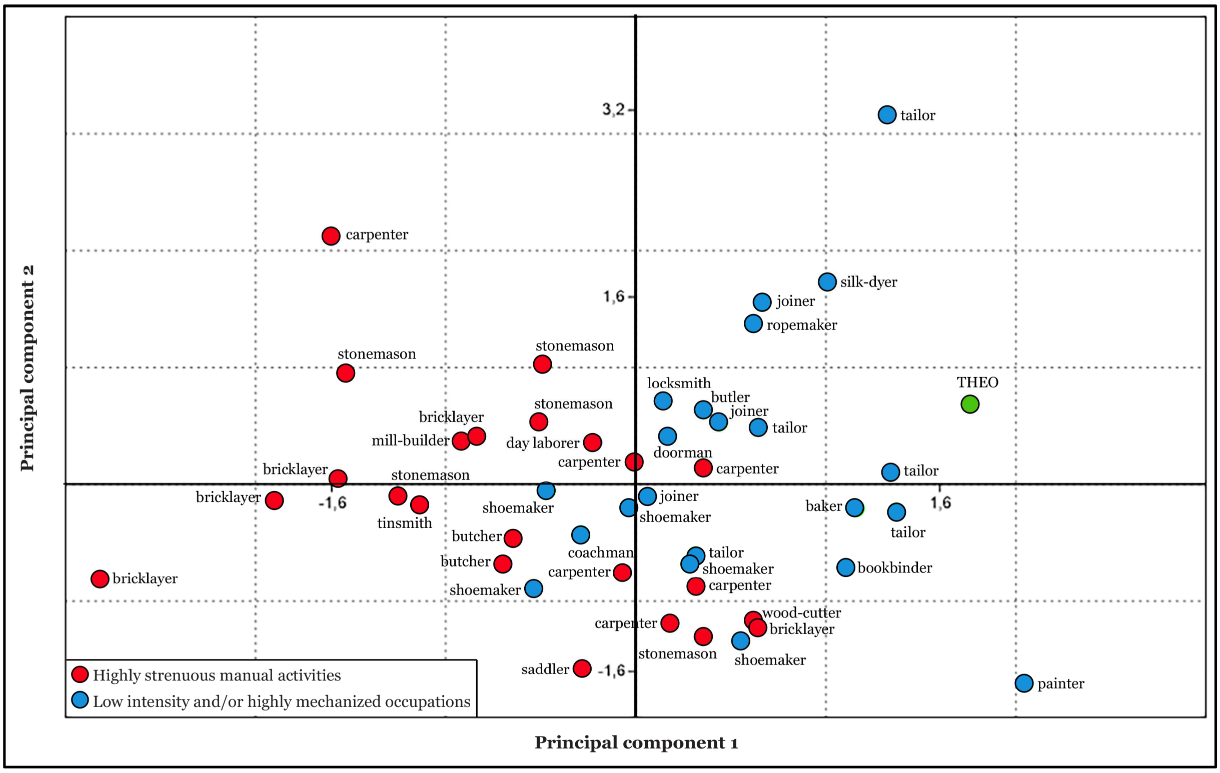

Figure 7. Scatter plot of the principal component analysis on raw entheseal 3D measurements without *a priori* group categorization. Only the hand entheses preserved in the specimen “Theo” were utilized (figure from Paper II). Occupations were classified (colored) based on the intensity of their lifelong manual activities, equivalent to the information presented in **Figure 6**. The score of the specimen “Theo” is indicated with a green dot (Hotz et al., 2017a).

Geometric morphometric analysis

The results of this analysis demonstrated that landmark placement was repeatable both within and between observers, indicating that the developed methodology for reconstructing entheseal form (size and shape) is repeatable. Furthermore, entheseal size and shape were significantly correlated in all three entheses. Particularly, larger phalangeal entheses were also much steeper in shape, while the greater allometric relationship was presented by the muscle attachment site of the *opponens pollicis.* For this enthesis, almost 27% of total shape variation was related to size differences across specimens. Based on subsequent analyses of different regions within this entheseal area, allometric variation occurs because larger areas present a narrow and elongated ridge which extends towards or below the bone midshaft (**Figure 8**). By contrast, entheseal shape variation did not significantly coincide with the values of age-at-death (below 50 years) or bone length.

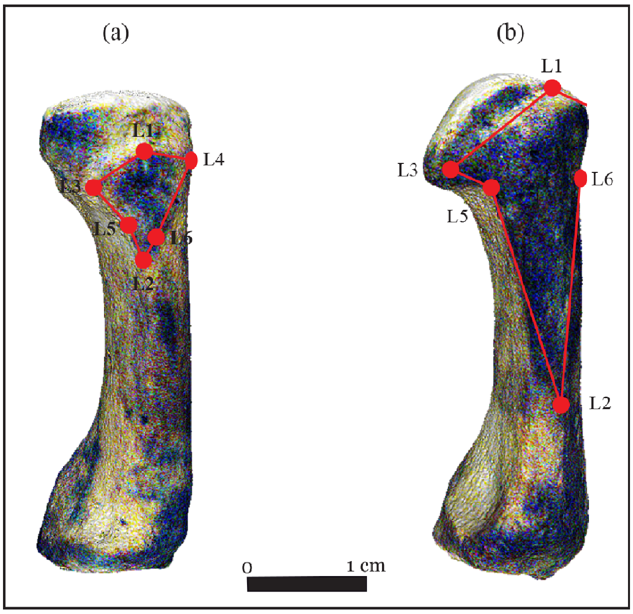

Figure 8. An example of a relatively small and a large entheseal surface of *opponens pollicis* (lateral view of the bone). In the depicted 3D models, the color histogram is equalized and expanded to fit all possible colors, as described in Karakostis & Lorenzo (2016) (figure from Paper III). The position of the fixed landmarks (L) is depicted in **Figure 3**.

Histological analysis

The most important observations of this study suggested that entheseal regions with elevated bone surface (either in the center or periphery of entheses) comprise greater levels of total calcified fibrocartilage. As a consequence, the individual entheses presenting non-pathological bone elevation in their marginal areas showed greater total concentrations of calcified fibrocartilage. In contrast, the individual entheses without marginal bone elevation showed substantially lower total values of calcified fibrocartilage. Finally, it should be mentioned that the enthesis of the *opponens pollicis* presented generally lower levels of total calcified fibrocartilage concentration than the phalangeal enthesis (**Figure 9**).

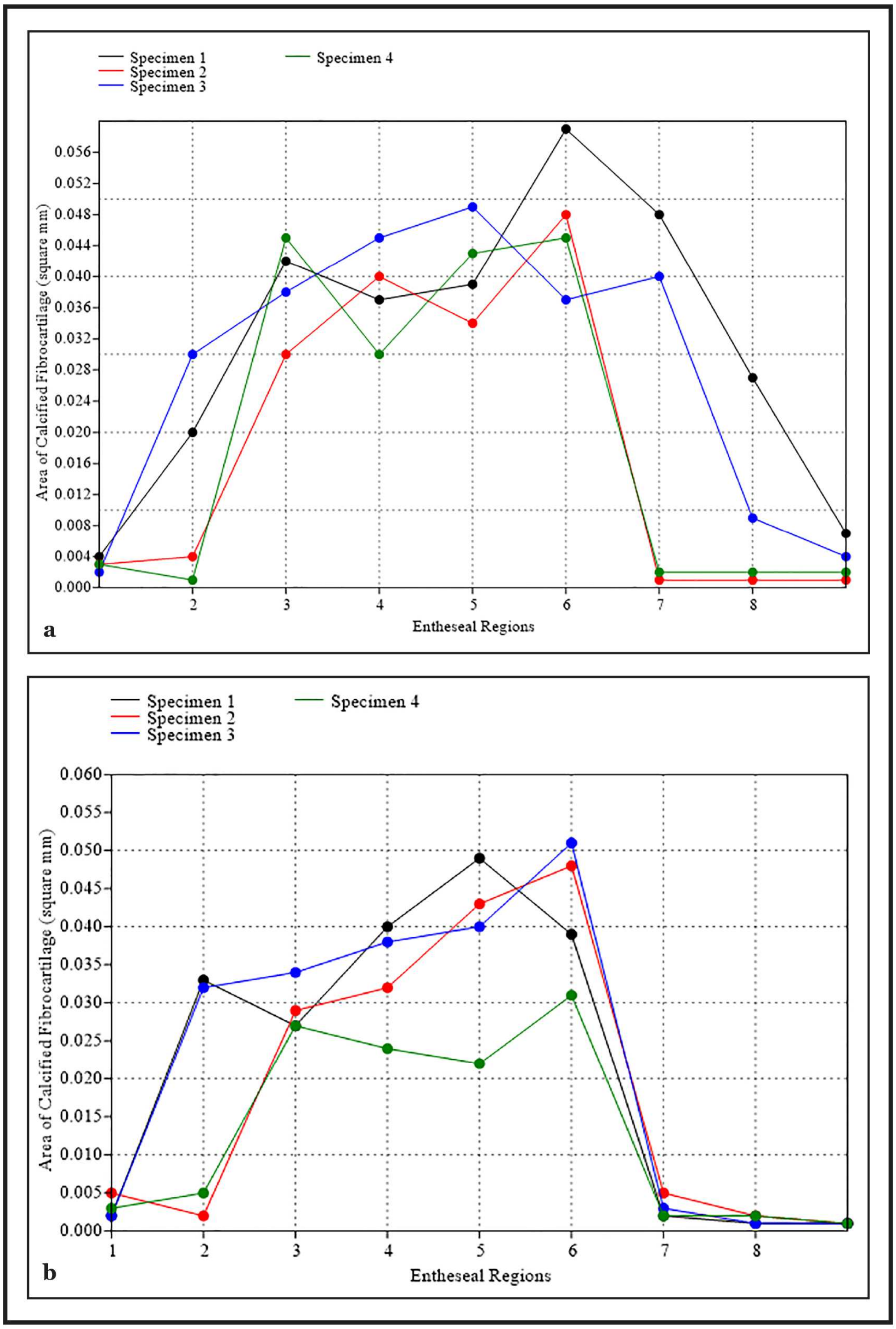

Figure 9. Plot demonstrating the mean area of calcified fibrocartilage across the nine regions of each individual enthesis, for the common insertion point of *abductor pollicis / flexor pollicis brevis* (a) and the insertion area of *opponens pollicis* (b). The values of all regions with flatter bone outline were below 0.01 square millimeters (figure from Paper IV).

Discussion and Conclusions

Agreement among the three methodological approaches

The three methodological approaches applied in this cumulative PhD thesis address different aspects of the same research question. Their results are highly complementary, forming together an originally complete picture of the relationship between biomechanical stress and the form of manual muscle attachments.

Particularly, the primary study identified a consistent relationship between the nature of occupational activity and the morphometric relationship among specific entheses. After controlling for the effects of overall size, this relationship explained almost one third of total variation (30.6%) in the sample. The patterns presenting a functional signal were not correlated with any other factor assumed to influence bone remodeling and entheses (Rauch, 2005; Foster et al., 2012; Henderson et al., 2017). The success of this study indicates that future works on occupational stress markers should focus on compiling samples which are documented in detail for the individuals' lifelong activities (Alves Cardoso & Henderson, 2012). Furthermore, future studies should apply multivariate approaches of entheseal analysis, which focus on the individuals' pattern among different entheses rather than each entheseal form separately (Milella et al., 2015; Karakostis et al., 2017). This is likely because the size and shape of each enthesis separately may be affected by numerous and immeasurable factors of variability among humans, while physical activity mainly regulates the distribution of bone mineral across the skeleton and not its raw quantity per individual (Bennell et al., 1997; Palombaro, 2005). Finally, the results of the precise methodology utilized in this study (Karakostis & Lorenzo, 2016) indicates that future work can substantially improve the precision of their analyses by utilizing high-resolution 3D surface scans and the digital process of entheseal delineation recommended by Karakostis & Lorenzo (2016).

Understanding whether size and shape differences coincide in entheses is essential, as metric variation among animal species is strongly correlated with different levels of muscle forces (Deymier-Black et al., 2015), while the proportion of entheseal bone extension in human evolution is directly associated to greater muscle moment arm and biomechanical efficiency (Maki & Trinkaus, 2011; Richmond et al., 2016). For this purpose, the geometric morphometric analysis of this PhD research established a consistent association between hand entheseal size and shape, by demonstrating that larger entheseal areas present greater proportional surface extension. This was achieved based on the development of the first semi-automated methodology of entheseal shape analysis with significant intra-observer and inter-observer precision. The efficiency of this method sets the basis for future analyses which can rely on both aspects of 3D form (size and shape) for approaching the factors regulating entheseal variation. Future work can also address the correlation between the 3D forms of different entheses and occupational activity. However, as this would require the use of multiple planes of reference (i.e., the use of landmark coordinates from multiple 3D models in the same analysis), such studies should first focus on refining the current geometric morphometric methods for shape analysis.

The analysis of the microstructure of hand entheses found histological support linking entheseal form to biomechanical stress. By observing that entheses with greater proportional extension presented greater concentrations of a direct indicator of biomechanical stress (calcified fibrocartilage) (e.g., Evans et al., 1991; Beaulieu et al., 2015), this analysis demonstrated that greater entheseal size and proportional extension are associated with higher biomechanical strain. The original observations of this small-scale pilot study can comprise a reasonable argument for future anthropological studies to use proportional bone elevation as a comparative measure of cumulative biomechanical stress. Additionally, future histological research should focus on further investigating the factors of entheseal variation based on larger sample sizes, provided that all cadaveric specimens would meet the essential criteria outlined in this PhD thesis.

Possibilities and limitations in using hand entheses to infer activity in the past

The agreement found among quantitative, qualitative, and histological analyses of entheseal form indicates that biomechanical stress is an observable factor of variation in human entheses. However, it should be highlighted that this conclusion does not signify that skeletal entheses can be used for predicting the exact lifelong occupational activities of unidentified individuals. Nevertheless, as demonstrated also in the comparative case study of the unidentified specimen “Theo”, the multivariate analysis of hand entheses can be used to predict essential background information regarding the general nature and intensity of an individual’s habitual manual activities, always with reference to other specimens from the same context or population. Moreover, entheseal analyses can only provide reliable proof of an individual's habitual behavior if their predictions come in full agreement with the associated historical and/or archaeological evidence.

For example, assuming that an archaeological context under study involves several individuals of distinct socioeconomic statuses (e.g., based on reliable burial evidence) and this community is safely associated with specific activity patterns (e.g., mining or agriculture), the comparative multivariate methodology developed here can be used for detecting highly consistent correlations between certain entheseal patterns (reflecting sustained power or precision grips) and specific socioeconomic statuses. In that way, it can contribute to reliable assessments regarding social distribution of labor, always on the basis of the respective historical and/or archaeological evidence. Overall, the analysis of hand entheses can contribute to supporting reasonable links between the cultural, behavioral, and biological aspects of unidentified individuals with respect to their archaeological contexts (Jurmain et al., 2012). It should be mentioned that the same concept could be applied in the field of forensic anthropology, for the purposes of human identification (Galtes et al., 2007).

Previous research on entheses as occupational stress markers

Previous biomechanical research has proposed that the form of entheses is influenced by the outcome of two opposing forces caused by body weight and muscle contraction (Oxnard, 1991; Oxnard, 2004). Given that the hand is the least-bodyweight-bearing anatomical element of humans (Toezeren, 2000), one could assume that the effects of muscle forces are greater for the hand entheses compared to other parts of the human skeleton. If that concept is valid, future work reconstructing muscle behavior from skeletal remains would benefit from further focusing on the muscle markings of the hand and their association with physical activity.

Several previous studies have questioned the effect of physical activity on entheses (Zumwalt, 2006; Djukic et al., 2015; Rabey et al., 2015; Williams-Hatala et al., 2016; Wallace et al., 2017). The results of these previous methods, however, are likely biased by their methodological choices. Particularly, previous experimental analyses (e.g., Zumwalt, 2006; Rabey et al., 2015; Wallace et al., 2017) used small samples of non-primate species, in spite of the fact that the mechanisms of bone remodeling can differ substantially among species (Bagi et al., 2005). Moreover, the exact threshold of biomechanical stress required for inflicting measurable bone formation is not yet known for each species (Zumwalt, 2006). Furthermore, human bone formation (due to lifelong bone remodeling) requires an average of four to eight months (Ganda, 2013), while previous experimental research based on biochemical markers found no increase in athletes over one year of systematic physical exercise (e.g., Bennell et al., 1997). In spite of this essential fact, no previous anthropological studies presented documented information on the long-term physical activities of the samples analyzed (Alves-Cardoso & Henderson, 2012). More importantly, all the above-mentioned studies were entirely based on bivariate analyses of correlation focusing on the form of each enthesis separately, without investigating the multivariate relationship among different entheses, which has been shown to present an observable functional signal (Milella et al., 2015; Karakostis et al., 2017).

Special reference should be made to a previous study on two hand entheses (*opponens pollicis* and *opponens digiti minimi*), which reported that entheseal size is not correlated with specific dimensions of the corresponding muscle (Williams-Hatala et al., 2016). The sample of this study utilized old specimens (77.9 ± 12 years), despite the widely accepted observation that both the entheses and their respective musculotendinous systems undergo substantial degenerative changes after the age of 50 to 60 years (Niinimaki, 2011; Milella et al., 2012). Furthermore, Williams-Hatala et al. (2016) did not take into consideration the possible differences between bone and soft tissue in their reaction to biomechanical stress. For instance, previous studies have found observable change in muscle dimensions within two months of systematic exercise (Ahtiainen et al., 2003; Ogasawara et al., 2013; Brummit and Cuddeford, 2015), whereas bone formation occurs after an average of four to eight months (Ganda, 2013). In fact, contrary to previous hypotheses (Wallace et al., 2017), this process could even be slower for entheseal bone surfaces as the structure of fibrocartilaginous attachment areas involves gradients which reduce stress concentration by dissipating tendon forces across the area of attachment (Benjamin et al., 2006). In consideration of the above information, it is more likely that entheseal forms do not reflect the muscle dimensions at the time of death but rather the cumulative effect of lifelong bone remodeling. This interpretation can explain the facts that older individuals present larger entheseal surfaces in all age-groups (Noldner & Edgar, 2013; Karakostis et al., 2017). Furthermore, the fact that long-term physical exercise regulates the distribution of bone mineral across the skeleton can account for the greater development of specific entheses within individuals (Karakostis et al., 2017).

Concluding remarks and future directions

This cumulative PhD thesis investigated the effect of physical activity on entheseal surfaces by performing four separate studies, which relied on three distinct methodological approaches. The first work identified a clear functional signal in hand entheses based on a precise multivariate 3D analysis of extensively documented individuals (Karakostis et al., 2017). Subsequently, in the second analysis, the same methodology and reference sample were used for predicting the occupational characteristics of an unidentified individual (Hotz et al., 2017a; 2017b). The third project put forth a precise method for assessing entheseal 3D shape. This demonstrated that larger entheses comprise greater proportional extension, which is indicative of greater biomechanical efficiency for the corresponding muscle (Maki & Trinkaus, 2011; Richmond et al., 2016). The final study performed a microscopic histological analysis of entheseal microstructure, which verified that entheses with greater proportional elevation show greater evidence of biomechanical stress. Overall, the conclusions of these studies combined present original evidence that the form of hand entheses is affected by habitual muscle forces and the lifelong process of bone remodeling. This is in agreement with the conclusions of previous experimental research which demonstrated that physical activity regulates the pattern of the bone mineral's distribution across different skeletal areas (Lohman et al., 1995; Heinonen et al., 1996; Bennell et al., 1997; Palombaro, 2005).

Based on these results, future research can utilize hand entheses in comparative analyses across individuals of the same historical context for inferring the nature of their manual physical activities, provided that all occupational predictions rely on reliable historical and/or archaeological information. Furthermore, the combined use of this project's multivariate methodology and unique reference sample can provide the long-awaited soil for accurately reconstructing habitual manipulative behaviors in the human fossil record. This would allow anthropologists to address some of the fundamental research questions regarding human evolution (c.f., in the Introduction).

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PAPERS

Paper I:

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Occupational manual activity is reflected on the patterns among hand entheses

Fotios Alexandros Karakostis[1], Gerhard Hotz[2], Heike Scherf[1], Joachim Wahl[1,3] & Katerina Harvati[1,4]

[1] Paleoanthropology, Senckenberg Centre for Human Evolution and Paleoenvironment, University of Tübingen, 72070 Tübingen, Germany.

[2] Natural History Museum of Basel, 4051 Basel, Switzerland.

[3] Osteology, State Office for Cultural Heritage Management Baden-Württemberg, 78467 Konstanz, Germany.

[4] DFG Centre for Advanced Studies 'Words, Bones, Genes, Tools: Tracking linguistic, cultural and biological trajectories of the human past'.

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Correspondence to:

Fotios Alexandros Karakostis

Eberhard Karls University at Tübingen,

Senckenberg Center for Human Evolution and Paleoecology,

Rumelinstrasse 23, Tübingen 72070, Germany.

Telephone number: +4970712976516

E-mail address: afkarakostis@hotmail.com

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ABSTRACT

Objectives: In anthropological sciences, entheses are widely utilized as occupational stress markers. However, the reaction of entheseal surfaces to mechanical loading is not well understood, while previous studies on entheses relied on the individuals' occupation-at-death. Past research by one of us has identified two patterns among hand entheses, proposing that they reflect two synergistic manual muscle groups. Here, we investigate the association between these patterns and habitual manual activity using an extensively documented skeletal sample and a three-dimensional system of quantification.

Materials and Methods: The hand bones utilized belong to 45 individual skeletons from mid-19th century Basel. These were male adults (18 to 48 years old) who were not directly related, showed no pathological conditions in the hands, and whose occupational activities during their lifetime were clearly documented and could be evaluated according to historical sources. The patterns of entheses were explored using principal component analysis on both raw and size-adjusted variables. The influence of age on the results was assessed through correlation tests.

Results: The analysis showed that the previously proposed patterns of entheses are present in our sample. Individuals with the same or comparable occupations presented similar entheseal patterns. Age and overall entheseal size, which were correlated, did not considerably affect the results.

Discussion: Individuals that were involved in intense manual labor during their lifetime presented a distinctive pattern of hand entheses, consistent with the application of high grip force. By contrast, individuals with less strenuous and/or highly mechanized occupations showed an entheseal pattern related to the thumb intrinsic muscles.

Although habitual physical activity is recognized as an important factor affecting the skeleton (Wolff, 1892; Scherf et al., 2013; Scherf et al., 2015), its influence is still poorly understood. In anthropology, entheses, the areas where ligaments, muscles, or tendons attach to bone, are widely considered as musculoskeletal stress markers when reconstructing the activity profiles of past populations (Foster et al., 2012). As the hand performs a vital role in everyday human activities (Marzke et al., 1998), it has been the subject of intense study when trying to reconstruct the behavior of fossil hominins and past human populations (e.g., Niewoehner, 2001; Almecija et al., 2010). In an osteological context, hand entheses are the sole direct sources of information about hand musculature. Identifying a strong association between habitual manual behavior and hand entheses, therefore, could provide the foundation for reconstructing occupational and habitual activity patterns based on human skeletal remains, thus enabling a greater understanding of the evolution of human manipulative capabilities.

Past work by one of us on entheseal 3D areas (Karakostis and Lorenzo, 2016) found two morphometric patterns among hand entheseal surfaces and suggested that they reflect two synergistic groups of muscles (Fig. 1): one group typically contracts during hand movements associated with sustained high grip force, while the second one cooperates for positioning the thumb relative to the palm and fingers (Table 1) (Maier and Hepp-Reymond, 1995; Marzke et al., 1998; Clarkson, 2000; Goislard de Monsabert et al., 2012; Karakostis and Lorenzo, 2016). However, the exact factors affecting entheseal form are highly controversial. Several recent studies have questioned the relationship between muscle recruitment and entheseal form, concluding that occupational stress may not in fact be reflected on the morphology of entheseal surfaces (Zumwalt, 2006; Djukic et al., 2015; Rabey et al., 2015; Williams-Hatala et al., 2016). By contrast, others focused instead on the statistical relationships among different entheseal patterns, proposing that these patterns (rather than the entheseal forms themselves) could provide important information on physical activity (Milella et al., 2015; Karakostis and Lorenzo, 2016).

[Figure 1 here]

[Table 1 here]

Previous research on the utility of entheses as occupational stress markers relied on the specimens' occupation-at-death (e.g., Alves Cardoso and Henderson, 2010; Alves Cardoso and Henderson, 2013; Lopreno et al., 2013). However, it has been demonstrated that information on specimens' occupation-at-death cannot provide an adequate basis for associating entheseal surfaces with physical activity (Alves Cardoso and Henderson, 2013).

Indeed, assessing the relationship between bone entheses and occupation would require a sample which is documented for the activities of individuals over the last years before death. For this purpose, this study will investigate the factors influencing the morphometric patterns of hand entheses based on a skeletal sample which is extensively documented for the occupational activities of specimens during their active working life (Hotz et al., 2012). The entheseal areas of these individuals will be analyzed using a precise three-dimensional (3D) system of quantification of entheseal surface area from high resolution surface scans (Karakostis and Lorenzo, 2016).

Subsequently, the results will be discussed on the basis of detailed historical sources and studies, which outline the manual activities of the occupations represented in our sample. Based on the previous hypothesis of two synergistic muscle groups (Karakostis and Lorenzo, 2016), we predict a distinctive pattern of hand entheses for individuals who systematically applied sustained high grip force during their lifetime. By contrast, we expect individuals involved in substantially less strenuous manual activities to present a pattern which reflects recruitment of the intrinsic thumb muscles.

MATERIALS AND METHODS

Sampling strategy

Our sample comprises part of the anthropological collection "Spitalfriedhof Saint Johann", housed at the Natural History Museum of Basel (Switzerland). This material was selected because of its unusually extensive documentation, which provides information not only about the sex and exact biological age, but also about the socioeconomic status, medical profile, and detailed occupational activities of each specimen during their active working life in Basel (Hotz et al., 2012). Information on each individual's occupation is available in the town archives of the city of Basel and it derives from multiple legal institutions of the city (i.e., the Police, the Spitalfriedhof St. Johann Hospital, and the town's city hall) (Hotz et al., 2012). Table 2 lists the occupations represented in our sample, accompanied by citations of historical sources describing their daily activity patterns in mid-19th century European cities.

[Table 2 here]

A total of 45 individuals, who lived in Basel between 1804 and 1865, were analyzed. They originated from the wider region around the city and the vast majority worked in Basel only after 1840. Based on their income and social status, they all belonged to the middle or low socioeconomic classes (Lorenceau, 2001; Hotz et al., 2012). The individuals analyzed were selected on the basis of six criteria, including state of preservation, pathology, sex, age, relatedness, and availability of detailed occupational information. All specimens selected preserved their hand bones intact and without pathological or taphonomic alterations (Villotte et al., 2010). Their medical documentation also did not report pathological conditions related to their hands. Sex and old biological age are also considered to be important factors of entheseal variation (Foster et al., 2012): it has been reported that after around the age of 50, entheseal surfaces are subject to extensive age-related changes (Myszka and Piontek, 2013). For this reason, our sample comprised only male adult individuals between 18 and 48 years of age. We further assessed the effect of age on our results statistically (see below). It should be mentioned that, in 19th century Basel, males of the middle or low socioeconomic classes usually started working at the age of 14 (Wadington, 1890). Thus, the duration of the individuals' active working life is directly related to their biological age. Moreover, in order to avoid the potential bias of genetic relatedness among individuals of the sample, we used its extensive documentation to verify that none of the selected specimens belonged to the same immediate family (c.f., Acknowledgements).

The selection of hand entheseal surfaces analyzed was based on the previous results establishing the association between manual muscle synergies and entheseal patterns (Karakostis and Lorenzo, 2016). The nine entheseal surfaces contributing to these two patterns are depicted in Figure 1. They are located on the surfaces of six hand elements, including the three thumb bones (first metacarpal, proximal phalanx, and distal phalanx), the index proximal phalanx, and two bones of the fifth hand ray (fifth metacarpal and fifth proximal phalanx). In two cases, the same entheseal area is associated with two muscles (*abductor pollicis* / *flexor pollicis brevis* and *abductor digiti minimi* / *flexor digiti minimi*). In this study, only right hand bones were used, in order to control for the potential effects of bilateral asymmetry on entheseal patterns. A total of 270 bones were analyzed, comprising 405 entheseal surfaces.

3D scanning and measurement

High resolution 3D models of the bones were developed using a Breuckmann SmartScan structured-light scanner (Breuckmann Inc., Baden, Germany) with 125 FOV, an automatic turntable, as well as the accompanying Optocat software package (Breuckmann Inc.). Measurement accuracy was 9 μm. Full triangulation was selected. For each bone, scans were taken from 20 different angles along an arc of 360 degrees. Subsequently all scans were aligned and merged into one 3D model. This was extracted as a single "ply" file and imported into the software "Meshlab version 1.3.3" (CNR-INC, Rome, Italy), in order to isolate the entheseal surface areas investigated.

The methodology followed for delineating the exact borders of hand entheseal surfaces is described elsewhere (Karakostis and Lorenzo, 2016). In that previous work by one of us (F.A.K.), the method applied presents statistically non-significant intra- and inter-observer error (maximum mean error was 0.60%). Briefly, the borders of entheseal areas were delineated on the surface models according to elevation, coloration, and surface complexity. Subsequently, the delimited entheseal areas were isolated from the rest of the bone surface and measured in square millimeters, using the Meshlab software package's tools. All measurements were collected by the same observer (F.A.K.). Throughout the quantification of entheseal surface areas, F.A.K. had no access to the occupational profile of the individuals selected.

Statistical analyses

All analyses were conducted in the IBM SPSS software package (IBM Inc., Armonk, NY, USA; version 24 for Windows). For identifying morphometric patterns among hand entheseal surfaces, a principal component analysis (PCA) was performed using all 45 individuals and the surface areas of the nine entheses as variables. A correlation matrix was selected because the nine variables had different scales (Field, 2013). The number of principal components (PCs) plotted was determined based on both the scree-plot technique and the Kaiser criterion (Jackson, 1991).

Given that the assignment of different occupations to wider occupational categories is considered to be often influenced by each researcher's perspective (Lopreno et al., 2013), our statistical analyses were not based on any *a priori* categorization of professions (Tabachnick and Fidell, 2001). Nevertheless, for the purpose of highlighting our results, individuals with the same occupation were highlighted in the resulting plots using different colors.

Previous research has reported strong correlation between entheseal 3D size and body size (Nolte and Wilczak, 2013). In order to evaluate whether the overall entheseal size of individuals influenced the observed patterns of entheses, a second PCA was conducted using size-adjusted variables. These variables were calculated by dividing each raw measurement by the geometric mean of all nine entheseal measurements for each individual. Then, the outcome was log-transformed on the basis of natural logarithms (e.g., Almecija et al., 2010).

For verifying that the two identified entheseal patterns are not substantially associated with biological age (within the range of 18 to 48 years), we assessed the relationship between age-at-death and the PCs of both PCAs (the one on raw measurements and the one on size-adjusted variables). These tests were carried out using the Pearson's correlation coefficient (r) with an alpha level of 0.05. Significant r-values between 0.40 and 0.60 demonstrate moderate positive association, whereas r-values over 0.60 indicate strong positive correlation (Campbell, 2006).

As far as statistical assumptions are concerned, the sampling adequacy of the dataset was verified using the Kaiser-Meyer-Olkin test. The outcome was 0.81 (“meritorious”) (Field, 2013). Multivariate normality of the variables was assessed using the Doornik and Hansen test (Doornik and Hansen, 2008). The presence of significant outliers was diagnosed using modified Z-scores, while multivariate outliers were detected using Mahalanobis squared distances (Iglewicz and Hoaglin, 1993; Field, 2013). The linear relationship between variables was assessed using bivariate scatterplots and a Bartlett’s test of sphericity was run to evaluate whether variables are suitable for data reduction (Field, 2013). Based on the results of the statistical procedures mentioned above, all assumptions of PCA and Pearson's correlation test were met (Tabachnick and Fidell, 2001; Field, 2013).

RESULTS

Patterns between hand entheses

A total of two PCs were plotted, which together accounted for the 65.76% of variance in the sample (Table 3). The first principal component (53.02% of the sample’s variance) reflected overall entheseal surface size variation, given that all its factor loadings were positive. The loadings were also comparable among variables, suggesting that all nine entheseal surfaces had a similar contribution to metric variation across individuals. The second principal component (12.74% of the sample's variance) represented variation in the proportions among entheseal measurements. Positive values on this component were related to individuals with proportionally larger entheseal surfaces of the *abductor digiti minimi* / *flexor digiti minimi*, *flexor pollicis longus*, *extensor pollicis brevis*, and *extensor carpi ulnaris.* The latter two entheses had the highest factor loadings. Negative scores on the component represented individuals with relatively larger entheses of the thumb intrinsic muscles (i.e., *opponens pollicis*, *abductor pollicis* / *flexor pollicis brevis*, *adductor pollicis*) and the first dorsal *interosseus*. The three entheseal surfaces of the thenar muscles had similar factor loadings (Table 3).

[Table 3 here]

As demonstrated in the plot of Figure 2, the vast majority of individuals with the same occupation were located in the same side of PC2 (positive or negative), thus sharing a similar pattern between their hand entheseal surfaces. In order to highlight this observation, the workers of each occupation were colored distinctively in the PCA plot (Fig. 2). On PC2, the most striking similarity among individuals with the same work was observed for the groups of carpenters (PC scores ranged between -0.1 and 0.6), shoemakers (ranged between -1.3 and 0.2), and joiners (ranged between -0.8 and 0.2). The ranges of PC scores were greater for bricklayers (-0.1 to 2.30) and tailors (-0.2 to -2.4). It should be mentioned that most overlapping occupations seem to be of comparable nature (c.f., in the Discussion). For instance, bricklayers, carpenters, and stonemasons were all heavy construction manual workers (Yeats, 1872; Winpenny, 1990; Alves Cardoso and Henderson, 2012), while the wood-cutter (specialized carpenter) was within the range of carpenters and the mill-builder (specialized builder) overlapped with the bricklayers (Fig. 2).

[Figure 2 here]

The effect of overall size and biological age

A second PCA was carried out using size-adjusted variables, in order to estimate the influence of overall entheseal size on the results. The three first PCs represented the 63.08% of the total sample's variance. The two morphological tendencies identified in the PCA of raw measurements (PC2) were also observed in the PCA of size-adjusted variables (Table 3). In the latter, these patterns were associated with PC1, which represented the 30.57% of the total sample's variance. The factor loadings of this component were all substantially higher than those of PC2 in the first PCA, emphasizing the occurrence of the two proposed morphological trends among hand entheses (Karakostis and Lorenzo, 2016). Nevertheless, the factor loading of the enthesis of the first palmar *interosseus*, which was almost zero on PC2 of the previous PCA, appears here (on PC1 of the second PCA) to be marginally negative (Table 3). Overall, the mean difference in specimen PC scores between PC2 of the first PCA and PC1 of the second PCA was 0.24. As demonstrated in the plot of Figure 3, this slight change does not reduce the similarity observed among specimens with the same or relevant occupation.

[Figure 3 here]

The factor loadings of the remaining two components (PC2 and PC3) did not reflect the two entheseal patterns investigated here (Table 3). Furthermore, they showed no association with the occupational profile of individuals, which is the focus of this study. Particularly, variation in PC2 (18.76%) is mainly related to the proportion between the entheses of *flexor pollicis longus* and the first dorsal *interosseus*, while the score of specimens on PC3 (13.74%) is mainly regulated by the proportion between the enthesis of *opponens pollicis* and those of *adductor pollicis* and *extensor pollicis brevis*.

The correlation tests showed that the values of biological age presented moderate positive association with the scores of PC1 of the PCA on raw measurements (Table 4). This PC represents overall size variation in the sample (Table 3). By contrast, the values of biological age did not significantly coincide with the scores of all remaining PCs of both PCAs (p-value > 0.05). These included the two PCs whose factor loadings reflected the two hypothesized patterns of hand entheses (i.e., PC2 of the first PCA and PC1 of the second PCA).

[Table 4 here]

DISCUSSION

The historical literature provides a plethora of evidence surrounding the physical requirements of the occupations represented in our sample (Table 2). This information allows us to identify the occupations which required systematic application of sustained high grip force, within the historical context of our sample. Since the beginning of the 19th century, Basel was considered as the most industrialized city of Switzerland and a mass producer of its own mechanical equipment, which had already replaced intense manual force in multiple urban occupations (Mergnac and Bertrand, 2004; Connor, 2007; Berend, 2013). By the mid-19th century, this revolutionary change of working conditions in European cities is reported for multiple crafts, including bakers (Webster, 1845; Rochelle, 2001), locksmiths (Yeats, 1872; Roper, 1976), ropemakers (Chapman, 1857), shoemakers (Phillips, 1817; Mergnac and Bertrand, 2004), silk-dyers (Hurst, 1892), tailors (Urquhart, 1881; Mergnac and Bertrand, 2004), and joiners (Yeats, 1872; Anonymous, 1908; Shivers, 1990). By contrast, the historical sources clearly describe certain urban occupations as highly demanding handcrafts within the industrialized environment of the mid-19th century, underlining the strenuous and repetitive use of manual strength for performing a variety of tasks (Wrigley, 1972; Winpenny, 1990). This was the case -among others- for urban butchers (Hennicke, 1866), carpenters (Yeats, 1872; Anonymous, 1908; Winpenny, 1990), bricklayers (Benjamin, 1827; Yeats, 1872; Anonymous, 1908), saddlers (Robertson, 1850; Beatie, 1981), stonemasons (Yeats, 1872; Anonymous, 1908), tinsmiths (Hall & Carpenter, 1886; Demer, 1978; Winpenny, 1990), as well as unspecialized day laborers (Anonymous, 1908; Mergnac and Bertrand, 2004).

Based on these historical sources (Table 2), individuals can be divided into workers involved in highly demanding manual activities and individuals with less strenuous and/or highly mechanized manual tasks. When the individuals of each occupational group are colored differently in our PCA plot on raw measurements (Fig. 4), this categorization provides a rather clear separation between the two groups of workers, with highly demanding occupations presenting distinctively higher positive scores on PC2. This difference between the two categories is also evident in Table 5, which contains the descriptive statistics with respect to each occupational category defined. The four entheseal areas associated with high grip force (Fig. 1) have a larger mean size in individuals involved in demanding manual labor, while the mean size of the thenar muscles' entheses is larger in individuals with less strenuous professions (Table 3). It is worth mentioning that the vast majority of occupations represented in our sample were categorized similarly in previous works on entheseal change, which separated individuals based on the intensity of their physical activities (e.g., Villotte et al., 2010; Alves Cardoso and Henderson, 2012). Previous research on entheseal change has recommended that different occupational categories should present comparable mean age-at-death (Alves Cardoso and Henderson, 2012). As demonstrated in Table 5, mean age is very similar between the two categories of occupations defined here.

[Figure 4 here]

[Table 5 here]

The purpose of this study was to assess the relationship between habitual manual activity and patterns of hand entheses, while controlling for as many confounding factors as possible in an anthropological sample (Foster et al., 2012). Our results verify the occurrence of the two previously described morphometric patterns of hand entheses (Karakostis and Lorenzo, 2016) in our sample, showing that these are not associated with interpopulation variation, sex, age (between 18 and 48 years), pathology, or close relatedness. In humans, the raw size of entheseal 3D areas has been reported to strongly correlate with body size (Nolte and Wilczak, 2013) as well as bone length (Karakostis and Lorenzo, 2016). This indicates that the overall size of entheseal areas, like any other bone part (Rauch, 2005), is highly dependent on the size of individuals. Furthermore, our correlation tests identified a significant moderate relationship between age-at-death and overall entheseal size (Table 4). Previous research has also reported a significant effect of age on entheseal change (e.g., Foster et al., 2012; Milella et al., 2012) and 3D size (Nolte and Wilczak, 2013), probably due to age-related degenerative processes in combination with lifelong accumulation of mechanical stress. In our analysis, when the effect of overall entheseal size was removed, the two observed entheseal patterns accounted for almost one third of the sample's total variance (Table 3). On this basis, body size and age-at-death could hardly be the regulating factors of the two observed entheseal patterns. This can be also supported by Figures 2 and 4 (PCA on raw measurements), where multiple individuals with extensive size differences (scores on PC1) presented almost the same entheseal pattern (scores on PC2).

The two entheseal patterns reported here (Table 3) are almost identical to the ones described in the previous research which proposed that they reflect two synergistic muscle groups (Fig. 1), based on a sample from medieval Burgos in Spain (Karakostis and Lorenzo, 2016). This similarity suggests that different population can present comparable patterns of hand entheses. It should be mentioned that this previous study utilized both male and female individuals (Karakostis and Lorenzo, 2016).

Previous research has questioned the impact of physical activity on human entheseal size and shape (Zumwalt, 2006; Djukic et al., 2015; Rabey et al., 2015; Williams-Hatala et al., 2016). However, the results of these past studies may be strongly influenced by their methodological choices. Two of these (Djukic et al., 2015; Rabey et al., 2015) did not include a 3D analysis of entheseal surface areas, which could provide complete information on their form. Moreover, other works (Zumwalt, 2006; Rabey et al., 2015) utilized experimental models involving non-primate animals, even though it is not known whether the mechanisms of entheseal change differ between humans and other mammals. Another study (Williams-Hatala et al., 2016) found no correlation between the size of the entheseal areas of *opponens pollicis* and *opponens digiti minimi* and specific dimensions of the corresponding muscles. However, the specimens used in that work were of advanced age (an average of 77.9 ± 12 years). The mechanisms of entheseal change are highly influenced by old age (Myszka and Piontek, 2013), while muscle dimensions substantially decrease after the age of 50 (Doherty, 2001; Deschenes, 2004).

In this study, the observed differences among specimens with the same occupation could be due to numerous other variables affecting human behavior and bone morphology (Maier and Hepp-Reymond, 1995; Marzke et al., 1998), including inter-individual genetic variability, nutrition, hormone levels, or even individual hand preference (Rauch, 2005; Foster et al., 2012). In spite of the multiple and complex factors at play, our approach was able to identify a functional signal in the patterns between hand entheses. On this basis, future research on occupational stress markers would substantially benefit from the compilation and study of skeletal samples which are documented for the active working life of individuals over a considerable period of time before death. Although the sample size used in this study is larger than in the majority of previous works on 3D models of entheseal surface areas (Zumwalt, 2005; Zumwalt, 2006; Noldner and Edgar, 2013; Williams-Hatala et al., 2016) – with the exception of one study which focused on a single entheseal surface (Nolte and Wilczak, 2013) and our previous work on a medieval non-documented sample (Karakostis and Lorenzo, 2016) – future work on increased sample sizes will also help to further establish the functional signal observed here. The conclusions of our work suggest that habitual manual activity has an observable effect on the morphometric patterns among hand entheses. On this basis, future application of our quantitative 3D approach to the analysis of entheseal patterns in the human fossil record could further our understanding of the evolution of tool making behavior and subsistence strategies among hominins. In bioarchaeology, the analysis of hand bone entheses could become an essential tool for reconstructing the manual activities, division of labor, and social structure of past populations.

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Table 1. The muscles related to the analyzed entheses and their function.

Muscles	**Primary function**	**Insertion site analyzed**
Abductor pollicis	Abducts the thumb	Radial base of the first proximal phalanx
Flexor pollicis brevis	Flexes the first metacarpophalangeal joint	Radial base of the first proximal phalanx
Adductor pollicis	Adducts the thumb	Ulnar base of the first proximal phalanx
First dorsal *interosseus*	Abducts the second finger	Radial base of the second proximal phalanx
First palmar *interosseus*	Draws second finger towards the 3rd finger	Ulnar base of the second proximal phalanx
Oponnens pollicis	Abducts, rotates, and flexes the thumb	Radial diaphysis of the first metacarpal
Extensor carpi ulnaris	Extends the wrist, adducts hand	Ulnar base of the fifth metacarpal
Flexor pollicis longus	Flexes the first distal phalanx	Palmar diaphysis of the first distal phalanx
Extensor pollicis brevis	Extends the thumb	Dorsal base of the first proximal phalanx
Abductor digiti minimi	Abducts the fifth finger	Ulnar base of the fifth proximal phalanx
Flexor digiti minimi	Flexes the fifth finger	Ulnar base of the fifth proximal phalanx

Table 2. Professions of individuals with citations of historical literature reporting their manual physical activities in mid-19th century European cities (c.f., in the Discussion).

Occupations involving sustained high-grip force		Less strenuous and/or mechanized occupations	
Occupations	**Number**	**Occupations**	**Number**
Bricklayer (Benjamin, 1827; Yeats, 1972; Anonymous, 1908)	5	**Baker** (Webster, 1845; Rochelle, 2001)	1
Butcher (Hennicke, 1866)	2	**Bookbinder** (Zaehnsdorf, 1880)	1
Carpenter (Yeats, 1872; Anonymous, 1908; Winpenny, 1990)	6	**Butler** (Beeton, 1861)	1
Day laborer (Anonymous, 1908; Mergnac and Bertrand, 2004)	1	**Doorman** (Bonnin and DeVillanova, 2006)	1
Mill builder (Benjamin, 1827)	1	**Coachman** (McShane and Tarr, 2007)	3
Saddler (Robertson, 1850; Beatie, 1981)	1	**Joiner** (Yeats, 1872; Anonymous, 1908; Shivers, 1990)	1
Stonemason (Yeats, 1872; Anonymous, 1908)	5	**Locksmith** (Yeats, 1872; Roper, 1976)	1
Tinsmith (Hall & Carpenter, 1866; Demer, 1978; Winpenny, 1990)	1	**Missionary** (Schlatterer, 1916)	1
Wood-cutter (Yeats, 1872; Anonymous, 1908; Winpenny, 1990)	1	**Painter** (Kugler and Head, 1854)	1
		Rope-maker (Chapman, 1857)	1
		Shoemaker (Phillips, 1817; Mergnac and Bertrand, 2004)	4
		Silk dyer (Hurst, 1892)	1
		Tailor (Urquhart, 1881; Mergnac and Bertrand, 2004)	5

Table 3. Eigenvalues and factor loadings of the two principal component analyses.

Variables utilized	Principal component	Eigenvalue	% of variance	Factor loadings[a]								
				OP	ABP/FPB	ADP	EPB	FPL	DI1	PI1	ADM/FDM	ECU
Raw measurements	PC 1	4.78	53.02	0.70	0.87	0.62	0.63	0.71	0.73	0.82	0.75	0.69
	PC 2	1.15	12.74	**– 0.39**	**– 0.37**	**– 0.48**	**0.48**	**0.14**	**– 0.18**	**0.04**	**0.35**	**0.47**
	Total		65.76									
Size-adjusted variables	PC 1	2.75	30.57	**– 0.56**	**– 0.82**	**– 0.54**	**0.62**	**0.23**	**– 0.42**	**– 0.29**	**0.53**	**0.71**
	PC 2	1.69	18.76	0.27	– 0.05	0.42	– 0.16	0.81	– 0.73	– 0.34	– 0.34	0.03
	PC 3	1.24	13.75	– 0.54	0.07	0.47	0.59	– 0.08	0.19	– 0.36	– 0.40	– 0.22
	Total		63.08									

[a] The factor loadings in bold are those of the principal components associated with the two investigated entheseal patterns.

Table 4. Correlations between biological age and the principal components of each principal component analysis (PCA) performed

Analysis	**Principal Components**[a]	**Biological age**	
		p-value	**r-value**
PCA on raw size	1st (overall size)	< 0.01	0.47
	2nd	**0.79**	**0.04**
PCA on size-adjusted variables	**1st**	**0.71**	**-0.05**
	2nd	0.73	0.05
	3rd	0.18	0.20

[a] The two principal components in bold are those associated with the investigated patterns between hand entheses (Karakostis and Lorenzo, 2016).

Table 5. Descriptive statistics for entheseal measurements (in square millimeters) and age at death (in years).

Bone	Measurements[a]	Occupations involving sustained high-grip force				Less strenuous and/or mechanized occupations			
		Range	Mean		Standard Deviation	Range	Mean		Standard Deviation
			Statistic	Standard Error			Statistic	Standard Error	
First proximal phalanx	ABP / FPB	82.67	82.02	4.74	22.72	92.92	86.34	5.04	23.65
	ADP	64.29	69.37	3.91	18.74	64.12	73.26	3.99	18.70
	EPB	55.93	67.24	3.45	16.56	59.90	44.56	2.93	13.74
Second proximal phalanx	DI1	113.44	121.97	6.34	30.38	117.28	120.07	6.21	29.14
	PI1	125.59	92.40	6.44	30.91	95.03	84.26	5.29	24.81
Fifth proximal phalanx	ADM / FDM	70.75	84.05	3.88	18.59	89.07	69.68	4.08	19.12
First metacarpal	OP	63.74	66.18	3.31	15.87	61.33	73.30	4.14	19.41
Fifth metacarpal	ECU	127.79	133.76	6.30	30.19	131.39	100.10	7.08	33.19
First distal phalanx	FPL	57.64	51.61	3.39	16.24	51.22	43.49	3.07	14.38
Age at death		30	28.91	1.79	8.57	26	27.14	1.57	7.37

[a.] ABP/FPB: *abductor pollicis / flexor pollicis brevis* (common insertion area for both muscles); ADP: *adductor pollicis*; EPB: *extensor pollicis brevis*; DI1: first dorsal *interosseus*; PI1: first palmar *interosseus*; ADM/FDM: *abductor digiti minimi / flexor digiti minimi* (common insertion area for both muscles); OP: *opponnens pollicis*; ECU: *extensor carpi ulnaris*; FPL: *flexor pollicis longus*.

Figure Legends

	Abbreviations
ABP	*abductor pollicis*
ADM	*abductor digiti minimi*
ADP	*adductor pollicis*
DI1	first dorsal *interosseus*
ECU	*extensor carpi ulnaris*
EPB	*extensor pollicis brevis*
FDM	*flexor digiti minimi*
FPB	*flexor pollicis brevis*
FPL	*flexor pollicis longus*
OP	*opponens pollicis*
PI1	first palmar *interosseus*

Figure 1. The nine entheseal surfaces of the two synergistic muscle groups described in previous research (Karakostis and Lorenzo, 2016). Each color (green or blue) represents one of the two observed entheseal patterns. For purposes of better demonstration, all five metacarpals and proximal phalanges are included in the figure. In two cases, the same enthesis corresponds to two different muscles ("ABP/FPB" and "ADM/FDM").

Figure 2. Scatter plot of the principal component analysis on raw entheseal 3D measurements without *a priori* group categorization. Individuals with the same occupation were highlighted. The two side figures demonstrate which entheseal areas are proportionally larger in individuals with higher scores on the second principal component (entheses in blue) and individuals with lower ones (entheses in green). Individuals with lower scores on the second principal component

present proportionally larger entheses of the four thumb intrinsic muscles and the first dorsal *interosseus* (green areas in the side figures). By contrast, individuals with higher scores on this component show relatively larger entheses of the muscles *abductor digiti minimi* / *flexor digiti minimi*, *flexor pollicis longus*, *extensor pollicis brevis*, and *extensor carpi ulnaris* (blue areas in the side figures).

Figure 3. Scatter plot of the principal component analysis on size-adjusted 3D entheseal measurements without *a priori* group categorization. Individuals with the same occupation were highlighted. The two side figures demonstrate which entheseal areas are proportionally larger in individuals with higher scores on the first principal component (entheses in blue) and individuals with lower ones (entheses in green). Individuals with lower scores on the first principal component present proportionally larger entheses of the four thumb intrinsic muscles, the first dorsal *interosseus*, and the first palmar *interosseus* (green entheses in the side figures). By contrast, individuals with higher scores on this component show relatively larger entheses of the muscles *abductor digiti minimi* / *flexor digiti minimi*, *flexor pollicis longus*, *extensor pollicis brevis*, and *extensor carpi ulnaris* (blue entheses in the side figures).

Figure 4. Scatter plot of the principal component analysis on raw entheseal 3D measurements without *a priori* group categorization. Occupations were classified (colored) based on the intensity of their manual activities, according to historical sources (c.f., Table 2). The two side figures demonstrate which entheseal areas are proportionally larger in individuals with higher

scores on the second principal component (entheses in blue) and individuals with lower ones (entheses in green) (c.f., in the legend of Figure 2).

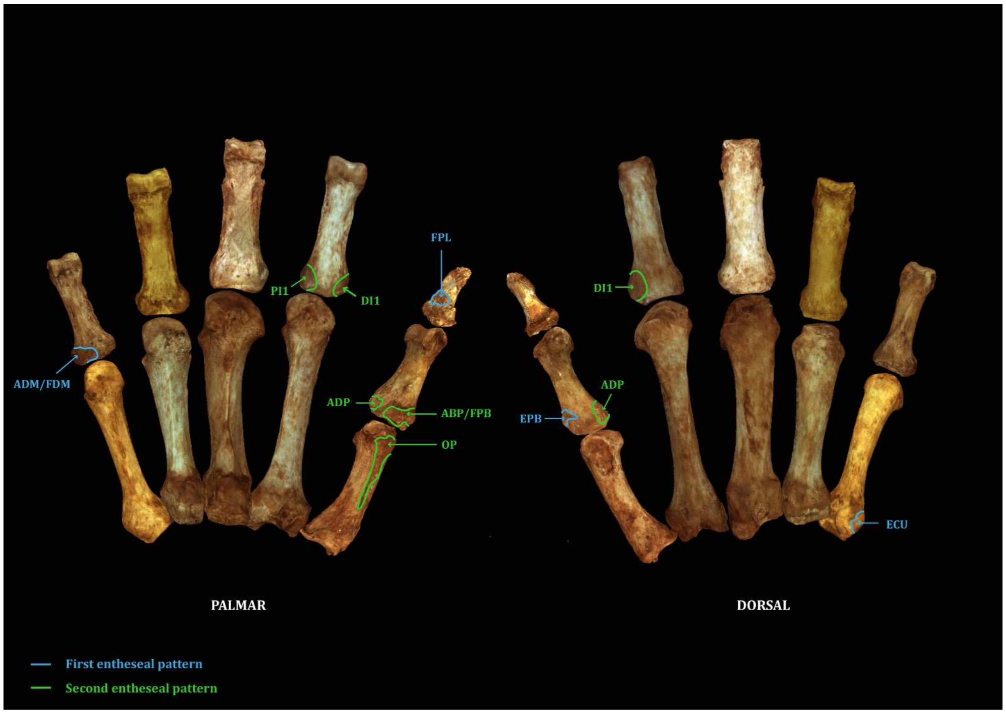

Figure 1. The nine entheseal surfaces of the two synergistic muscle groups described in previous research (Karakostis and Lorenzo, 2016). Each color (green or blue) represents one of the two observed entheseal patterns. For purposes of better demonstration, all five metacarpals and proximal phalanges are included in the figure. In two cases, the same enthesis corresponds to two different muscles ("ABP/FPB" and "ADM/FDM").
Figure 1
296x209mm (300 x 300 DPI)

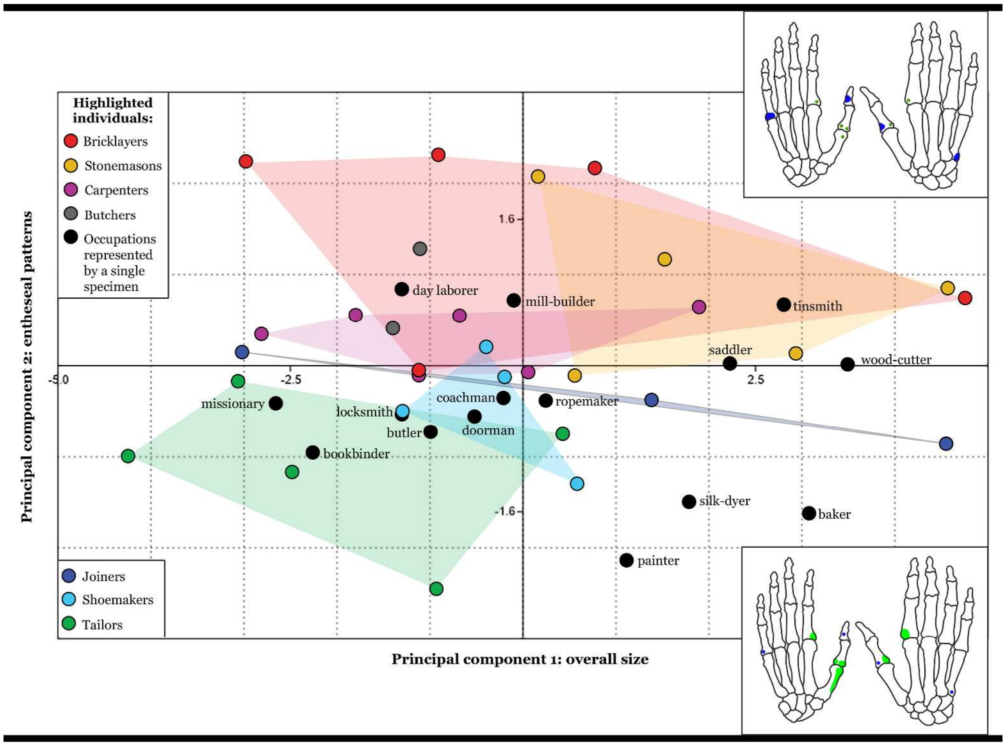

Figure 2. Scatter plot of the principal component analysis on raw entheseal 3D measurements without a priori group categorization. Individuals with the same occupation were highlighted. The two side figures demonstrate which entheseal areas are proportionally larger in individuals with higher scores on the second principal component (entheses in blue) and individuals with lower ones (entheses in green). Individuals with lower scores on the second principal component present proportionally larger entheses of the four thumb intrinsic muscles and the first dorsal interosseus (green areas in the side figures). By contrast, individuals with higher scores on this component show relatively larger entheses of the muscles abductor digiti minimi / flexor digiti minimi, flexor pollicis longus, extensor pollicis brevis, and extensor carpi ulnaris (blue areas in the side figures).
Figure 2
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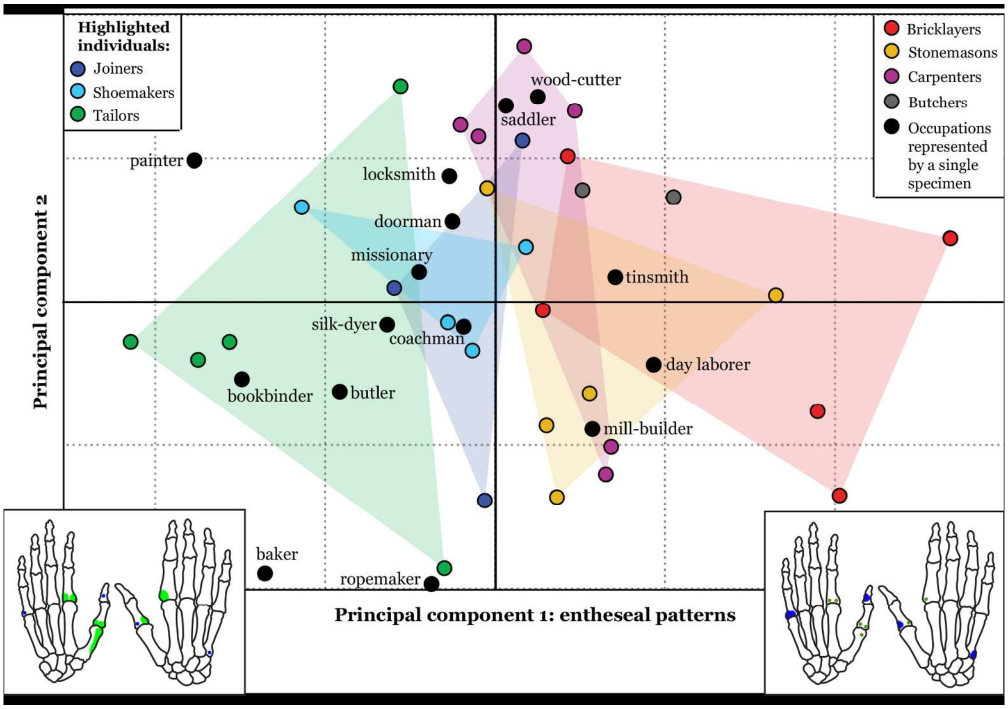

Figure 3. Scatter plot of the principal component analysis on size-adjusted 3D entheseal measurements without a priori group categorization. Individuals with the same occupation were highlighted. The two side figures demonstrate which entheseal areas are proportionally larger in individuals with higher scores on the first principal component (entheses in blue) and individuals with lower ones (entheses in green). Individuals with lower scores on the first principal component present proportionally larger entheses of the four thumb intrinsic muscles, the first dorsal interosseus, and the first palmar interosseus (green entheses in the side figures). By contrast, individuals with higher scores on this component show relatively larger entheses of the muscles abductor digiti minimi / flexor digiti minimi, flexor pollicis longus, extensor pollicis brevis, and extensor carpi ulnaris (blue entheses in the side figures).

Figure 3

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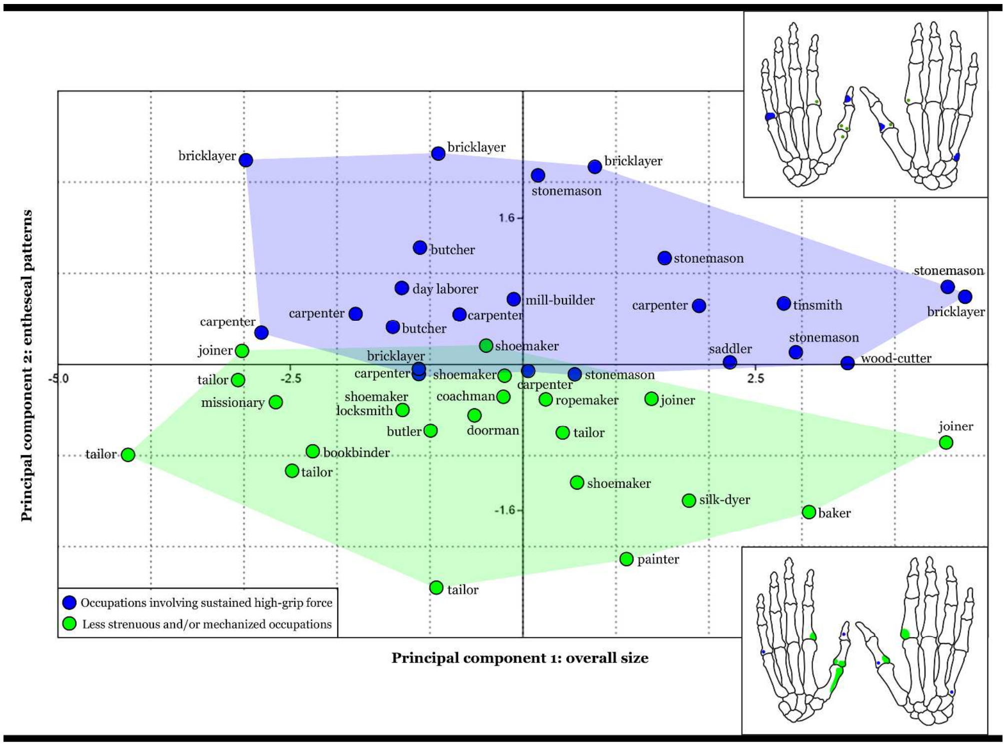

Figure 4. Scatter plot of the principal component analysis on raw entheseal 3D measurements without a priori group categorization. Occupations were classified (colored) based on the intensity of their manual activities, according to historical sources (c.f., Table 2). The two side figures demonstrate which entheseal areas are proportionally larger in individuals with higher scores on the second principal component (entheses in blue) and individuals with lower ones (entheses in green) (c.f., in the legend of Figure 2).
Figure 4
295x218mm (300 x 300 DPI)

Theo der Pfeifenraucher

Ein genealogisch-naturwissenschaftliches Identifizierungsprojekt

Gerhard Hotz, Stefanie Doppler, Marie-Louise Gamma, Diana Gysin, Odette Haas, Guido Helmig, Ludwig Huber, Simon Kramis, Fotios Alexandros Karakostis, Liselotte Meyer, Geneviève Perréard Lopreno, Jürgen Rauber, Lutz Roewer, Jessica Rothe, Albert Spycher, Ursula Wittwer-Backofen und Marina Zulauf-Semmler

in Memoriam
Sepp Uebelhart (13.11.1927 – 19.10.2015) und
Paul Meier (28.3.1926 – 20.7.2017)

Résumé

« Theo le fumeur de pipe » est le nom d'une personne qui vivait à Bâle au 18ième siècle et qui était fumeur de pipe passionné. Theo est mort dans la première moitié du 19ième siècle et a été enterré dans le cimetière des pauvres en face de l'église Saint Théodore à Kleinbasel. On a commencé à s'intéresser en 1984 à Theo dont le nom dérive de l'église Saint Théodore, lorsque le service archéologique de la ville de Bâle-Ville a exhumé le squelette de Theo lors de l'assainissement de la canalisation. Des étudiants de l'université de Bâle ont examiné le squelette en 2004 dans le Musée d'Histoire naturelle et ont été frappé par les trous ovales dans sa dentition. Pendant les années l'embouchoir de la pipe s'était fait un trou dans le dentier du fumeur de pipe. Des collaborateurs du musée ont décidé en 2007 à cause de ce constat extraordinaire de découvrir qui était ce fumeur de pipe et quel était son vrai nom. Ils voulaient savoir quel métier il avait exercé et pourquoi il était mort beaucoup trop tôt à seulement 30 ans. Un groupe de naturalistes, de généalogistes, d'historiens et de scientifiques participatives s'est mis à détecter les traces. Comparable à une analyse criminalistique, maints éléments d'une mosaïque ont été rassemblés pour dévoiler l'identité de Theo jusqu`à ce que Theo apparaisse presque comme une personne réelle pour les chercheurs. Des chercheurs du monde entier ont parti-

cipé à la recherche interdisciplinaire des traces. Maintenant on est peut-être tout près d'éclaircir le mystère. Les généalogistes du projet scientifique participative ont réussi à trouver des descendants potentiels de Theo. Des prélèvements de salive de descendants potentiels de Theo sont comparés actuellement avec l'ADN des os de Theo. Si les marqueurs génétiques correspondent, l'identité de Theo sera relevée. Du point de vue scientifique, cela serait une sensation et montrerait et prouverait que la coopération interdisciplinaire et des projets de recherche citoyen peuvent réussir

Zusammenfassung

„Theo der Pfeifenraucher" ist der fiktive Name einer Person, die Ende des 18. Jahrhunderts in Basel lebte und ein passionierter Pfeifenraucher war. Theo starb in der ersten Hälfte des 19. Jahrhunderts und wurde im Armenfriedhof vis-à-vis der Theodorskirche in Kleinbasel beerdigt. Der Blick der Forschung richtete sich 1984 auf Theo, dessen Name sich von der Theodorskirche ableitet, als die Archäologische Bodenforschung Basel-Stadt im Zuge einer Leitungsgrabung Theos Skelett exhumierte. Studierende der Universität Basel untersuchten 2004 im Naturhistorischen Museum Basel sein Skelett und waren von dessen auffälligen und oval geformten Löchern im Gebiss fasziniert. Das Mundstück der Pfeife hatte sich kontinuierlich über die Jahre ins Gebiss des Pfeifenrauchers eingeschliffen. Mitarbeitende des Museums entschlossen sich 2007 aufgrund dieses aussergewöhnlichen Befunds, dem Pfeifenraucher auf die Spur zu kommen und herauszufinden, wie Theo in Wirklichkeit geheissen hatte, was für einen Beruf er ausgeübt hatte und warum er mit nur 30 Jahren viel zu jung verstorben war. Ein Team von Naturwissenschaftlern, Genealogen, Historikern und Bürgerwissenschaftlern machte sich auf die Spurensuche. Vergleichbar einer kriminalistischen Analyse wurden Mosaiksteinchen um Mosaiksteinchen zu Theos Identität zusammengetragen, bis Theo fast als greifbare Person den Forschenden gegenüberstand. Wissenschaftlern aus der ganzen Welt beteiligten sich an der interdisziplinär angelegten Spurensuche. Nun steht möglicherweise die Auflösung des Rätsels unmittelbar bevor. Den Genealogen des Bürgerforschungsprojekts BBS gelang es, potentielle Nachfahren von Theo zu finden. Die Speichelproben von potentiellen Nachfahren Theos werden zurzeit mit der alten DNA aus Theos Knochen verglichen; stimmen die genetischen Marker überein, kann Theos Identität entschlüsselt werden. Wissenschaftlich gesehen wäre das eine mittlere Sensation und würde zeigen, wie erfolgreich interdisziplinäre Zusammenarbeit und Bürgerforschungsprojekte sein können

Einleitung

Menschliche Skelette üben seit jeher eine grosse Faszination auf uns aus, erinnern uns doch die knöchernen Überreste an unsere eigene Vergänglichkeit. Diese Begeisterung war auch im Vortrag „Theo der Pfeifenraucher – Eine genealogisch-anthropologische Spurensuche“ anlässlich der Hauptversammlung der Schweizerischen Gesellschaft für Familienforschung (SGFF) im Mai 2017 in Basel spürbar. Das Interesse der Vereinsmitglieder wurde sicherlich auch durch das Vorliegen neuer naturwissenschaftlicher, genealogischer und molekulargenetischer Forschungsergebnisse zum Basler Theo verstärkt. Gerade mit naturwissenschaftlicher Analytik ist eine weitere Ebene zur oben erwähnten Faszination „menschliches Skelett“ hinzugekommen, lassen sich doch aufgrund unterschiedlicher naturwissenschaftlicher Methoden Informationen zur Identität, Verwandtschaft, Herkunft, Aussehen, Gesundheit und Lebensgewohnheiten zu prähistorischen und historischen Personen gewinnen. Das menschliche Skelett stellt insofern ein einzigartiges Bioarchiv dar, dessen vollständige Entschlüsselung mittels naturwissenschaftlicher Analytik bei weitem noch nicht abgeschlossen ist.[1] Diesbezüglich leisten genealogische Forschungen einen wichtigen Beitrag zur Unterstützung anthropologischer Methodenentwicklung.[2]

Bewegen wir uns im historischen Zeitraum und liegen schriftliche Quellen im Kontext der untersuchten Skelette vor, stellen die knöchernen Überreste unserer Vorfahren eine Schnittstelle zwischen Natur- und Geisteswissenschaften dar.[3] Das Bioarchiv Skelett liefert auf der individuellen Ebene Informationen, die sich selten aus den historischen Quellen erschliessen lassen.[4] Umgekehrt können die historischen Quellen die naturwissenschaftlichen Ergebnisse in optimaler Weise ergänzen und eine Kontextualisierung ermöglichen. Von besonderer Bedeutung sind in diesem Bezug die mikrohistorisch und genealogisch orientierten Forschungsbereiche.[5] Die Kombination von „menschlichen Skeletten“ und „zugehörigen historischen Quellen“ birgt ein enormes wissenschaftliches Potential, welches im optimalen Fall, nämlich beim Vorliegen iden-

[1] So untersucht Gabriela Mani-Caplazi im Rahmen ihrer Dissertation (IPNA, Universität Basel) Zähne der identifizierten Skelette des Spitalfriedhofs Basel, um Anzahl und Zeitpunkt von Schwangerschaften in den Zahnzementschichten festzustellen. Diese Grundinformationen wurden durch das Genealogen-Team des Bürgerforschungsprojekts (BBS) geschaffen, siehe: Mani et al. 2017: eingereicht.

[2] Karakostis et al. 2017; Mani et al. 2017: eingereicht; Ingold et al. 2018: eingereicht; Hotz et al. 2015: 9-13.

[3] Hotz und Steinke 2012: 105-138.

[4] Ryser 2016: 1-69.

[5] Opgenoorth und Schulz: 2001.

tifizierter Skelette, zu einem interdisziplinären Ausschöpfen des wissenschaftlichen Potentials führen kann.[6]

Identifizierte Skelette aus historischen Zeiten liegen in der Regel vor allem von Personen aus den gehobenen Bevölkerungskreisen vor, da diese vielfach über Grabstätten in Kirchen verfügten. Solche Grabstätten überdauern unter Umständen die Zeit und können bei Sanierungsarbeiten der Kirchen geöffnet oder sogar exhumiert werden.[7] Wir sprechen hier nur so berühmte Beispiele wie Jörg Jenatsch, Goethe und Schiller an.[8] Insofern stellt das Identifizierungsprojekt „Theo der Pfeifenraucher" eher eine Ausnahme dar, da hier ein „kleiner Mann", ein „Nobody" aus der sozialen Unterschicht identifiziert, werden soll.[9] Gerade seine Herkunft aus der sozialen Unterschicht und die damit verbundenen Lebensbedingungen stehen im Fokus der Forschungen um Theo. Theo der Pfeifenraucher weist zudem eine weitere Besonderheit auf: In diesem Projekt kommunizieren in ergänzender Weise natur- und geisteswissenschaftliche Disziplinen. Den genealogischen Forschungen kommt in diesem Bezug eine Schlüsselfunktion zu, stellen sie doch eine wichtige Komponente der Grundlagenforschungen zur Identifizierung und zur sozialen Herkunft der betroffenen Personen dar.

Für die Forschungen zu Theo stellt die Stadt Basel archivalisch gesehen einen Glücksfall dar – das Staatsarchiv Basel-Stadt verfügt über einen ungewöhnlich reichhaltigen Schatz schriftlicher und bildlicher Quellen des 18. und vor allem des 19. Jahrhunderts, welcher für die Erforschung sowohl der städtischen Sozialgeschichte, als auch für genealogische Recherchen geradezu ideal ist.[10]

Ausgangslage

Im Rahmen eines Praktikums seitens des Instituts für prähistorische und naturwissenschaftliche Archäologie (IPNA, Universität Basel) am Naturhistorischen Museum Basel untersuchten 2004 die Studierenden Simon Kramis (Anthropologe) und Fabian Link (Historiker) ein Skelett aus dem ehemaligen Ersatzfriedhof bei der Kirche St. Theodor in Kleinbasel, dessen Skelett die Ar-

[6] Hotz et al. 2016: 121-131; Seifert 2012: 115-124; Haidle 1997: 1-160.

[7] Hotz et al. 2017a: 139-143.

[8] Ullrich 2004: 1-336; Janosa 2014: 1-209.

[9] Hotz et al. 2017b: 65-71.

[10] An dieser Stelle sei dem Team des Staatarchivs Basel-Stadt für die vielen freundlichen Gespräche, Anregungen und Unterstützungen gedankt, insbesondere Esther Baur, Hermann Wichers, Sabine Strebel, Krishna Das Steinhauser, Patricia Eckert, Barbara Gut, Daniel Hartmann, Peter Hofer, Michaela Liechti, Daniel Kress und Andreas Barth.

chäologische Bodenforschung Basel-Stadt im Rahmen einer Leitungsgrabung im Winter 1984 exhumiert hatte.[11] Ins Auge stachen sofort zwei oval geformte Lücken im Gebiss des jung verstorbenen Mannes. Handelte es sich hier um einen Pfeifenraucher? Hatte sich das harte, aus Keramik geformte Mundstück der Pfeife durch den jahrelangen Tabakgenuss in die Zwischenräume der Zähne eingeschliffen? Die aus Keramik geformten Mundstücke waren offenbar weitaus härter als der Zahnschmelz des menschlichen Gebisses.

Abb. 1: Deutlich hat Theos Passion Pfeife zu rauchen zwei oval geformte Lücken in seinem Gebiss hinterlassen (Foto: Gerhard Hotz).

Dieser aussergewöhnliche Tatbestand weckte ihr Interesse, mehr über diese Person herauszufinden. Wer war er gewesen? War er verheiratet und welchen Beruf hatte er ausgeübt? Warum war er in einem solch jugendlichen Alter gestorben? Das Skelett erhielt den Namen „Theo der Pfeifenraucher“, benannt nach der Theodorskirche und seiner offensichtlichen Vorliebe für das Schmauchen einer Tabakpfeife.[12]

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[11] Wir bedanken uns bei Guido Lassau, Christoph Matt und Christian Stegmüller und dem ganzen Team der Archäologischen Bodenforschung Basel-Stadt für die vielfach gewährte Unterstützung.

[12] Namenspate zum Skelett aus der Grabgrube 19 war Basil Thüring, Leiter der Geowissenschaften am Naturhistorischen Museum Basel. Er prägte den griffigen Titel „Theo der Pfeifenraucher“.

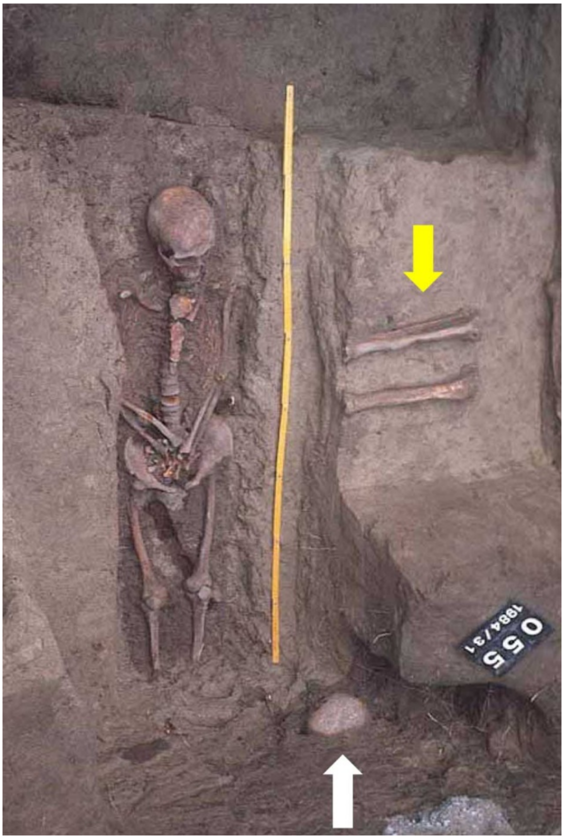


Abb. 2: Theos knöcherne Überreste liegen eingebettet zwischen den Gräbern 17–20. In der unteren Bildmitte befindet sich das Skelett Theos in Grab 19, das etwas früher angelegt wurde als Grab 20 zu seinen Füssen (weisser Pfeil). Bei Anlegung der gleich wie bei Theo orientierten Gräber wurden die Bestattungen der älteren, nicht so tief ausgeschachteten und um 90° abweichend orientierten Belegungsphase, gestört. Auf dem Foto ist deshalb von Grab 17 nur noch die Beinpartie erkennbar (gelber Pfeil). Foto: Archäologische Bodenforschung Basel-Stadt. Theodorskirchplatz (A), 1984/33, Sektor III.

Theo der Pfeifenraucher fand seine letzte Ruhestätte in einem kleinen nur während 54 Jahren vom 5. Oktober 1779 bis 27. April 1833 genutzten Ersatzfriedhof, der gegenüber dem regulären Kirchhof in einem ehemals als Rebacker genutzten Areal lag. In diesem Friedhof wurden vor allem Angehörige der sozialen Unterschicht bestattet. Personen aus der Oberschicht fanden ihre letzte Ruhestätte vorwiegend in den Grabstätten in der Pfarrkirche, Kapelle oder im Kirchhof selbst.

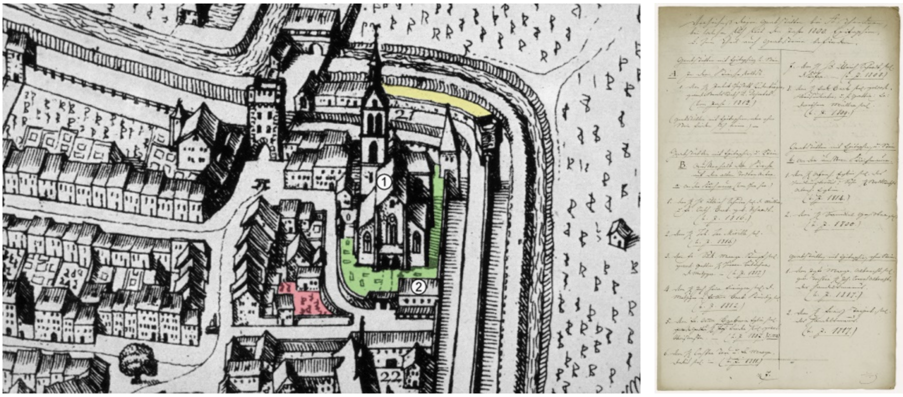


Abb. 3: Links: Zwei Areale (pink und gelb eingefärbt) dienten als Ersatzfriedhöfe. Theo wurde im Ersatzfriedhof (pink) vis-à-vis des regulären Kirchhofs (grün eingefärbt) bestattet. Begüterte Personen fanden ihre letzte Ruhestätte in der St. Theodorskirche (1) oder der zugehörigen Kapelle (2) (Bild Archäologische Bodenforschung Basel-Stadt). Rechts: Das Steinbuch der Theodorskirche erlaubte alle potentiellen Kandidaten zu Theo auszuschliessen, wenn sich eine Angabe zu einem Grab in der Kirche (1), der Kapelle (2) oder im Kirchhof (grün eingefärbt) fand (StABS Bauacten JJ 52).

Diese soziale Segmentierung liess sich aufgrund des „Verzeichnis der Grabstätten bei der Theodorskirche“, dem sogenannten Steinbuch[13] nachvollziehen. Im Steinbuch wurden die Familiengräber und deren Lokalisierung beschrieben mit zugehörigen Epitaphien. Leider liessen sich keine alten Gräberpläne zum Ersatzfriedhof im Staatsarchiv Basel-Stadt finden. Im 18. und 19. Jahrhundert wurden keine Gräberpläne angefertigt, da in den Friedhöfen nicht nach einer räumlichen Abfolge in Reihen bestattet wurde, wie das heute der Fall ist, sondern Gräber wurden dort angelegt, wo gerade Platz war oder die Möglichkeit der Zugehörigkeit zu einer Familiengrabstätte bestand.[14] Aber im Staatsarchiv Basel-Stadt fand sich das Beerdigungsregister[15] zur Kirchgemeinde St. Theodor.[16] Im Beerdigungsregister wurden alle in Kleinbasel verstorbenen Personen von den jeweiligen Pfarrherren namentlich mit Angaben zu Beruf, der Herkunft und dem Sterbealter einzeln aufgelistet. Für den Zeitraum 5.10.1779 bis

[13] StABS Bauacten JJ 52, Verzeichnis der Grabstätten bei der Theodorskirche.

[14] Perréard Lopreno et al. 2017: im Druck.

[15] StABS Kirchenarchiv CC 16, 1 bis 3 und Kirchenarchiv CC 17.

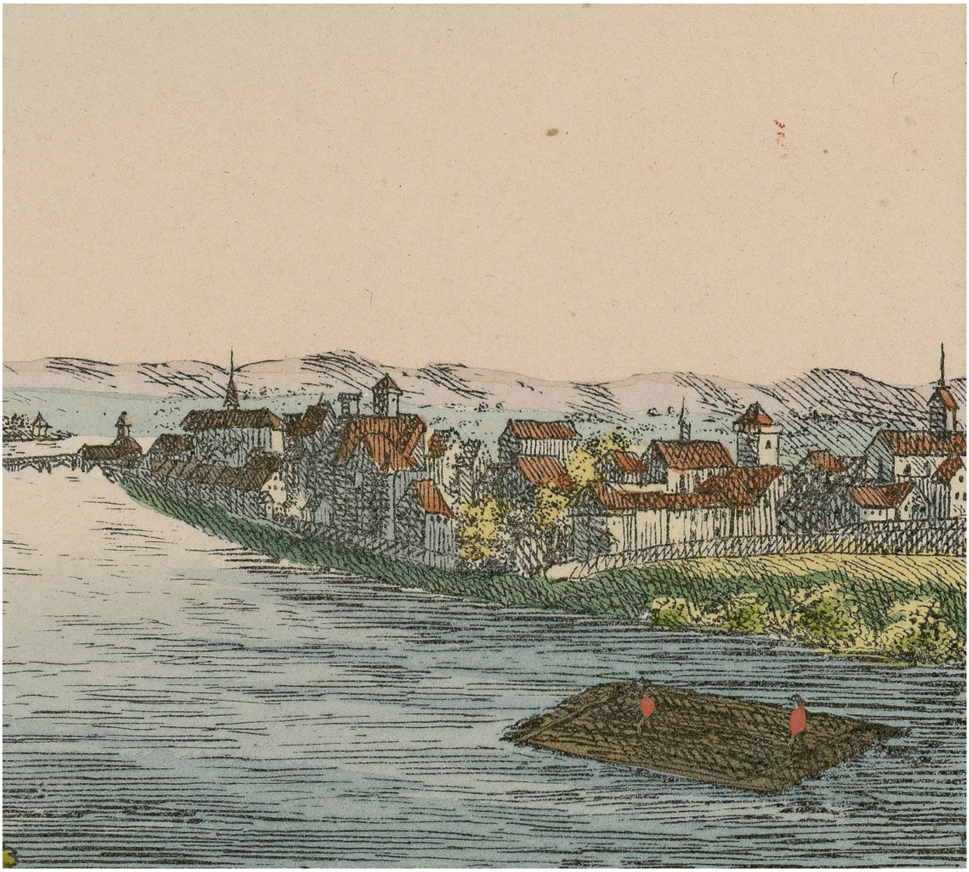
[16] Die Kirchgemeinde St. Theodor besitzt die beiden ältesten Taufbücher Europas, welche ab 1490 in Latein und ab 1529 in Deutsch geführt wurden. Aus Versehen wurden die beiden Bücher im 19. Jahrhundert mit einem Nachlass verkauft. Die Originale befinden sich in London in der British Library.

Abb. 4: Ansicht von Basel rheinabwärts. Rechts im Bild (gegenüberliegende Seite) Kleinbasel mit der Kirche St. Theodor am rechten Bildrand. In Kleinbasel lebte Theo und in unmittelbarer Nähe fand er auch seine letzte Ruhestätte (kolorierte Radierung, um 1800. StABS BILD Visch. A 12).

27.4.1833 lagen „nur“ 4‘334 mögliche Kandidaten vor, die alle in den Friedhöfen um die Theodorskirche, in der Kirche oder Kapelle selbst ihre Grabstätte besassen. Einer von ihnen musste Theo sein.

Genealogie, Identifizierung und Alltagsgeschichte

Bei der Durchsicht des Beerdigungsregisters und anderer historischer Archivalien zum Ersatzfriedhof, der sich aufgrund der sozialen Zugehörigkeit der dort Bestatteten auch als „Armenfriedhof“ bezeichnen lässt, reifte ein ehrgeiziges



Vorhaben heran. Wenn nur 4‘334 Personen als potentielle Kandidaten für das Skelett von Theo dem Pfeifenraucher und nur ein schmales Zeitfenster von 54 Jahren als Bestattungszeitraum in Frage kommen, sollte es doch möglich sein, herauszufinden, wie dieser Theo in Wirklichkeit geheissen, welchen Beruf er ausgeübt und wie sein Alltag ausgesehen hatte. Diese Zuversicht wurde durch eine vor 28 Jahren vorgenommene Identifizierung der Skelettserie des Basler Spitalfriedhofs genährt. Damals konnten fast 800 Bestattungen ehemaliger Patienten des Bürgerspitals aufgrund historischer und anthropologischer Quellen identifiziert werden.[17] Die Patienten und Patientinnen waren im Zeitraum von 1845 bis 1868 verstorben und sind auf dem naheliegenden Spitalfriedhof

[17] Hotz und Cueni 2003: 1-6.

beigesetzt worden.[18] Warum sollte dies nicht auch im vorliegenden Fall ebenfalls möglich sein? Es ging aber im Fall von Theo um weitaus mehr, als um eine blosse historische Personenidentifizierung. Es sollte die Geschichte eines einfachen Mannes erzählt werden, der im Armenfriedhof seine letzte Ruhestätte gefunden hatte. Eines Mannes aus der sozialen Unterschicht – also eines Vertreters einer Personengruppe, die selten markante Spuren in der dokumentierten Geschichte hinterlässt. Dem sollte nun in Form einer Buchpublikation[19] Rechnung getragen werden.

Für dieses Vorhaben standen sein Skelett mit all den in den Knochen noch schlummernden Informationen und ein bedeutender historischer Schatz in Form von schriftlichen und bildlichen Quellen zur Verfügung. Drei unterschiedliche Quellengattungen, Skelett, Schriftquellen und Bilder, sollten zu diesem Zweck erforscht werden.

Ein solch ambitioniertes Unterfangen war nur in interdisziplinärer Zusammenarbeit mit einem Team von Experten aus verschiedenen Fachbereichen durchführbar. Zudem mussten sämtliche Informationen aus dem Sterberegister erfasst und einer genealogischen Analyse zugänglich gemacht werden. Zahlreiche andere Dokumente, verfasst in schwer lesbarer deutscher Kurrenthandschrift, mussten ebenfalls transkribiert werden, um die Bestattungsgeschichte der Kirche St. Theodor zu erforschen und um dem pfeifenrauchenden „Nobody“ auf die Schliche zu kommen. Ein Riesenaufwand, der sowohl die Möglichkeiten, als auch die Ressourcen des Naturhistorischen Museums Basel bei weitem überstieg. Was war zu tun? Welche Möglichkeiten kamen infrage?

Das Bürgerforschungsprojekt – ein Experiment mit offenem Ausgang

Hier entschloss sich das Museum ein Experiment zu wagen. In einer kleinen Ausstellung, die im Juni 2007 im Hochparterre des Museums eröffnet wurde, sollte die historisch interessierte Bevölkerung Basels auf Theo den Pfeifenraucher aufmerksam gemacht und zur Spurensuche und Mitarbeit im Forschungsprojekt aufgerufen werden. Parallel zur Ausstellungs- und Medienarbeit wur-

[18] Hotz und Scholz 2015: 52-55.

[19] Obwohl Theo bis anhin noch nicht identifiziert wurde, erschien im Christoph Merian Verlag 2010 das Buch „Theo der Pfeifenraucher – Leben in Kleinbasel“. Die Herausgeber nutzten einen „Kunstgriff“ um diese fehlende Identifikation auszugleichen und es wurden an Stelle des Lebenslaufs von Theo, die drei Topkandidaten „Christian Friedrich Bender, Achilles Itin und Peter Kestenholz“ vorgestellt und das soziale Leben der Kleinbasler Unterschicht beschrieben. Dies hatte den Vorteil, dass die Sozialgeschichte viel breiter ausgeführt werden konnte. Siehe hierzu: Hotz et al. 2010: 1-236, und die einzelnen im vorliegenden Beitrag zitierten Kapitel.

den Forschungskooperationen mit Kaspar von Greyerz und Lucas Burkart seitens des Departements für Geschichte der Universität Basel und anderen Institutionen gesucht, galt es doch, die verschiedenen Kompetenzen ins Boot zu holen.[20] Medial fand die kleine Ausstellung über die Landesgrenzen hinaus grosse Aufmerksamkeit, zumal strategisch geschickt, kontinuierlich über den Forschungsprozess berichtet und der Kreis von mit Theo identifizierbaren Personen schrittweise reduziert werden konnte. Das Team von freiwilligen Mitarbeitenden wuchs kontinuierlich und umfasste Genealogen und historisch interessierte Bürgerwissenschaftler. In der Endphase des Bürgerforschungsprojekts „Theo der Pfeifenraucher" zählte das Team fünfzig Mitarbeitende. Alles Personen, welche die schwer entzifferbare alte deutsche Kurrentschrift lesen und transkribieren konnten.

Eine knöcherne Spurensuche

Vergleichbar einer kriminalistischen Spurensuche wurde das Profil von Theo mittels aufwändiger naturwissenschaftlicher Analytik Schritt für Schritt erforscht. Dabei stellten sich Fragen wie: Erlauben Theos Knochen Rückschlüsse auf seinen frühen Tod oder auf seine berufliche Tätigkeit? War Theo ein „Basler" oder wanderte er von auswärts ein? Mosaikstein um Mosaikstein fügten sich die Informationen zu einem detaillierten Profil. Eine Gesichtsrekonstruktion verhalf dem Gesuchten zudem zu einem Phantombild.

Dieses Profil sollte den Wissenschaftlern erlauben, aus dem Pool der 4`334 Verdächtigen den Gesuchten herauszufiltern. Dazu erfassten die Genealogen Alfred und Karin Schweizer die gesamten Informationen aus den Beerdigungs-, Tauf- und Eheregistern unabhängig von Geschlecht und Sterbealter in einer genealogischen Datenbank.[21] Diese Datenbank berücksichtigte in Form von

[20] In diesem Zusammenhang wurden drei Masterarbeiten zu Theo dem Pfeifenraucher geschrieben, die in die Buchpublikation als eigenständige Kapitel einflossen: Fasol 2009: 1-108; Guyer: 2009: 1-106; Senn 2009: 1-108. Zwei weitere historische Dissertationen seitens der Universitäten Basel und Bern bereicherten den historischen Lebensalltag zu zwei zentralen Themen: Sterben und Freizeit. Siehe hierzu: Zihlmann-Märki 2010a: 1-445; Zihlmann-Märki 2010b: 210-217, und Raciti 2006: 1-100; Raciti 2010: 140-151; Raciti 2013: 1-423.

[21] Alfred und Karin Schweizer erstellten und erstellen in langjähriger genealogischer Forschungsarbeit die sogenannte „Historische Personendatenbank Basel", kurz „HiPeBa" genannt. Die Datenbank umfasst über 200`000 historische Personen aus der Region Basel, mit genealogischen Grundinformationen und Quellenangaben. Diese wissenschaftlich und stadtgeschichtlich gesehen wertvolle Datenbank ist unter http://homepage.swissonline.ch/seelentag/HiPeBa allen Forschenden online zugänglich. Siehe: Duthaler 2012: 33.

Abb. 5: So dürfte Theo kurz vor seinem Tod ausgesehen haben. Vollplastische Gesichtsrekonstruktion von Gyula Skultéty †, Basel.

Wahrscheinlichkeitsvektoren die unterschiedlichen Profilmerkmale zu den 4`334 möglichen Theo-Kandidaten, wie Geschlecht, Sterbealter, Bestattungsplatz, geografische Herkunft, berufliche Tätigkeit und Krankheiten, um nur einige Merkmale zu nennen. Alle Personen weiblichen Geschlechts erhielten zum Beispiel im Merkmal „Geschlecht" die Wahrscheinlichkeit Null zugewiesen. Ein weiteres wichtiges Merkmal stellen die Berufe[22] dar, bei welchen zum Beispiel aufgrund anzunehmender Geruchsbelästigungen Pfeifenrauchen naheliegend war. Berufe wie Gerber oder Metzger erhielten im Theo-Profil eine leicht höhere Wahrscheinlichkeit zugewiesen. So verdiente der potentielle Theo-Kandidat Johann Bieler seinen Lebensunterhalt als Metzger. Sein ältester Bruder Jakob Bieler wurde am 25. Januar 1781 aktenkundig. Er wurde für wiederholtes Rauchen während der Arbeit als Metzger mit 15 Schilling gebüsst. [23] Dabei standen keine hygienischen Überlegungen hinter dieser Bestrafung; Rauchen galt schlicht als moralisch verwerflich und ebenso als Geldverschwendung.[24]

Berufe wie Zimmerleute und Schreiner, bei denen Pfeifenrauchen aufgrund von Brandgefährdung eher eine geringere Wahrscheinlichkeit aufwies, erhielten im Theo-Profil eine leicht geringere Wahrscheinlichkeit zugewiesen, da wir davon ausgingen, dass Theo seine Pfeife über eine längere Lebensspanne geraucht hatte. Die beiden oval geformten Lücken im Gebiss weisen auf eine

[22] Spycher-Gautschi 2010a: 132-139; Spycher-Gautschi 2010b: 1-206.
[23] Hotz 2010: 81.
[24] Kramis 2007: 41-44.

Abb. 6: Metzgermeister Johannes David-Bienz (1755-1829) gönnt sich eine Pause und schmaucht entspannt eine Pfeife. Seitens der Obrigkeit hätte ihm das eine saftige Strafe eingetragen. Jakob Bieler wurde 1781 mit 15 Schilling für eine solche Handlung gebüsst (Aquarell von Wilhelm Oser, um 1820. StABS PA Oser, 632, D4).

solch andauernde Nutzung hin.[25] Diese Datenbank erlaubte es, im Verlauf der Forschungen neue Ergebnisse zu berücksichtigen, das Profil stets zu aktualisieren und so den potentiellen Kandidatenkreis um Theo den Pfeifenraucher schrittweise zu reduzieren.

Zentrales Merkmal war natürlich die Angabe zum Geschlecht, das aufgrund der vollständig erhaltenen Beckenknochen zweifelsfrei feststand. Theo war männlichen Geschlechts. Der Kandidatenkreis reduzierte sich von 4`334 Personen beiderlei Geschlechts auf noch 2`069 Männer und Knaben. Zweites Hauptmerkmal war Theos Sterbealter. Je genauer die Sterbealtersschätzung aufgrund der Knochen und Zähne ausfiel, umso stärker konnte der Personenkreis um Theo reduziert werden. Nun ist eine Sterbealtersschätzung aufgrund eines Skeletts keine einfache Angelegenheit. Die Knochen erlauben eine Schät-

[25] Pfeifenlöcher werden nach fünf bis zehn Jahren intensiven Rauchens ausgebildet (Kramis 2010: 60).

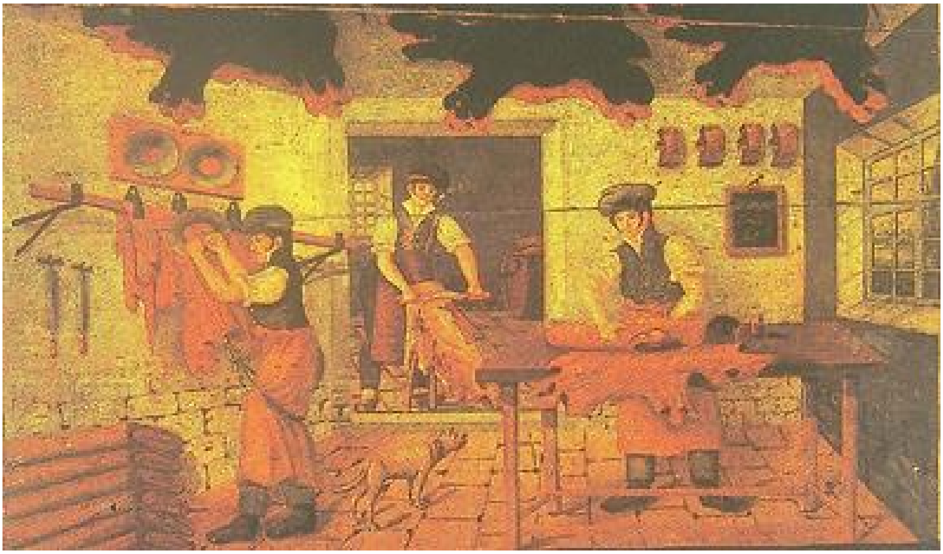


Abb. 7: Gerber beim Bearbeiten von Tierhäuten. Möglicherweise um den Gestank zu überdecken, rauchten zwei der drei abgebildeten Handwerker (Liestal um 1820. Ölfarbe auf Holz, Historisches Museum Basel, Foto Nr. C1924, Inv. Nr. 1900.162. Foto: M. Babey).

zung des sogenannten biologischen Alters, welches den biologischen Entwicklungszustand des Skeletts wiedergibt. Die genealogisch eruierten Altersangaben aus den Registern ergeben aber das sogenannte chronologische, sprich kalendarische Alter. Biologisches und chronologisches Alter können unter Umständen deutlich voneinander abweichen, vor allem wenn eine Person an einer Stoffwechsel- oder Nierenkrankheit litt oder im hohen Alter verstarb. Bei Theo kamen unterschiedliche Methoden zum Einsatz, da die Sterbealtersschätzungen in der Regel zuverlässiger ausfallen, je zahlreicher altersaffine Merkmale am Skelett berücksichtigt werden. So führte Ursula Wittwer-Backofen eine sogenannte zahnzementchronologische Analyse an einer Zahnwurzel Theos durch.[26] Jährlich lagern sich eine helle (im Sommer) und eine dunkle (im Winter) Zementschicht an den Wurzeln an. Die in Kunstharz eingegossene Zahnwurzel wird mit einem diamantbesetzten Sägeblatt in sehr dünne Scheiben, histologische Schnitte, zerteilt. Anschliessend werden unter dem Durchlicht-Mikroskop an den histologischen Schnitten in zeitkonsumierender Arbeit die einzelnen Zementschichten ausgezählt.

[26] Wittwer-Backofen et al. 2002: 119-129.

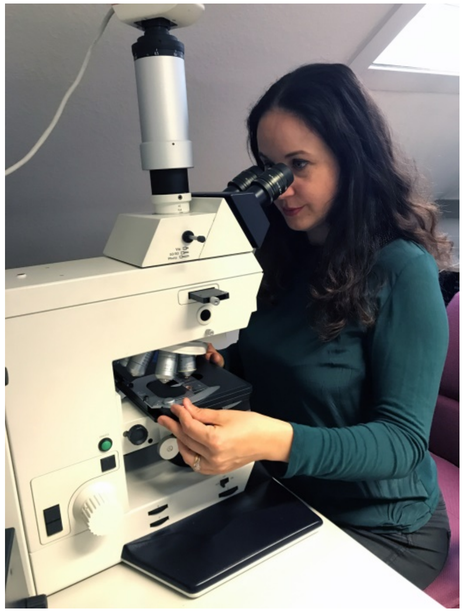


Abb. 8: Gabriela Mani-Caplazi am Durchlicht-Mikroskop beim Analysieren von Stressanomalien an Zahnwurzeln (Foto: Petra Urban, Freiburg i. Br.).

Diese Methode gilt als zuverlässig, aber leider kommt es immer wieder zu hohen Abweichungen, die wissenschaftlich noch nicht erklärt werden können. Als weiterer interessanter Punkt erlaubt diese Methode durchlebte Stressphasen aufgrund dicker ausgeprägter und unterschiedlich mineralisierter Zementschichten festzustellen. Solche Stressanomalien können aufgrund von schweren Krankheiten, langanhaltenden Hungerkrisen[27] und der physiologischen Belastung durch Schwangerschaften gebildet werden (siehe Fussnote 1). Nach der zementchronologischen Analyse (TCA) starb Theo zwischen dem 28. und 33. Lebensjahr, und als 16-Jähriger durchlebte er eine massive Stressphase (siehe Abb. 9 linke Seite, weisse Pfeile).[28] Ein ähnliches Ergebnis resultierte aus einem dünnen Schnitt durch Theos Oberschenkelknochen. Unter dem Durchlicht-Mikroskop konnte Stefanie Doppler sogenannte Haltelinien (Wachstumsstopplinien) feststellen. Es handelt sich dabei um mineraldichte weisse Linien im Knochen, die auf langandauernde Hungersnöte, Stress oder Krankheiten verweisen können. Bei Theo konnte die Wissenschaftlerin zwei solche Linien feststellen, die beide zwischen dem 17. und 21. Lebensjahr entstanden waren (siehe Abb. 9 rechte Seite, weisse Pfeile). Ob die Zahnzementanomalie mit 16 Jahren und die erste Wachstumsstopplinie im 17. Lebensjahr aus ein und demselben Stressereignis in Theos Leben resultierten, ist Thema neuer Forschungen.

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[27] Zur grossen Hungerskrise von 1816/17 siehe Krämer 2015.

[28] Wittwer-Backofen 2010: 43.

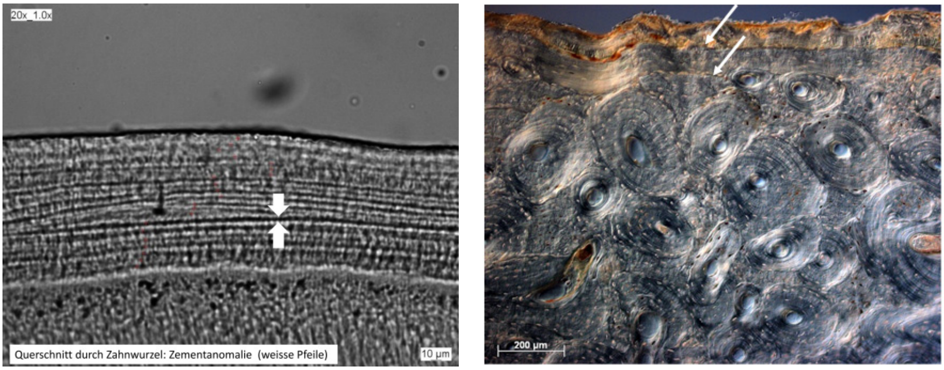

Abb. 9: Foto links: Zwei weisse Pfeile markieren eine dick ausgeprägte Zementschicht, eine sogenannte Stressanomalie. Sie zeigt, dass Theo im 16. Lebensalter eine massive Stressphase durchlebte (Foto: Ursula Wittwer-Backofen, Freiburg i.Br.). Foto rechts: Mikroskopische Untersuchungen am Oberschenkelknochen beweisen, dass Theo zwischen dem 17. und 21. Lebensalter zwei weitere Stressphasen (weisse Pfeile) durchstand (Foto: Stefanie Doppler, München).

Dieses zahnzementchronologisch ermittelte Sterbealter wurde auch durch andere altersaffine Merkmale am Skelett bestätigt. Alfred und Karin Schweizer überprüften bei allen Männern, die zwischen dem 21. und 49. Lebensjahr verstarben, die Geburtsdaten und damit die im Beerdigungsregister angegebenen Altersangaben. Dieses Vorgehen wird als Geburtsverifizierung bezeichnet und ist bei demografischen Arbeiten ein übliches Vorgehen. Sie konnten Altersabweichungen bis zu zehn Jahren feststellen. Beruhend auf dieser geburtsverifizierten Liste wurden alle Männer jünger als 26 und älter als 34 Jahre aus dem Pool ausgeschlossen. So reduzierte sich der Personenkreis von 2`069 auf noch 134 verbleibende Kandidaten.[29] Eine erfreuliche Reduktion, aber immer noch eine zu grosse Anzahl möglicher Kandidaten. Vier im Sterberegister verzeichnete namenlose Wasserleichen konnten bei diesem Prozedere nicht berücksichtigt werden, da zu diesen im Rhein ertrunkenen Männern keine Altersangaben vorlagen. Es wäre schon ein Riesenpech, wenn der Pfeifenraucher ausgerechnet zur Gruppe der ertrunkenen und anonymen Personen gehören sollte – dann wäre eine Spurensuche aussichtslos. Die Chancen stehen bei immerhin 4 zu 2`069. Beruhigend zu wissen, dass Theos Skelett keinerlei Anzeichen von für an Wasserleichen typischen Schleifspuren am Schädel aufweist. Wasserleichen, die über Tage hinweg von der Strömung getrieben im Niederwasser über den kiesigen Flussgrund schleifen, können an Stirn oder Hinterhaupt entsprechende typische Abschliffspuren aufweisen.

[29] Hotz 2010: 78-82.

Abb. 10: Schädel einer bei Basel Ende des 19. Jahrhunderts aus dem Rhein gezogenen Wasserleiche. Der Rheinschotter hatte durch das über den Untergrund Schleifen ein grosses Loch am Hinterhaupt verursacht. Solche Löcher sind typisch für Wasserleichen (NMB-2649, Foto: Gerhard Hotz, Basel).

Bei weiteren sechzehn Männern fanden sich im Steinbuch Hinweise für ein Grab in der Theodorskirche – hier handelte es sich um Männer aus der sozialen Oberschicht. Nach all diesen Ausschlussschritten verblieben immer noch 118 mögliche Kandidaten zum Theo-Personenkreis. Seitens der historischen Quellen hatte sich damit das Reduktionspotential erschöpft. Es konnten in den schriftlichen Quellen keine weiteren Informationen gefunden werden, die einen zusätzlichen Ausschluss erlaubt hätten. Für eine weitere Reduktion musste nun mit archäologischen und naturwissenschaftlichen Methoden gearbeitet werden. So zeigte sich im Armenfriedhof ein auffälliges Phänomen. Die bis anhin Nordwest-Südost-orientierten Gräber erfuhren ab einem uns unbekannten Zeitpunkt eine Umorientierung um 90 Grad im Gegenuhrzeigersinn und zusätzlich wurden die Grabgruben tiefer in der Erde angelegt.

Abb. 11: Lage der 1984 im ehemaligen Armenfriedhof aufgedeckten Gräber in der Leitungstrasse (graue Fläche) des Quartierwärmeverbundes. Theos Skelett ist rot hervorgehoben. Die Skelette der älteren Phase sind grün, die der jüngeren Phase blau markiert. Theo gehörte zur blauen und damit jüngeren Gräbergruppe. Zeichnung: Christian Stegmüller. Massstab 1:250.

Offensichtlich musste es einen Grund geben, der zu einer kompletten Neuorientierung der Gräber führte. Der Archäologe Guido Helmig stellte diese Neuorganisation im Armenfriedhof in Zusammenhang mit der grossen Typhusepidemie von 1814 (siehe auch Abb. 2, Grabfoto von Theo).[30] Nach 1814 mussten in Basel Gräber tiefer angelegt werden und es gab ein allgemeines Verbot,

[30] Helmig et al. 2010: 34.

in den Kirchen zu bestatten (welches immer wieder umgangen wurde). Damit wurde die jüngere Gräbergruppe (blaue Skelette, siehe Abb. 11), zu der Theo gehörte, nach 1814 datiert. Damit konnten alle Personen, die vor 1814 verstorben waren, ausgeschlossen werden und der Kandidatenpool reduzierte sich auf noch 25 Männer, die alle im Alter von 26 bis 34 Jahren verstarben.

Abb. 12: ‹Das schreckliche Nervenfieber› 1814 herrschte in Basel eine durch das Militär der Alliierten verursachte Typhusepidemie. Zahlreiche Basler Bürger und Bürgerinnen erlagen der Seuche. Dies führte zu einer Neuordnung des Bestattungsreglements (Tuschezeichnung von Jeremias Burckhardt, Privatbesitz, vgl. Eugen A. Meier: Aus dem alten Basel. Basel 1970).

Eine weitere hervorragende Reduktionsmöglichkeit bietet die Strontiumisotopen-Analyse. Mit dieser Methode lassen sich mit einer gewissen Wahrscheinlichkeit die geografische Herkunft von Menschen bestimmen, und damit auch Einwanderungsbewegungen nachvollziehen. Über unsere Ernährung gelangen die stabilen Sr^{87}- und Sr^{86}-Isotope in unseren Stoffwechsel und werden in Knochen und Zähnen angereichert. Das relative Verhältnis Sr^{87} zu Sr^{86} ist ortsspezifisch und wird durch den örtlich vorliegenden Untergrund vorgegeben. Eine Schwachstelle der Methode liegt in der Tatsache, dass gleicher geologischer Untergrund zu identischen Isotopensignaturen Sr^{87}/Sr^{86}–Verhältnissen führt. Alistair Pike konnte bei Theo anhand der ersten drei Backenzähne nachweisen, dass Theo in der Region Basel geboren, aufgewachsen und bis mindestens zum 14. Lebensalter in der Region gelebt hatte.[31] Bis zu diesem Lebensalter archivieren die Zähne im Schmelz die Isotopensignatur, danach ist die Ausbildung der Zahnkrone abgeschlossen und der Informationsträger versiegelt. Theo war also ein Basler.

Für uns war dieses Ergebnis eine grosse Enttäuschung. Wäre Theo ein Fremder gewesen, hätten wir seine Identität aufgrund der Herkunftsangaben im Beerdigungsregister mit grosser Wahrscheinlichkeit feststellen können. Einziger Vorteil an dieser Sachlage: Wenn Theo ein Basler war, liessen sich mit grösserer Wahrscheinlichkeit historische Quellen wie Erbschaftsinventare[32] oder Polizeirapporte zu seiner Person finden. Auch dürfte es einfacher sein, noch lebende Nachfahren in der Region Basel zu finden, als wenn sie über die ganze Welt verstreut leben.

Einen weiteren Ansatz bot eine neue von Geneviève Perréard entwickelte Methode. Sie untersuchte mittels computertomografischen Analysen die Asymmetrie und Robustizität von Theos Armknochen und verglich Theos Daten mit den Daten einer identifizierten Skelettserie[33] aus Genf, bei welchen die beruflichen Tätigkeiten bekannt waren. Die Analysen schliessen für Theo einen Beruf mit schwerster körperlicher Belastung, wie z. B. einem Steinhauer, aus und verweisen ihn in Richtung Berufe mit geringer körperlicher Belastung und einer eher feinmotorischen Tätigkeit.[34] Im Sommer 2016 untersuchte Fotios Alexandros Karakostis mit einer neu entwickelten Methode die Handknochen von Theo. Mittels 3D-Lichtscan des Handskelettes und aufgrund von Vergleichsdaten der identifizierten Skelettserie Basel-Spitalfriedhof[35] liess sich das von Geneviève Perréard 2010 erarbeitete Ergebnis bestätigen und sogar verfeinern. Nach Fotios Alexandros Karakostis Analysen ist Theos berufliche Tätigkeit im Umfeld feinmotorischer Berufe, wie z. B. wie Bäcker, Schneider, Seidenfärber oder Seiler zu suchen.

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[31] Hotz und Pike 2010: 65.

[32] In Erbschaftsinventaren hofften wir Angaben zu einem möglichen Tabakkonsum/-vorrat zu finden. Tabak hatte als Konsumgut seinen Preis. Bei einem passionierten Raucher wie Theo, konnte bei seinem Ableben unter Umständen ein allfälliger Tabakvorrat durchaus in seinem Erbschaftsinventar eine Erwähnung gefunden haben. Leider fand sich in keinem der recherchierten Inventarien eine solche Angabe. Weiterführende Information zu dieser wenig bekannten Quelle finden sich in: Zulauf-Semmler et al. 2016: 171-181; Zulauf-Semmler et al. 2017: 65-70.

[33] Série de squelettes identifiés vaudois (collection SIMON), Université de Genève.

[34] Perréard Lopreno 2010: 57.

[35] Karakostis et al. 2017: im Druck.

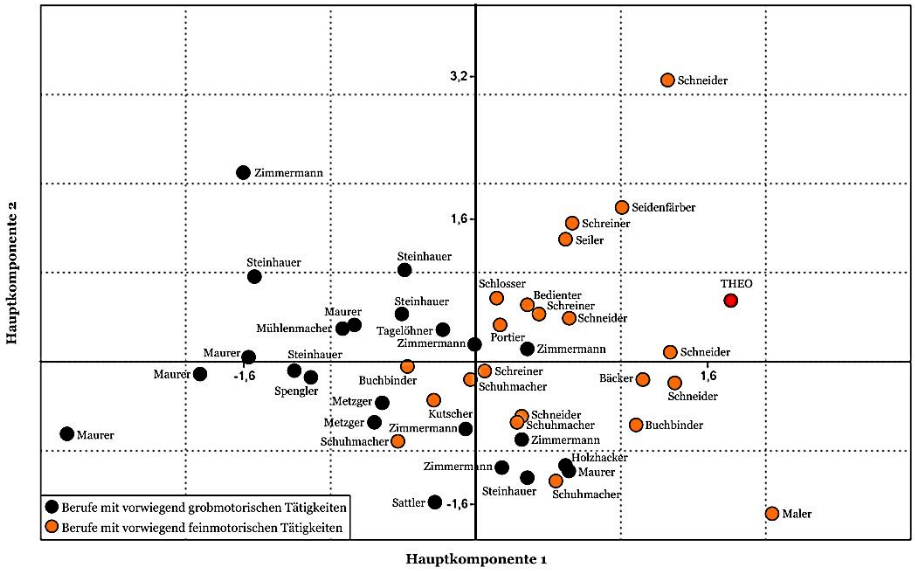

Abb. 13: Theos (roter Punkt, nahe des rechten Bildrandes) berufliche Tätigkeit dürfte eher bei feinmotorisch tätigen Handwerkern (orange Punkte) wie Bäcker, Schneider, Seidenfärber oder Seiler zu suchen sein. Ergebnis der 3D-Lichtscans von Fotios Alexandros Karakostis (Grafik: Fotios Alexandros Karakostis, Tübingen).

DNA und Speichelabgleich

Diese und weitere hier aus Platzgründen nicht angeführte Analysen wurden in der Wahrscheinlichkeits-Datenbank von Alfred und Karin Schweizer berücksichtigt. Personen, die den beschriebenen beruflichen Tätigkeiten oder anderen Kriterien entsprachen, erhielten im Theo-Profil eine leicht erhöhte Wahrscheinlichkeit. Auf diese Weise konnte der Personenkreis um Theo auf 12 verbleibende Topkandidaten reduziert werden. Diese zwölf Topkandidaten beinhalten mit einer Wahrscheinlichkeit von 96% den gesuchten Theo.

Damit war endlich die Grundlage erarbeitet, die den finalen Schritt erlaubte: Ausgehend von Theos alter DNA und mittels eines DNA-Vergleichs von Theo-Nachfahren, liesse sich überhaupt die definitive Beweisführung antreten, den Gesuchten gefunden zu haben.

Zu diesem Zweck musste aber die alte DNA aus Theos Skelett extrahiert werden. Dies setzt eine sehr empfindliche und aufwändige Analytik voraus. Ebenso benötigt man noch intaktes Knochenmaterial, welches die DNA der verstorbenen Person konservieren konnte. Kurt Alt und seinem Team gelang es, aus der Zahnhöhle eines Backenzahns die mitochondriale DNA (mtDNA) zu

ID-Nr.	Vorname	Name	Herkunft	Alter	Geburts-jahr	berufliche Tätigkeiten
1	Christian Friedrich	Bender	Bouxwiller (F)	33	1784	Glasermeister
2	Achilles	Itin	Buckten (BL)	31	1786	Vater: Stadtsoldat
3	Peter	Kestenholz	Lupsingen (BL)	29	1789	Pfannenflicker
4	Johann Jacob	Gessler	Basel	32	1782	Weissgerber
5	Johann	Merian	Hölstein (BL)	30	1784	Vater: Seiler
6	Niklaus	Lang	Basel	28	1794	Handelscommis
7	Johann Jakob	Schmid	Müllheim (TG)	33	1782	Mühlenmacher
8	Valentin	Kunz	Todtnau (D)	33	1789	Seifensieder
9	Franz Georg	Perrot	Biel (BE)	26	1793	Handelscommiss
10	Friedrich	Wohnlich	Basel	31	1783	Weissbäcker
11	Jakob	Hediger	Rothenfluh (BL)	27	1789	Fabrikarbeiter
12	Johann	Bieler	Basel	32	1781	Vater: Metzger

Tab. 1: Kandidaten-Liste zu Theo dem Pfeifenraucher. Diese 12 Namen beinhalten mit einer Wahrscheinlichkeit von 96% die zutreffende Identität zu unserem gesuchten Pfeifenraucher (modifiziert nach Hotz 2010, Seite 82).

isolieren.[36] Dieses hervorragende Ergebnis hatte eine Schattenseite: die mtDNA wird von der Mutter auf ihre Kinder vererbt, aber nur ihre Töchter geben die Erbinformation an die nächste Generation weiter. Das bedeutete für das Genealogenteam, dass lebende Nachfahren über acht Generationen über die mütterliche Linie gesucht werden müssen. Eine anspruchsvolle genealogische Spurensuche, die seitens der Genealoginnen und Genealogen Marina Zulauf, Diana Gysin, Ursula Fink und Beat Stadler gemeistert wurde. Ein rechercheintensiver Weg war zu beschreiten, da bei jeder neuen Generation die Frauen den Namen ihres Ehemanns annahmen und genealogische Register in der Regel nach der männlichen Linie orientiert sind. Hier blieb nur eine breitgefächerte und detailreiche Personensuche übrig. Die Spurensuche führte unter anderem bis nach Argentinien.[37] Bei acht Kandidaten starben die Linien auf der mütterlichen Seite aus oder es konnten keine Nachfahren gefunden werden, da die historischen Quellenlagen in Teilen der Schweiz, Deutschlands oder Frankreichs, um nur einige zu nennen, nicht immer optimal waren. Bei einem potentiellen Kandidaten verstarb der letzte Nachfahre 1985 und sein Grab wurde 2008 auf dem Hörnli in Basel aufgehoben – diese Spur konnte nicht mehr weiterverfolgt werden. In monatelangen Recherchen konnten zum potentiellen Kandidaten Johann Bieler, dem oben erwähnten Metzger, Nachfahren gefunden werden. Die Nachfahren gaben ihre Speichelprobe im Rahmen des Forschungsprojekts ab – und leider ergab sich zu unserem grossen Bedauern ein eindeutig negatives Ergebnis. Aber durch diesen negativen Ausschluss

[36] Roth und Alt 2010: 66-68.
[37] Zulauf et al. 2010: 83-86.

konnte der Kreis der potentiellen Kandidaten von zwölf auf noch elf verbleibende Kandidaten reduziert werden und Theo bleibt noch immer ohne Namen.

Wie geht es weiter?

Wissenschaftliche Methoden verbessern sich fortlaufend und neue Methoden ermöglichen neue Fragestellungen. 2010 konnte ein Teil der mitochondrialen DNA isoliert werden. Sieben Jahre später erlaubt die fortgeschrittene Technologie der Molekulargenetik mittels des sogenannten „Next Generation Sequencing" das gesamte Genom eines Menschen zu entschlüsseln. Den Berliner Forensikern Lutz Roewer und Jessica Rothe ist es nun gelungen, einen Teil der nukleären DNA von Theo aus je einer Probe aus dem Oberschenkelknochen und dem Wadenbein zu isolieren. Dazu wurde die Oberfläche des Knochens abgeschliffen, um mögliche Kontaminationen durch zuvor stattgefundene Berührungen von Wissenschaftlern auszuschliessen. Dann wurden die Knochen in ungefähr 0.5 mm kleine Würfelchen geschnitten und anschliessend zusätzlich mit Ethanol gesäubert.

Die Knochenstückchen wurden unter Verwendung von flüssigem Stickstoff in einer Schwingmühle zu Mehl pulverisiert. Dieses Mehl wurde schliesslich für die DNA-Extraktion verwendet.[38] Mit Hilfe einer Multiplex PCR Analyse[39] wurden die verschiedenen autosomalen und Y-chromosomalen „Short Tandem Repeats" (STRs)[40] typisiert. Um eine Kontamination mit Fremd-DNA auszuschliessen, wurden insgesamt fünf Extraktionen der verschiedenen Knochenstückchen durchgeführt um jedes Mal dasselbe Ergebnis zu erhalten. Läge eine Verunreinigung durch Fremd-DNA vor, würde man eine solche Übereinstimmung nicht erhalten. Neben den autosomalen STR Markern[41] konnten insge-

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[38] Rothe et al. 2015: 90–97; Haas et al. 2013: 610-617.

[39] Die PCR (Polymerase-Ketten-Reaktion) ist ein Verfahren zur Vervielfältigung eines ausgewählten DNA-Abschnittes. Die Sequenz des vervielfältigten DNA-Abschnitts kann anschließend durch weitere Verfahren analysiert werden. In einer Multiplex PCR werden Einzel-PCRs miteinander kombiniert, so dass gleichzeitig mehrere DNA Abschnitte vervielfältigt werden können. Vervielfältigung ist insofern wichtig, weil eine genügend grosse Zahl Kopien für den Nachweis erforderlich sind.

[40] STR Marker (short tandem repeats) sind Wiederholungen kurzer Sequenzmotive (meist 3 oder 4 Basenpaare lang), welche in nicht codierenden Bereichen des Erbguts auftreten. Die Anzahl der Wiederholungen ist sehr variabel. Personen können sich somit in der Anzahl der Wiederholungen der STR Marker unterscheiden, was zu einer Variabilität zwischen verschiedenen Personen führt. Da das Y-Chromosom unverändert (mit Ausnahme von Mutationen) an die männlichen Nachkommen vererbt wird, definieren Y-STR Marker die männliche Linie.

[41] Autosomale STRs sind Marker die sich auf den autosomalen Chromosomen des Erbguts befinden. Autosomen gehören nicht zu den Geschlechts-Chromosomen. Sie treten paarweise auf und können sich im Gegensatz zum Y-Chromosom rekombinieren. Das menschliche Genom besteht

samt 27 Y-STRs bei Theo analysiert werden. Damit stand ein Set väterlicherseits vererbter DNA-Marker des Y-Chromosoms, also die väterliche Linie von Theo, für die weiteren genealogischen Forschungen zur Verfügung.


Abb. 14: Foto links: Jessica Rothe arbeitet im DNA-Labor mit Schutzkleidung, damit keine „Fremd-DNA“ die zu untersuchenden Proben verunreinigen (Foto: IRM Berlin) Foto rechts: Aus einem Knochen wird eine Probe herausgesägt (Foto: IRM Berlin).

Aufgrund der elf verbleibenden Theo-Kandidaten recherchieren nun die Genealoginnen und Genealogen auf der genealogisch leichter zu erforschenden männlichen Linie nach lebenden Nachfahren. Einen Nachteil beinhalten die Forschungen über die männliche Linie. Bei den Recherchen über die weibliche Linie bleibt die Mutterschaft immer zweifelsfrei nachweisbar. Bei Recherchen über die männliche Linie hingegen müssen wir mit sogenannten „Kuckuckskindern“ rechnen. In jeder Gesellschaft tritt ein bestimmter Anteil von illegitimen Kindern auf. Selten werden illegitime Kinder in den historischen Quellen namentlich so benannt. Damit bleibt bei einem negativen Speichelabgleich ein Restzweifel bestehen. Wir können nicht mit letzter Sicherheit wissen, ob nicht ein illegitimes Kind zum Abbruch der männlichen Linie geführt haben könnte und damit das negative Ergebnis bewirkt hatte. Auch Mutationen, die im Verlauf der acht Generationen zwischen Theos Tod und Heute geschehen konnten, könnten eine Identifizierung erschweren.

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aus 22 autosomalen Chromosomenpaaren und den zwei Geschlechtschromosomen X, X oder X, Y. Die autosomalen STR Marker bilden den „genetischen Fingerabdruck“, welcher eine Person eindeutig identifizieren kann.

Erste Ergebnisse der genealogischen Recherchen

Das Genealoginnen-Team hat nach intensiven Recherchen bereits zu unserem ersten Topkandidaten Peter Kestenholz noch lebende Nachfahren gefunden. In Absprache mit dem Basler kantonalen Datenschutzbeauftragten Beat Rudin nahmen wir mit dem Nachfahren Walter Kestenholz persönlich Kontakt auf. Walter Kestenholz ist ein Urururgrossneffe von Peter Kestenholz. Zwischen den beiden liegen fünf Generationen. Das Interesse seitens Walter Kestenholz war gross und er gab gerne seine Einwilligung zu einem DNA-Abgleich mittels Speichelprobe. Bei einem solchen Speichelabgleich werden strikt nur die erwähnten 27 Marker untersucht. Restproben werden nach Ablauf des Forschungsprojekts ebenfalls vernichtet. Die genetischen Analysen der Speichelprobe wurden am Institut für Rechtsmedizin der Universität Basel durch Daniel Dion durchgeführt, der anschliessend die Ergebnisse nach Berlin weiterleitete.[42]

Die Analyse des in väterlicher Linie vererbten Y-Chromosoms aus der Speichelprobe von Walter Kestenholz ergab, dass an den 27 Markern insgesamt 17 Mutationen auftreten, welche die beiden Personen deutlich unterscheiden. Wenn man die Y-Haplotypen[43] von Theo (Vorfahr) und Walter Kestenholz (Nachfahr) in das YHRD Programm „Kinship“
(siehe https://yhrd.org/kinship/check) eingibt und die Wahrscheinlichkeit für/gegen patrilineare Verwandtschaft ausrechnen lässt, kommt man auf Zahlen die eindeutig zeigen, dass der letzte gemeinsame Vorfahr von Theo und Walter Kestenholz nicht innerhalb der letzten 100 Generationen gelebt haben kann.

Walter Kestenholz ist also kein Nachfahre von Theo dem Pfeifenraucher und damit kann Peter Kestenholz von der Kandidaten-Liste gestrichen werden.

Die Suche geht weiter – nun wird der nächste Kandidat Achilles Itin auf noch lebende Nachfahren recherchiert.[44]

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[42] Wir möchten an dieser Stelle dem Institut für Rechtsmedizin Basel einen grossen Dank für die tolle Kooperation aussprechen, insbesondere Eva Scheurer, Daniel Dion und Sarah Kron.

[43] Die Haplogruppe definiert die Position des untersuchten Y-Chromosoms im menschlichen Stammbaum. Aufgrund der Korrelation von Mutationsereignissen und der zeitlichen Abfolge der Ausbreitung des Menschen über alle Kontinente besitzt jede Haplogruppe eine begrenzte geographische Verbreitung mit einem Zentrum und einem Verbreitungsfeld, in dem die Häufigkeit mit Abstand vom Zentrum graduell abnimmt.

[44] Wir danken Hans-Rudolf Schulz und Fritz Häsler seitens des Bürgerforschungsprojekts Basel-Spitalfriedhof (BBS), und Daniela Brändlin seitens des Naturhistorischen Museums für ihr sorgfältiges Korrekturlesen.

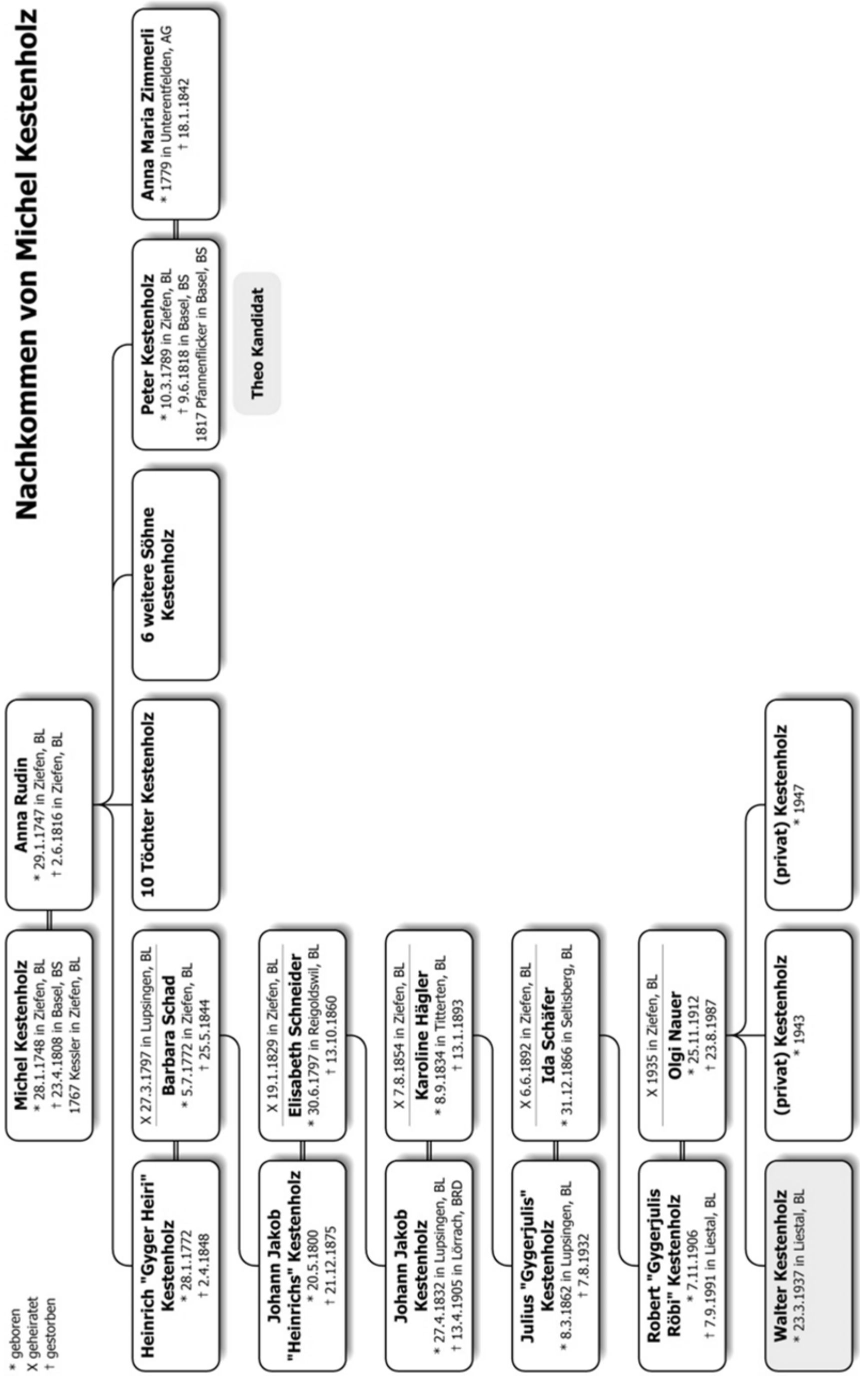

Abb. 14: Stammbaum von Peter Kestenholz, dessen Ururgrossneffe 1937 in Basel geboren wurde (Stammbaum: Jürgen Rauber, Rheinfelden 2017).

Schlussbemerkung

Unser Beitrag hat sich drei Ziele vorgenommen. Einerseits will er das Projekt „Theo der Pfeifenraucher" einem grösseren genealogisch interessierten Publikum nahe bringen. Andererseits die Leserschaft über die zunehmende Bedeutung genealogischer (und mikrohistorischer) Recherchen für die anthropologisch-naturwissenschaftlich orientierten Forschungen informieren. Zahlreiche Grundlagen wurden seitens der Genealogie für die naturwissenschaftlich-anthropologische Analytik erarbeitet.[45] Ebenso möchten wir der Leserschaft die Arbeitsweisen der Naturwissenschaften mit einigen konkreten Beispielen näher bringen. Hervorheben wollen wir, dass genealogische Recherchen seitens der Geschichtsforschung zunehmend Beachtung finden. Diese Tendenz ist bei den universitären Standorten zur Wirtschafts- und Sozialgeschichte in Deutschland[46] stärker wahrnehmbar ist als hier in der Schweiz. Trotzdem kann man auch seitens der Schweizer Geschichtsforschung ein deutlich zunehmendes Interesse an genealogischen Fragestellungen feststellen.

Unser Beitrag richtet sich an Genealoginnen und Genealogen, im spezielle an die Mitglieder der Schweizerischen Gesellschaft für Familienforschung (SGFF), mit einem Aufruf zum Personenkreis der Theo-Kandidaten. Sollten Sie zu den in der Tabelle 1 aufgelisteten Kandidaten über genealogische Informationen verfügen, würden wir uns über eine Kontaktaufnahme sehr freuen. Zudem wendet sich unser Beitrag an alle Interessierten, sich aktiv bei den Projekten des Bürgerforschungsprojekts (BBS, siehe www.ipna.unibas.ch/bbs) oder bei den Recherchen zu Theo dem Pfeifenraucher zu beteiligen. Sei dies bei der Unterstützung von Transkriptionen, Datenerschliessungen, Datenbereinigung oder genealogischen Recherchen – Unterstützungen sind herzlich willkommen. Interessierte mögen sich mit den Projektleitern Dr. Gerhard Hotz (gerhard.hotz@bs.ch) oder Marina Zulauf (Marina.Zulauf@unibas.ch) in Verbindung setzen.

Autorinnen und Autoren

Gerhard Hotz ist Kurator am Naturhistorischen Museum und Lehrbeauftragter für Archäo-Anthropologie am IPNA an der Universität Basel. Er leitet zusammen mit Marina Zulauf-Semmler das Bürgerforschungsprojekt Basel-

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[45] Siehe zum Beispiel die laufenden Dissertationen von Gabriela Mani-Caplazi (IPNA, Universität Basel und Universität Freiburg i.Br.) und die Dissertation von Fotios Alexandros Karakostis (Universität Tübingen und IPNA, Universität Basel).

[46] Als Beispiel sei die Martin-Luther-Universität in Halle erwähnt: Lehrstuhl für Wirtschafts- und Sozialgeschichte am Institut für Geschichte.

Spitalfriedhof (BBS). Marina Zulauf-Semmler ist freiwillige wissenschaftliche Mitarbeiterin am IPNA und leitet die genealogischen Recherchen. Marie-Louise Gamma, Diana Gysin, Odette Haas, Ludwig Huber und Marina Zulauf-Semmler sind GenealogInnen des Bürgerforschungsprojekts und verantworten die zeitaufwändigen Recherchen zu Theo. Jürgen Rauber und Albert Spycher sind ebenfalls freiwillige Mitarbeiter des Bürgerforschungsprojekts. Jürgen Rauber ist für die Visualisierung der genealogischen Ergebnisse verantwortlich. Albert Spycher forscht im komplexen Bereich des Zunftwesens und der beruflichen Tätigkeiten in historischen Zeiten. Liselotte Meyer ist freiwillige Mitarbeiterin des Naturhistorischen Museums und von Beginn an bei den Theo-Forschungen involviert. Guido Helmig ist Archäologe im Ruhestand und war seitens der Archäologischen Bodenforschung Basel-Stadt für die archäologischen Fragestellungen zu Theo zuständig. Simon Kramis ist Anthropologe und Doktorand an der Universität Basel (IPNA) und forscht zu römischen Skelettdeponierungen aus Augsta Raurica (Augst, BL). Fotios Alexandros Karakostis ist Anthropologe und Doktorand an der Universität Tübingen und forscht zu biomechanischen Einflüssen beruflicher Tätigkeiten auf das menschliche Handskelett. Geneviève Perréard Lopreno ist externe Mitarbeiterin und Anthropologin am Laboratoire d'Archéologie préhistorique et Anthropologie der Universität Genf und forscht im Bereich von Enthesiopathien und biokulturellen Einflüssen am menschlichen Skelett. Stefanie Doppler ist Biologin, aktuell in der medizinischen Forschung tätig und arbeitet in der Klinik für Herz- und Gefäßchirurgie des Deutschen Herzzentrums in München. Lutz Roewer ist Leiter der Molekulargenetik am Institut für Rechtsmedizin und Forensische Wissenschaften der Universitätsmedizin Berlin und wurde von Jessica Rothe, wissenschaftliche Mitarbeiterin, bei den molekulargenetischen Analysen zu Theo unterstützt. Lutz Roewer war auch für die DNA-Analysen zu Jörg Jenatsch aus dem Kanton Graubünden verantwortlich.[47] Ursula Wittwer-Backofen ist Professorin für Anthropologie an der Universität Freiburg im Breisgau und führte die zahnzementchronologischen Analysen an Theos Gebiss durch.

Weitere Mitarbeiterinnen und Mitarbeiter

In diesem Beitrag konnten nicht alle Forschenden namentlich erwähnt oder als Autorinnen und Autoren aufgeführt werden. Ebenso konnten wir nicht alle beteiligten Institutionen erwähnen. Wir möchten allen beteiligten Personen und Institutionen des Forschungsprojekts „Theo der Pfeifenraucher" einen grossen Dank aussprechen. Insbesondere möchten wir zwei Freiwilligen Mitar-

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[47] Siehe Janosa 2014: 1-209.

beitern der ersten Stunde gedenken: Dies sind Joseph (Sepp) Uebelhart (13.11.1927 – 19.10.2015) und Paul Meier (28.3.1926 – 20.7.2017). Beide lebten und forschten in Basel und gehörten zum ersten Viererteam, welches Quellen zu Theo transkribierte. Ebenso möchten wir allen freiwilligen Mitarbeitenden des Bürgerforschungsprojekts Basel-Spitalfriedhof einen grossen Dank für die Unterstützungen aussprechen. Dies sind namentlich: Margaretha Avis (Therwil), Yvonne Bächle (Basel), Heiner Bangerter (Basel), Erich Bär (Meggen), Werner Betz (Basel), Marian Bielser (Pratteln), Susan und Roger Blatter (Bottmingen), Noemi Bönzli (Basel), Heidi Bösch (Basel), Claudia Briellmann (Basel), Thomas Briellmann (Basel), Maritta Bromundt (St. Gallen), Kathrin Decrue (Muttenz), Katharina Matt Eder (Basel), Verena Fiebig-Ebneter (Frenkendorf), Ursula Fink (Basel), Hans Peter Frey (Basel), Angelo Gianola (Basel), Verena Grunauer (Riehen), Fritz Häsler (Birsfelden), Ingrid Hefti (Allschwil), Dascha und Michael Herber (Basel), Ursula Hirter (Basel), Annemarie Hitz † (Basel), Bernd Holtze † (Weil am Rhein DE), Rolf Hopf (Basel), Vesna Horvat (Basel), Hanns Walter Huppenbauer (Affoltern am Albis), Lara Indra (Basel), Hiroko Känel (Rüfenacht BE), Michaela Klaus (Basel), Karim Kleb (Umiken AG), Christine Küpfer (Basel), Rosemarie Kuhn (Basel), Marie Kumpf (Köln), Fabienne Klumpp (Basel), Victor Meier (Reinach), Franziska Meili (Regensdorf), Christoph Meissburger (Basel), Véronique Muller (Strasbourg), Jörg Müller (Bern), Urs Müller (Basel), Laura Muser (Basel), Lolita Nikolova (Salt Lake City, USA), Jessica Pabst (Allschwil), Cécile Rollé (Basel), Heinz Ruegg (Maisprach), Gudrun Rubli (Murten), Felicitas Ruch (Maisprach), David Roth (Basel), Sabine Reimund (Konstanz), Semira Ryser (Basel), Bruno Santschi (Arlesheim), Yvonne und Lukas Schaub (Oltingen), Susi Schläpfer (Binningen), Christina Schmidt (Freiburg i.Br.), Hans-Rudolf Schulz (Riehen), Beatrice Schumacher (Basel), Ursula Siegrist (Jegenstorf), Christel Sitzler (Riehen), Beat Stadler (Basel), Anita Stocker (Rheinsulz), Christian Thommen (Basel), Verena Thöni (Bern), Edgar Uebelhart (Basel), Helena Vogler (Bösingen), Anja Walther (Basel), Gisela Weiche † (Bern), Susanne Weyermann (Dornach), Holger Wittig (Basel) und Daniel Zulauf (Pratteln).

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Dr. Gerhard Hotz ist Kurator für Anthropologie am Naturhistorischen Museum Basel und Lehrbeauftragter für Archäo-Anthropologie an der Universität Basel. Er ist der Projektleiter der Forschungen zum Spitalfriedhof Basel, in welches auch das gleichnamige Bürgerforschungsprojekt eingeschlossen ist. Das Forschungsprojekt ist am Institut für Prähistorische und Naturwissenschaftliche Archäologie (IPNA) verortet.

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A repeatable geometric morphometric approach to the analysis of hand entheseal three-dimensional form

Fotios Alexandros Karakostis[1], Gerhard Hotz[2], Heike Scherf[1], Joachim Wahl[1,3] & Katerina Harvati[1,4]

[1] Paleoanthropology, Senckenberg Centre for Human Evolution and Paleoenvironment, University of Tübingen, 72070 Tübingen, Germany.

[2] Natural History Museum of Basel, 4051 Basel, Switzerland.

[3] Osteology, State Office for Cultural Heritage Management Baden-Württemberg, 78467 Konstanz, Germany.

[4] DFG Centre for Advanced Studies 'Words, Bones, Genes, Tools: Tracking linguistic, cultural and biological trajectories of the human past'.

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Correspondence to:

Fotios Alexandros Karakostis

Eberhard Karls University at Tübingen,

Senckenberg Center for Human Evolution and Paleoecology,

Rumelinstrasse 23, Tübingen 72070, Germany.

Telephone number: +4970712976516

E-mail address: afkarakostis@hotmail.com

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ABSTRACT

Objectives: The purpose of this study was to put forth a precise landmark-based technique for reconstructing the three-dimensional shape of human entheseal surfaces, in order to investigate whether the shape of human entheses is related to their size. The effects of age-at-death and bone length on entheseal shapes were also assessed.

Materials and methods: The sample comprised high-definition three-dimensional models of three right hand entheseal surfaces, which correspond to 45 male adult individuals of known age. For each enthesis, a particular landmark configuration was introduced, whose precision was tested both within and between observers. The effect of three-dimensional size, age-at-death, and bone length on shape was investigated through shape regression.

Results: The method presented high intra-observer and inter-observer repeatability. All entheses showed significant allometry, with the area of *opponens pollicis* demonstrating the most substantial relationship. This was particularly due to variation related to its proximal elongated ridge. The effect of age-at-death and bone length on entheses was limited.

Discussion: The introduced methodology can set a reliable basis for further research on the factors affecting entheseal shape. Using both size and shape variables can provide further information on entheseal variation and its biomechanics implications. The low entheseal variation by age verifies that specimens under 50 years of age are not substantially affected by age-related changes. The lack of correlation between entheseal shape and bone length or age

implies that other factors may regulate entheseal surfaces. Future research should focus on multivariate shape patterns among entheses and their association with occupation.

INTRODUCTION

Entheses are defined as the areas of the bones where muscles attach (Jurmain & Villotte, 2010). The robusticity and/or pathology of these structures (i.e., enthesopathies) are often used as a basis for reconstructing physical activity patterns from human skeletal remains (Peterson & Hawkey, 1998; Jurmain, 1999; Jurmain et al., 2012; Villotte et al., 2016). Previous research has suggested that the thin cortical layer of fibrocartilaginous entheses undergoes shape changes throughout lifetime, as a consequence of cumulative biomechanical loading applied on their surfaces during muscle contraction (e.g., Benjamin et al., 2002; Benjamin et al., 2006; Schlecht, 2012). Based on this principle, the most commonly employed methods for assessing the stage of entheseal change rely on macroscopic observation of entheseal morphology, followed by the application of various ordinal scoring systems (e.g., Hawkey & Merbs, 1995; Mariotti et al., 2004; 2007; Villotte, 2009; Henderson et al., 2017). Nevertheless, these methods have often been associated with low measurement precision (Davis et al., 2013; Wilczak et al., 2016), with most previous works reporting a mean interobserver error of approximately 20% or above (Mariotti et al., 2004; 2007; Davis et al., 2013; Wilczak et al., 2016). Furthermore, the exact factors responsible for morphological differences between the stages of the applied scoring systems are not yet understood (Henderson & Alves Cardoso, 2013; Henderson et al., 2016; 2017), while the morphological criteria for each stage lack consistency among studies (Villotte et al., 2016). As a consequence, recent works have put forth an initiative to improve and further standardize the existing ordinal scoring systems (Mariotti et al., 2007; Henderson et al., 2016; 2017; Villotte et al., 2016)

Several recent studies investigated entheseal variation using measurements of their three-dimensional (3D) surface areas (Zumwalt, 2006; Noldner & Edgar, 2013; Nolte & Wilczak, 2013; Williams-Hatala et al., 2016; Karakostis et al., 2017). These quantitative approaches rely less on subjective evaluation and can present higher intra-observer and inter-observer repeatability (Karakostis & Lorenzo, 2016). However, most of these studies focused on the 3D size of entheses and not their 3D shape. In fact, no previous work has analyzed the shape of entheses using 3D landmark-based geometric morphometrics. These methods' semi-automated nature could potentially provide high repeatability in reconstructing and quantifying entheseal form (a term encompassing both size and shape), while the resulting shape variables can be used to statistically compare specimens in 3D size, shape, as well as allometry (i.e., the relationship between 3D size and shape) (Mitteroecker & Gunz, 2009). Furthermore, these methods provide visualization of statistically significant shape variation, where the exact level and direction of morphological differences among specimens can be objectively determined (Adams & Otarola-Castillo, 2013).

A previous study has linked the relative lengths of entheseal surfaces to their general morphological typology (proliferative, lytic, or "mixed") (Henderson, 2013). Moreover, another research on animal bones reported that the central portion of entheseal areas presents lower surface complexity than their periphery (Zumwalt, 2005). Other works have addressed the potential correlations between entheseal measurements (either 3D size or shape) and various muscle dimensions (e.g., Zumwalt, 2006; Deymier-Black et al., 2015; Williams-Hatala et al., 2016). However, there is no previous study demonstrating that entheseal development in 3D size is significantly correlated with entheseal changes in 3D shape. Therefore, it remains unclear whether there is any association between the qualitative (e.g., Hawkey & Merbs, 1995; Henderson et al., 2017) and the metric (e.g., Nolte & Wilczak, 2013; Karakostis et al., 2017) assessments of entheseal variation (Henderson & Alves Cardoso, 2013). To that end, the methods of geometric morphometrics could provide solid statistical evidence that entheseal surfaces present significant allometry. If shape variation in entheses significantly coincides with their size differences, future estimations of entheseal variation could utilize both aspects of entheseal form (size and shape) on the basis that these two vary together.

The application of geometric morphometrics could also shed light on some of the factors considered to substantially affect the shape of entheseal surfaces, such as biological age (Milella et al., 2012; Nolte & Wilczak, 2013). The traditional focus of anthropological research on entheses is to utilize them as indicators of occupational stress (Foster et al., 2012). However, a statistically significant effect of age-at-death on shape would suggest that entheseal morphology may be mainly regulated by age-related degenerative changes. Furthermore, bone length is a dimension which does not considerably change after development (Rauch, 2005), strongly correlates with body size (Krishan & Sharma, 2007; Pawar & Dadhich, 2012), and is thus not considerably influenced by lifelong bone remodeling (Rauch, 2005). On this basis, a strong correlation between entheseal shape and its corresponding element's bone length would suggest that shorter bones consistently comprise different entheseal shape than longer ones, irrespective of other factors, such as the bone's response to lifelong biomechanical stress.

For addressing the above hypotheses, the primary aim of this study is to put forth a novel landmark-based approach for measuring the 3D form (both size and shape) of human hand entheseal surface areas. The selection of this anatomical area was based on the fact that previous research has reported a close statistical association between certain hand entheses and lifelong occupational activities (Karakostis et al., 2017), while the degree of surface projection in certain hand entheses has been associated with the level of biomechanical efficiency (Maki & Trinkaus, 2011; Richmond et al., 2016). The intra-observer and inter-observer precision of this methodology will be evaluated through a repeatability analysis. The developed technique will be used to assess the relationship between the 3D size and shape of entheses. Subsequently, it will be used to statistically assess the effects of biological age and bone length on entheseal 3D shape.

MATERIALS AND METHODS

Sample

For the purposes of this study, we selected three entheseal surface areas of the right hand bones. These comprise the insertion area of *opponens pollicis* (OP) in the thumb metacarpal, the common attachment area of *abductor pollicis* and *flexor pollicis brevis* (ABP/FPB) in the thumb proximal phalanx, and the common entheseal surface of *abductor digiti minimi* and *flexor digiti minimi* (ADM/FDM) in the fifth proximal phalanx (Fig. 1). These particular entheses were selected because previous research showed that they can be delineated and quantified on the bone 3D surface with high repeatability (Karakostis & Lorenzo, 2016). Moreover, these three right hand entheses substantially contributed to two morphometric patterns of entheses reflecting fundamental manual muscle synergies (Karakostis et al., 2017). Until present, due to the very small size of hand entheses, the only ordinal scoring system proposed for them is binary, classifying entheseal areas into "present" (visible) or "absent" (non-visible) (Cashmore & Zakrzewski, 2011). However, such a classification relies on the raw size and visibility of the entheses and does not include particular information on their shape.

[Figure 1 here]

The material used comprises part of the anthropological collection "Basel-Spitalfriedhof", which is currently curated at the National History Museum of Basel (Switzerland). A total of 135 entheseal surfaces were analyzed, corresponding to 45 individuals who lived in Basel between 1804 and 1865. All individuals of the sample are extensively documented for their biological, medical, occupational, and socio-economic profiles (Hotz et al., 2012; see also Karakostis et al., 2017). The hand bones of this sample are in a remarkable state of preservation, presenting no taphonomic or pathological alterations. The criteria used for identifying enthesopathies are described in Villotte et al. (2010). Also, the documented medical records for these individuals verifies that they did not experience hand-related pathological conditions (c.f., Acknowledgements). This study focused on adult individuals with fully fused hand bones, on which fibrocartilaginous hand entheses (which are almost linked to the articular surfaces) were developed and measurable (Standring, 2008). These specimens in our sample were 18 years old or above. Given that entheseal form is known to be substantially affected by sex and advanced biological age (especially after approximately the age of 50; Foster et al., 2012; Milella et al., 2012), we only used male individuals between 18 and 48 years old. The mean biological age in the sample was 28 with a standard deviation of 8 years. Based on the archival documentation available for our collection (Hotz et al., 2012), which includes the immediate relatives of each individual (c.f., Acknowledgements), there was no direct family relation among the specimens comprising the sample.

3D reconstruction and delineation of entheseal areas

The specimens were scanned using a Breuckmann Smartscan structured-light scanner (Breuckmann Inc., Baden, Germany), with 125 FOV, and an automatic turntable. The measurement accuracy of this equipment is 9 μm. Full triangulation was selected. The Optocat software package (Breuckmann Inc.) was used for developing 3D models. Each specimen was scanned from 20 different angles along an arc of 360 degrees. Then, all 20 scans were aligned and merged into a single 3D model, which was extracted as a "ply" file.

The borders of the entheseal surfaces on the bone 3D models were located and digitally delineated in the software "Meshlab version 1.3.3" (CNR-INC, Rome, Italy). The methodology followed was introduced in previous work by one of us (F.A.K.), where it presented very high intra-observer and inter-observer repeatability (maximum mean error was 0.60%) (Karakostis and Lorenzo, 2016). In summary, entheseal borders were defined on the surface models based on elevation, surface complexity, and/or coloration. The delineated entheseal areas were then isolated from the surrounding bone surface and saved as a separate "ply" file. All entheses were processed by the same author (F.A.K.).

It should be mentioned that the exact location of OP's entheseal surface on the first metacarpal varies across anthropological studies. Older works placed the entheseal area of OP along almost the entire lateral aspect of the first metacarpal (Gray, 1918), while more recent sources depicted it as a smaller surface area, which is slightly more restricted to the distal part of the lateral diaphysis (e.g., Standring, 2008). In this study, the allocation of OP's enthesis followed the methodology of previous research by one of us (Karakostis and Lorenzo, 2016). In that previous work, the analyzed area involved the distinctive ridge located at the laterodistal margins of the wider insertion area of this muscle (Standring, 2008) (Fig. 1a). In dissection photographs by Gosling et al (2009), this particular bone area seems to be also associated with a tendinous insertion point for OP. Based on our observations, in some individual cases, the ridge extends towards the midshaft acquiring the form illustrated in Standring (2008) as well as Karakostis and Lorenzo (2016). By contrast, in other specimens, the enthesis appears to comprise a tubercle situated near the metacarpal head (e.g., Fig. 1a).

Landmark digitization and measurements obtained

The maximum length of all bones (first metacarpals, first proximal phalanges, and fifth proximal phalanges) was measured in millimeters by the first author (F.A.K.) directly on the 3D models of hand bones, using the tools of the Meshlab software package. After a period of two months, the three bone lengths of 20 randomly selected individuals were measured again by F.A.K. and another observer (c.f., Acknowledgements). A series of paired t-tests verified that length calculations were significantly repeatable (p-value > 0.05).

For all geometric morphometric analyses, the Geomorph package (version 3.0) of the R software (version 2.1.6) was utilized (Adams & Otarola-Castillo, 2013). Initially, the "ply" files consisting of the delimited entheses were imported into the program. Then, for each of the three entheseal surfaces analyzed (i.e., OP, ABP/FPB, and ADM/FDM), a set of fixed landmarks were digitized along the outline of the entheseal surface (its borders). The points selected were only those which could be obviously determined in all specimens and could provide a comprehensive summary of the entheseal outline (Webster and Sheets, 2010). The exact location of these fixed points is demonstrated in Figure 1 and described in Table 1. The number of fixed outline points in OP and ABP/FPB were six. By contrast, for ADM/FDM, only four points of the outline were clearly homologous across all specimens.

[Table 1 here]

For each specimen analyzed, the fixed points were used as a basis for calculating a set of surface semilandmarks. These were distributed equidistantly across the 3D surface through a "nearest-neighbor" approach, following the algorithm proposed in Mitteroecker & Gunz (2009). For ABP/FPB and ADM/FDM, a total of 15 surface semilandmarks were digitized, whereas 30 semilandmarks were used for OP. This is because the shape of the latter entheseal area was relatively more complex and required a higher number of semilandmarks to be adequately covered and described. Based on our observations, even though these sets of landmarks (both fixed points and semilandmarks together) could not cover all parts of the entheseal area (Figs. 1 and 2c), they appeared to provide a representative summary of its overall geometry. An example of the semilandmarks' position on each entheseal surface is depicted below, in the Results section (Figs. 2, 3, and 4). All landmarks and semilandmarks were computed by the same author (F.A.K.). In this study, curve semilandmarks were not utilized because the resulting shape change plots were less comprehensive and did not provide substantial further information. This seems to be due to a high degree of interindividual variability in the outline curve of entheseal surfaces.

Following the digitization of all fixed landmarks and surface semilandmarks on the entheseal areas, we performed Procrustes superimposition on the raw landmark data. This process centers all specimens at the origin, scales them to unit-centroid size, and rotates them around the origin (on the basis of a least-squares criterion) until the corresponding points across specimens are aligned as much as possible (Adams and Otarola-Castillo, 2013). In addition, the homologous position of semilandmarks was improved by allowing them to slide along tangent planes on the entheseal surfaces, using the minimum bending energy criterion (Ivan Perez et al., 2006). The main products of the superimposition process involve the Procrustes coordinates (shape variables) and the centroid size (size measure) for each individual entheseal area (Mitteroecker and Gunz, 2009). Previous studies have measured the 3D size of entheseal areas in square millimeters (Zumwalt, 2006; Noldner & Edgar, 2013; Karakostis et al., 2016; 2017; Williams-Hatala et al., 2016). These include a previous work on the same sample of hand entheses used here (Karakostis et al., 2017), where measurements were obtained using a highly precise method of 3D quantification (Karakostis & Lorenzo, 2016). To verify that the values of centroid size coincide with the raw measurements for these entheses, a Pearson's correlation test was performed for each entheseal area (Field, 2013).

Repeatability analysis

In order to evaluate the precision of landmark placement, we conducted a repeatability analysis (Singleton, 2002) consisting of two sessions and two observers (c.f., Acknowledgements). The first observer (F.A.K.) is familiar with entheseal analysis, whereas the second digitizer has not previously conducted research on entheses. Prior to the analysis, the second observer was given a three-day introduction on the exact position of each fixed landmark (Table 1 and Fig. 1) as well as the main criteria used for delineating the entheseal areas on the hand bones, as described in Karakostis & Lorenzo (2016). This brief training also included the systematic observation of dissection photographs indicating the exact borders of muscle attachment on the hand bone elements (Gosling et al., 2009).

The first session involved digitizations performed in two different time periods, separated from each other by five months. All three entheses of three randomly selected individuals (nine entheses) were utilized. Initially, for each of the nine entheseal areas, the landmarks were digitized ten times by the first observer (F.A.K.) and ten times by the second one (a total of 20 digitizations). After a period of five months, these nine entheses were digitized again by the same observers for another 20 times (ten for each observer). For each enthesis, the time interval between each digitization was one day. Therefore, for both observers, two digitizations were performed per day. Subsequently, using all 40 digitizations (20 per observer), the intra-observer and inter-observer errors were assessed for each of the nine entheseal areas separately (Singleton, 2002; von Cramon-Taubadel et al., 2007). In the second session, another nine randomly selected individuals (27 entheses) were added to the previous sample of three individuals. Thus, a total of 12 specimens (almost 27% of the sample) were tested together for each enthesis, which correspond to 36 entheseal areas (40 digitizations each). For the 27 entheses of the nine individuals (a total of 1080 digitizations), the landmark placements were performed over a period of 12 days (45 digitizations a day per observer) without time intervals between digitizations.

In both sessions, for each enthesis, Procrustes superimposition was performed on the raw coordinates of all 40 repetitions (twenty for each observer). Then, the Euclidean distance between each landmark's coordinates and the corresponding centroid (i.e., the distance defined as centroid radius) was computed for each landmark repeat (Singleton, 2002; von Cramon-Taubadel et al., 2007). Using the centroid radius of all repetitions, the mean deviation (error) and percentage error was computed for each landmark, both within (intra-observer error) and between the two observers (inter-observer error) (von Cramon-Taubadel et al., 2007). Finally, the average percentage error across all individual landmarks was calculated both within and between observers (Singleton, 2002).

This approach for estimating error was preferred because it evaluates prevision for each landmark separately, allowing for a statistical evaluation of precision in different areas of entheseal surfaces (Singleton, 2002; von Cramon-Taubadel et al., 2007). In this study, this was particularly useful because some specimens presented extensive allometric variance in specific areas or landmarks. It should be mentioned that previous research has noted two potential issues for this method (von Cramon-Taubadel et al., 2007). Particularly, the authors observed that the landmarks located closer to the centroid may consistently exhibit greater error than the rest, while error in any direction which is perpendicular to the centroid may be undetectable. However, in this study, the results demonstrated a similar and non-significant level of error for all landmarks, irrespective of their proximity to the centroid (c.f., Results). Furthermore, based on systematic observations on all specimens, the analyzed hand entheseal surface areas appear to be generally irregular and sloped. Therefore, we hypothesized that landmark placement error in any direction would also likely result in considerably different centroid radius. To evaluate this assumption, we performed a series of error simulations on three randomly selected surface areas (one for each enthesis). For each entheseal surface, ten landmarks were randomly selected among both fixed points and semilandmarks. The position of each landmark was then manually altered for a total of five times, towards five different locations surrounding the original landmark, always at an arbitrary Euclidean distance of 0.5 millimeters from it. For each landmark alteration, Procrustes superimposition and sliding were performed again on the entire sample (all landmarks of the enthesis for all 45 individuals) and the centroid radius for this particular landmark was computed. Finally, we calculated the percent difference between the centroid radii of the altered landmark and the original one. For all five alterations of the ten landmarks in each of the three entheses, this percent difference was above 5%, ranging between 5.4% and 9.1%. This result indicates that, in our sample, a very slight landmark placement error in any direction on the 3D surfaces would be detectable using the methodological approach involving the centroid radius (Singleton, 2002).

Statistical analysis

The effect of the entheses' size on their shape was measured using allometric regression, which is a multivariate regression of the Procrustes coordinates (shape variables) on the natural logarithm of centroid size (LogCS) (Mitteroecker et al., 2004; Mitteroecker and Gunz, 2009). In this study, centroid size was highly correlated with 3D raw surface size (c.f., Results). For each of the three entheseal surfaces, the amount of shape variation accounted for by the regression model was calculated as a percentage of allometric shape variation (Drake & Klingenberg, 2008). The statistical significance of allometry was assessed via permutation tests (1000 random permutations) using the Goodall's F method (Goodall, 1991; Adams & Nistri, 2010; Adams &

Otarola-Castillo, 2013). The shape scores resulting from the regression of shape on size were plotted against LogCS, in order to graphically demonstrate the multivariate association between size and shape (Drake & Klingenberg, 2008). Allometric shape variation was illustrated using plots depicting the size-related differences of landmark coordinates in Procrustes shape space (Adams & Otarola-Castillo, 2013). For increasing the interpretability of these plots, we incorporated wireframes connecting the fixed points of each enthesis (see below, Figs. 2, 3, and 4). Nevertheless, it should be underlined that the lines of these wireframes do not fully reflect the complex shape of the entheseal outlines. The same statistical method (shape regression) was utilized for assessing the association between entheseal shape variables (Procrustes coordinates) and the values of biological age as well as bone length (Mitteroecker & Gunz, 2009).

During the analysis of OP, it was observed that most landmarks of a specific part of the enthesis presented extensive variability in all regression processes. These were points located on the narrow ridge of the entheseal surface, proximally to a hypothetical outline curve connecting L5 and L6 (Table 1). At the same time, most landmarks on the proximal tubercle presented considerable variation as well. This condition was highly indicative of a probable "Pinocchio" effect, which occurs when large variation in certain landmarks is smeared out across other landmarks of the configuration (von Cramon-Taubadael et al., 2007). Therefore, further analysis was conducted for this particular enthesis. Specifically, following the methodological recommendations on this issue from a previous research on allometry using landmark-based geometric morphometrics (van der Linde and Houle, 2009), all shape analyses for OP were repeated for another three times, using a different subset of landmarks in each case. For each subset, the same analytical process was applied, including superimposition and shape regression. The first subset included only the eight landmarks of the entheseal proximal ridge (the eight most proximal landmarks of this enthesis, below a hypothetical outline curve between L5 and L6), while the second subset involved only the landmarks of the distal tubercle, which showed small – but existent- variability across specimens. These two processes allowed us to observe whether excluding the ridge landmarks causes the tubercle points to stop presenting significant shape change among specimens. Finally, the third subset involved all landmarks except for the fixed landmark L2 (Fig. 1), which presented the most extreme variability. This step could indicate whether the high shape variation of the ridge's surface semilandmarks is driven by that particular point's change in position.

RESULTS

The results demonstrated that the proposed landmark configurations (Fig. 1 and Table 1) are highly repeatable both within and between observers. For all 36 entheseal surfaces tested, all individual landmarks presented less than 4% of error. In average, the intra-observer error is slightly lower than the inter-observer one. Particularly, in the first session, the mean intra-observer error across landmarks was 1.7% (ranging between 0.1 and 2.8%), while the average inter-observer error was 3.1% (ranging from 0.1 to 3.8%). In the second session, the mean landmark error was 2.4% within observers (ranging from 0.1 to 3.4%) and 3.3% (ranging from 0.1 to 3.9%) between observers. Between the two time periods of the first session (separated by five months), the absolute difference in mean error was approximately 0.5%, both in the intra-observer and inter-observer analyses. Similarly, the level of experience was not a factor of considerable variation, as the mean difference between the two observers was less than 1% for all sessions and individual entheses. Overall, the three entheses presented very similar precision rates. Particularly, in both sessions, both within and between observers, the difference across the three entheses in mean error was always less than 1%.

Based on the three correlation tests, the centroid size is strongly and positively correlated with the 3D measurements of entheses in square millimetres. In all cases, the p-value was less than 0.01. The r-value was 0.93 for OP, 0.91 for ABP/FPB, and 0.90 for ADM/FDM. The output of the three allometric regression analyses is presented in Table 2. For all three entheseal surfaces, there was a significant positive relationship between LogCS and shape (p-value < 0.01). The highest proportion of shape variance explained by size was observed for OP (26.8%), followed by ABP/FPB (13.8%), and ADM/FDM (6.2%).

[Table 2 here]

For the two phalangeal entheses, the location of the centrally-placed surface semilandmarks can be observed in Figures 2c and 3c, respectively. For ABP/FPB, the central semilandmarks lie within a hypothetical rectangle formed by L1, L2, L4, and L5 (Fig. 2c), while the central semilandmarks of ADM/FDM take place around a hypothetical line connecting L1 to L2 (Fig. 3c). When observing the position of the same central semilandmarks in Figures 2b and 3b (using the fixed landmarks for orientation), it seems that both entheses show a relatively similar pattern of allometric shape change (Figs. 2b and 3b). In larger specimens, the central area of both entheseal surfaces (i.e., most of the centrally-placed semilandmarks) is proportionally more elevated, while the semilandmarks on the medial and lateral peripheral areas present less projecting positions, as the 3D surface descends more abruptly towards the outline of the entheses (defined by the fixed landmarks). Consequently, the overall shape of larger phalangeal entheses is steeper.

[Figure 2 here]

[Figure 3 here]

Regarding OP, when the landmarks on the proximal ridge of OP (the eight most proximal points of the enthesis) were analyzed separately, the proportion of variance was almost the same (26.4%), while the direction and level of their change was very similar (as in Fig. 4c). By contrast, the tubercle landmarks alone did not present significant allometry and their change in position was minimal after exclusion of the ridge landmarks (Table 2). This indicates that the previously observed differences in the distal tubercle (Fig. 4) were probably due to a "Pinocchio" effect, driven by the extensive variability of the proximal ridge's landmark points. Finally, even though the exclusion of the extreme point L2 from the dataset reduced substantially the proportion of shape variation by allometry, this remained statistically significant due to the inclusion of the ridge's seven surface semilandmarks (Table 2). Again, the level and direction of change for the proximal ridge semilandmarks was almost the same (as in Fig. 4c).

[Figure 4 here]

[Figure 5 here]

The resulting regression plot was almost identical between the analysis of all OP's landmarks and the analysis restricted to the eight proximal points of the ridge (Fig. 4a). In these plots, allometric shape variation in OP presented a clear morphological dichotomy. Larger entheseal surfaces comprised proportionally elongated proximal ridges (Fig. 4b). By contrast, smaller entheseal areas seemed to resemble the shape of a tubercle with limited extension proximally. In order to better demonstrate these extensive morphological differences in the outline shape of this enthesis, the 3D models of one small and one very large entheses are depicted in Figure 5, which shows how the elongated proximal ridge of the largest entheseal surfaces (Fig. 5b) can extend below the midshaft of the metacarpal.

[Table 3 here]

[Table 4 here]

Overall, the results of the shape regression analyses found no significant relationship between biological age (between 18 and 48 years) and entheseal shape (Table 3). Similarly, the shape of the two phalangeal entheses (ABP/FPB and ADM/FDM) was not associated with the corresponding element's length. By contrast, the shape of OP was significantly correlated with the first metacarpal's maximum length (Table 4). For this enthesis, the pattern of shape variation related to bone length was profoundly similar to the one observed for the allometric regression (using LogCS). This extensive similarity is depicted in Figure 6, which demonstrates shape variation attributable to LogCS (Fig. 6a) and bone length (Fig. 6b). However, the consequent analyses on the landmark subsamples revealed that variation in the tubercle points alone was very weakly correlated with bone length (Table 4). At the same time, even though the ridge landmarks alone presented a greater proportion of variation related to bone length (6.1%), this relationship was still not statistically significant (Table 4). In fact, omitting the fixed landmark L2 (the most proximal and extreme point for this enthesis) from the analysis also led to a non-significant association.

[Figure 6 here]

DISCUSSION

The high repeatability in landmark placement verifies that the proposed position of the fixed outline landmarks was straightforward for all specimens. This is also supported by the high precision rates observed for the second digitizer, who does not specialize on entheseal morphology. The slight mean differences between intra-observer and inter-observer error (1.4%

in the first session and 0.9% in the second one) suggest that the existing subjectivity of the developed technique is relatively limited. Furthermore, the results of the first session suggest that the level of repeatability can be consistent after a time interval of five months. The mean error was higher in the second session, in spite of the fact that landmark placement was performed without time intervals between digitizations. Overall, the observed amount of error (always less than 4%) is substantially lower than the error percentages reported for ordinal scoring systems (an average of 20% or above), which mainly rely on naked-eye observation (Davis et al., 2013; Wilczak et al., 2016). Given that the technique for delineating the entheseal surface on the bone is also highly precise (Karakostis and Lorenzo, 2016), future work could use the introduced methodology for further investigating the various potential factors influencing entheseal shape variation (Foster et al., 2012; Henderson et al., 2017).

There are also non-landmark-based methods which could potentially be used to analyze entheseal shape. However, the implementation of statistical testing in these methods is not yet fully developed (Katzenberg & Saunders, 2011). For instance, previous research has recommended the use of finite element analysis for analyzing functional morphology (e.g., Richmond et al., 2005). Nevertheless, it has been argued that the data deriving from this method are not suitable for statistical analyses of structures with high morphological variability (Khalaji et al., 2008; Weber et al., 2011). In fact, a recent work has concluded that further methodological refinement is necessary before implementing a statistical finite element analysis on 3D models for assessing intraspecies variation (Marce-Nogue et al., 2016). By contrast, the methods of 3D geometric morphometrics are currently able to identify and visualize patterns of statistically significant shape variation within a sample (Mitteroecker & Gunz, 2009). Another previous approach for analyzing entheseal shape complexity relied on the average fractal dimensions of extracted entheseal profiles, whose values ranged between 1 ("smooth") and 2 ("infinitely complex") (Zumwalt, 2005; 2006). However, this ordinal scoring system may not provide complete information on shape variability in multiple parts of the enthesis at the same time.

Our results identified a significant positive association between entheseal 3D shape and size. Given that a significant amount of entheseal shape differences is attributable to 3D size, utilizing both aspects of entheseal 3D form (size and shape) can strengthen future assessments of variation in human entheses. Also, provided that centroid size strongly coincides with raw 3D measurements (in square millimeters), both variables could be used to represent entheseal surface size. The allometric shape pattern observed for the two phalangeal entheses (Figs. 2 and 3) indicates that larger entheseal areas have a steeper shape with a proportionally extended central region. This morphology seems to reflect the general pattern of entheseal change outlined in observational qualitative methods (e.g., Hawkey and Merbs, 1995; Mariotti et al., 2007). Furthermore, previous histological studies have reported that the central regions of fibrocartilaginous entheses present higher concentrations of calcified fibrocartilage, which is directly associated with greater biomechanical stress (Shea et al., 2001; Sasaki et al., 2012; Beaulieu et al., 2015). If this information is considered together with the fact that biomechanical forces regulate the distribution of bone mineral across the bone (e.g., Bennell et al., 1997; Palombaro, 2005), a higher level of biomechanical loading at the centre of entheses would be expected to inflict greater bone formation in this area. This possibility could explain the fact that some entheses present a proportionally more projecting central portion than others (Figs. 2 and 3).

From a biomechanical perspective, greater extension of hand entheseal areas is linked to a greater moment arm and biomechanical efficiency for the corresponding muscle (e.g., Maki & Trinkaus, 2011; Richmond et al., 2016). At the same time, previous research on different animal species have concluded that the 3D size of entheses is strongly correlated with the levels of muscle forces subjected on their surface area (Deymier-Black et al., 2015; Rossetti et al., 2017). On this basis, the combined occurrence of a proportionally projecting 3D shape and a large 3D size (Figs. 2 and 3) in entheses is potentially related to greater biomechanical efficiency for the attaching muscle. This result encourages future research to utilize the allometric scores of entheses (representing the interaction between 3D size and shape) as a potential indicator of greater muscle moment arm.

In this study, the observed proportions of shape variation related to size (ranging between 6.9% and 26.4%) were comparable with the percentages reported in multiple previous osteological works, which used the same statistical approach to verify allometric relationships in other anatomical structures (e.g., White, 2009; Klingenberg and Marugan-Lobon, 2013;Mitteroecker et al., 2013; Yazdi, 2014; Klingenberg, 2016). In fact, proportions over 20% are often treated as direct indicators of substantial allometry (e.g., White, 2009; Mitteroecker et al., 2013), while some studies identify allometric relationships based on a percentage of 13% (Klingenberg and Marugan-Lobon, 2013) or even below 5% (Yazdi, 2014).

According to our results, the proportion of total shape variation explained by allometry varied substantially across the three entheseal surfaces. Initially, the highest percentage was presented by the enthesis with the most landmarks (OP), whereas the lowest proportion was observed for the enthesis with the least landmarks (ADM/FDM). Therefore, one could argue that this variation could be partly due to the different number of landmarks utilized for each enthesis, which may have affected the computation of Procrustes distances used for the Goodall's F test (Goodall, 1991). However, considering the extensive allometric shape change observed for the proximal ridge of OP (in an analysis based on only eight landmark points), it seems that allometry differs across entheses irrespective of the number of landmarks utilized. On this basis, it would be beneficial for entheseal studies to explore the allometry of other entheses in the human skeleton. Furthermore, further research on increased sample sizes is needed to further validate the correlations reported for the three hand entheses investigated in this study.

For all three entheses studied, there was a substantial remaining proportion of total shape variance which was not directly associated with size (Table 2). In fact, concerning the two phalangeal entheses (ABP/FPB and ADM/FDM), several specimens with extensive size differences presented very similar shape regression scores (Figs. 2a and 3a). This could be due to the effect of various complex factors potentially affecting entheseal shape, such as interindividual genetic variability, lifelong physical activity, hormone levels, or nutrition (Foster et al., 2012). In this study, age-at-death did not have a significant effect on entheseal shape (Table 3). However, the sample was comprised of individuals younger than 50 years of age and without pathological conditions in their hands. Considering the extensive effect of degenerative changes on entheseal morphology after around the age of 50 (Milella et al., 2012), this result would likely be different if older individuals were included in the analysis.

The values of bone length, which is strongly associated with genes and body size (Rauch, 2005; Krishan and Sharma, 2007; Pawar and Dadhich, 2012), did not coincide with shape variation in the two phalangeal entheses. Regarding OP, when all 36 landmarks were included, the elongated shape appeared to be more frequent in longer metacarpal bones (Table 4). However, based on the consequent analyses of the subsamples, one can observe that the slight significance observed in the first analysis (including all 36 landmarks) was probably influenced by the accumulated shape variation of the distal tubercle's points (Figs. 4b and 4c). These, however, were likely affected by a "Pinocchio" effect, which can explain why the entheses ceased to present shape variation by bone length when each subset (tubercle and ridge) was analyzed separately or when the extreme landmark L2 was omitted from the process (Table 4).

The allometric shape pattern observed for the proximal ridge of OP demonstrates that the aforementioned morphological dichotomy (c.f., in the Materials and methods) seems to be highly associated with the 3D size of the area. Larger entheseal areas usually contain elongated proximal ridges extending towards or over the metacarpal's midshaft (Fig. 5b), as illustrated in Standring (2008). By contrast, smaller entheses tend to resemble tubercles restricted in the distal part of the metacarpal, as shown in Figure 5b and previously described in Karakostis et al. (2016). As it can be observed in Figure 4c, several slid surface semilandmarks of smaller OP areas were concentrated in the proximal entheseal border (their middle-right part in the plot). This condition appears to be a result of the distinctive morphological dichotomy observed in the shape of this enthesis (Fig. 5). In larger entheseal areas, these specific equidistant surface semi-landmarks were initially placed across the entire surface of the proportionally long proximal ridge (Fig. 5b). By contrast, in the smaller entheses (which do not include a relatively elongated proximal portion), the corresponding semi-landmarks were restricted within a proportionally much shorter proximal ridge (Fig. 5a).

Previous research on the same anthropological sample established a statistical correlation between occupational activities and patterns among different hand entheses (Karakostis et al., 2017). However, the conclusions of that previous study highlighted that this association is only observable when the multivariate relationship among multiple hand entheses is analyzed and not merely the form of each entheseal structure separately. This study focused on putting forth a geometric morphometric analysis of the factors affecting the shape of each entheseal form separately. Future research could use the introduced methodology as a basis for exploring statistical patterns among the shapes (or forms) of different entheses and their potential relationship with occupational activity.

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Table 1. Description of the fixed landmarks' position for each entheseal surface analyzed.

Landmarks	***Opponens pollicis*** [a]	***Abductor pollicis / flexor pollicis brevis*** [a]	***Abductor digiti minimi / flexor digiti minimi*** [a]
L1	The most distal point of the entheseal surface (i.e., the closest to the distal articular surface of the first metacarpal).	In the lateral side of the enthesis, the most distal point of the outline (i.e., the closest to midshaft).	The most distal point of the entheseal surface, which is palmaromedially placed (i.e., the closest to midshaft).
L2	The most proximal point of the entheseal surface (i.e., the closest to midshaft).	In the palmar side of the enthesis, the most distal point of the outline (i.e., the closest to midshaft).	The most proximal point of the entheseal surface, which is in the medial side of the enthesis (i.e., the closest to the proximal articular surface).
L3	The point of the entheseal surface which is closest to the palmar aspect of the metacarpal diaphysis (distal side of the enthesis).	The point of the enthesis that is closest to the palmar aspect of the first proximal phalanx (palmar side of the enthesis).	The point of the entheseal surface that is closest to the dorsal aspect of the fifth proximal phalanx (medial side of the enthesis).
L4	The point of the entheseal surface that is closest to the dorsal aspect of the metacarpal diaphysis (distal side of the enthesis).	In the palmar side of the enthesis, the most proximal point (i.e., the closest to the proximal articular surface).	The most palmar point of the entheseal surface (palmar side of the enthesis).
L5	The angle of the outline's curve between L2 and L3, which separates between the distal - roughly rectangular- robust portion of the enthesis and its proximal narrow elongated part.	In the lateral side of the enthesis, the most proximal point (i.e., the closest to the proximal articular surface).	
L6	The angle of the outline's curve between L2 and L4, which separates between the distal - roughly rectangular- robust portion of the enthesis and its proximal narrow elongated part.	The most dorsal point of the entheseal surface (lateral side of the enthesis).	

[a] Each entheseal measurement is represented by the name of its corresponding muscle.

Table 2. Results of the three allometric regression analyses.

Entheseal surfaces [a]	Sum of squares	Mean squares	Degrees of freedom	% Explained variance	F-value	P-value
Opponens pollicis (all landmarks)	0.71	0.71	1	26.84	15.78	< 0.01
Opponens pollicis (tubercle)	0.05	0.05	1	2.78	1.23	0.27
Opponens pollicis (ridge)	0.59	0.59	1	26.40	15.45	< 0.01
Opponens pollicis (all landmarks except for L2)	0.22	0.22	1	11.21	5.43	< 0.01
Abductor pollicis / flexor pollicis brevis	0.39	0.39	1	13.77	6.71	< 0.01
Abductor digiti minimi / flexor digiti minimi	0.12	0.12	1	6.21	2.71	0.02

[a] Each entheseal measurement in this column is represented by the name of its corresponding muscle.

Table 3. Results of the regression of shape on age-at-death.

Entheseal surfaces [a]	**Sum of squares**	**Mean squares**	**Degrees of freedom**	**% Explained variance**	**F-value**	**P-value**
Opponens pollicis	0.04	0.04	1	1.35	0.59	0.70
Opponens pollicis (tubercle)	0.03	0.03	1	1.50	0.65	0.76
Opponens pollicis (ridge)	0.06	0.06	1	2.70	1.20	0.27
Opponens pollicis (all landmarks except for L2)	0.03	0.03	1	1.74	0.76	0.59
Abductor pollicis / flexor pollicis brevis	0.06	0.06	1	2.13	0.92	0.43
Abductor digiti minimi / flexor digiti minimi	0.02	0.02	1	0.85	0.35	0.89

[a] Each entheseal measurement in this column is represented by the name of its corresponding muscle.

Table 4. Results of the regression of shape on bone length.

Entheseal surfaces [a, b]	Sum of squares	Mean squares	Degrees of freedom	% Explained variance	F-value	P-value
Opponens pollicis	**0.22**	**0.22**	**1**	**8.22**	**3.85**	**0.01**
Opponens pollicis (tubercle)	0.04	0.04	1	1.20	0.88	0.53
Opponens pollicis (ridge)	0.14	0.14	1	6.10	2.79	0.11
Opponens pollicis (all landmarks except for L2)	0.07	0.07	1	3.80	1.69	0.10
Abductor pollicis / flexor pollicis brevis	0.13	0.13	1	4.67	2.06	0.06
Abductor digiti minimi / flexor digiti minimi	0.08	0.08	1	4.29	1.84	0.11

[a] Each entheseal measurement in this column is represented by the name of its corresponding muscle.

[b] Statistically significant results are in bold.

Figure Legends

Figure 1. The location of the entheseal surfaces on the bone 3D models (1) and the position of the fixed outline landmarks (L) on their outline (2). These were used for the calculation of surface semilandmarks (c.f. Figs. 2-4). For purposes of clearer demonstration, the color histogram of the 3D models is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016). The description of the fixed landmarks' anatomical position is provided in Table 1.

Figure 2. Plot of the shape regression scores against the logarithm of centroid size for the enthesis of *abductor pollicis / flexor pollicis brevis* (a)[a] and graphs depicting allometric shape variation from a proximopalmar (b) and a lateropalmar (c) view of the bone. In this study, centroid size was highly correlated with 3D raw size (in square millimeters). The proximopalmar view indicates which landmarks are more elevated, while the lateropalmar aspect shows which surface semilandmarks are placed centrally in the enthesis. The bottom figures explain the approximate location of landmarks (both fixed and sliding surface semilandmarks) on the bone 3D models. For clearer demonstration, the color histogram of the models is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016).

[a] For the purpose of highlighting the association between the regression scores and the logarithm of centroid size, a regression line was drawn.

Figure 3. Plot of the shape regression scores against the logarithm of centroid size for the enthesis of *abductor digiti minimi/ flexor digiti minimi* (a)[a] and graphs depicting allometric shape variation from a proximomedial (b) and a medial (c) view of the bone. In this study, centroid size was highly correlated with 3D raw size (in square millimeters). The proximomedial view indicates which landmarks are more elevated, while the medial aspect shows which surface semilandmarks are placed centrally in the enthesis. The two bottom figures explain the approximate location of landmarks (both fixed and sliding surface semilandmarks) on the bone 3D models. For clearer demonstration, the color histogram of the models is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016).

[a] For the purpose of highlighting the association between the regression scores and the logarithm of centroid size, a regression line was drawn.

Figure 4. Plot of the shape regression scores against the logarithm of centroid size for the enthesis of *opponens pollicis* (a)[a] and graphs depicting allometric variation (from a lateral view of the bone) in the position of outline fixed points (b) and surface sliding semilandmarks (c). The regression plot (a) was almost identical between the analysis with all 36 landmarks and the one focusing on the proximal ridge. In this study, centroid size was highly correlated with 3D raw size (in square millimeters). For the semilandmarks, vectors were used to demonstrate variation in surface semilandmarks (blue arrows) and fixed outline landmarks (red arrows). The two bottom figures explain the approximate location of landmarks (both fixed and sliding surface semilandmarks) on the bone 3D models. For clearer demonstration, the color histogram of the models is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016). After exclusion of the eight most proximal landmarks of the ridge (the points indicated with an asterisk), the remaining landmarks on the distal tubercle (over a hypothetical outline curve between L5 and L6) did not present significant shape variation. In all subsamples, the ridge landmarks presented approximately the same direction of shape change.

[a] For the purpose of highlighting the association between the regression scores and the logarithm of centroid size, a regression line was drawn.

Figure 5. An example of a relatively small and a very large entheseal surface of *opponens pollicis* (lateral view of the bone). In the depicted 3D models, the color histogram is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016). The position of the fixed landmarks (L) is explained in Table 1 and depicted in Figure 1.

Figure 6. Shape variation in the entheseal surface of *opponens pollicis* (lateral view of the bone) related to its size (a) and corresponding bone length (b). The position of the fixed landmark points (L) is described in Table 1 and depicted in Figure 1. After exclusion of the eight most proximal landmarks on the ridge (indicated with an asterisk), the remaining landmarks on the distal tubercle (over a hypothetical outline curve between L5 and L6) did not present significant shape variation. In all subsamples, the ridge landmarks presented approximately the same direction of shape change.

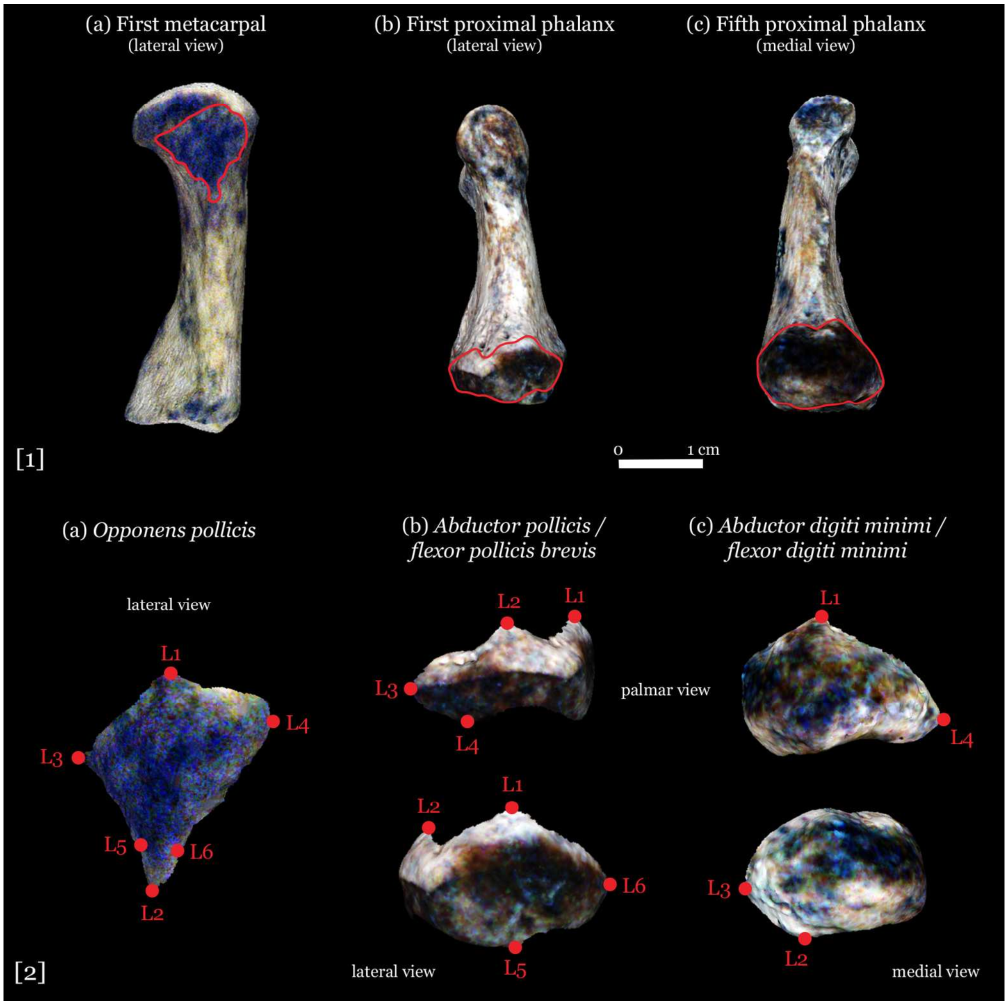

Figure 1. The location of the entheseal surfaces on the bone 3D models (1) and the position of the fixed outline landmarks (L) on their outline (2). These were used for the calculation of surface semilandmarks (c.f. Figs. 2-4). For purposes of clearer demonstration, the color histogram of the 3D models is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016). The description of the fixed landmarks' anatomical position is provided in Table 1.

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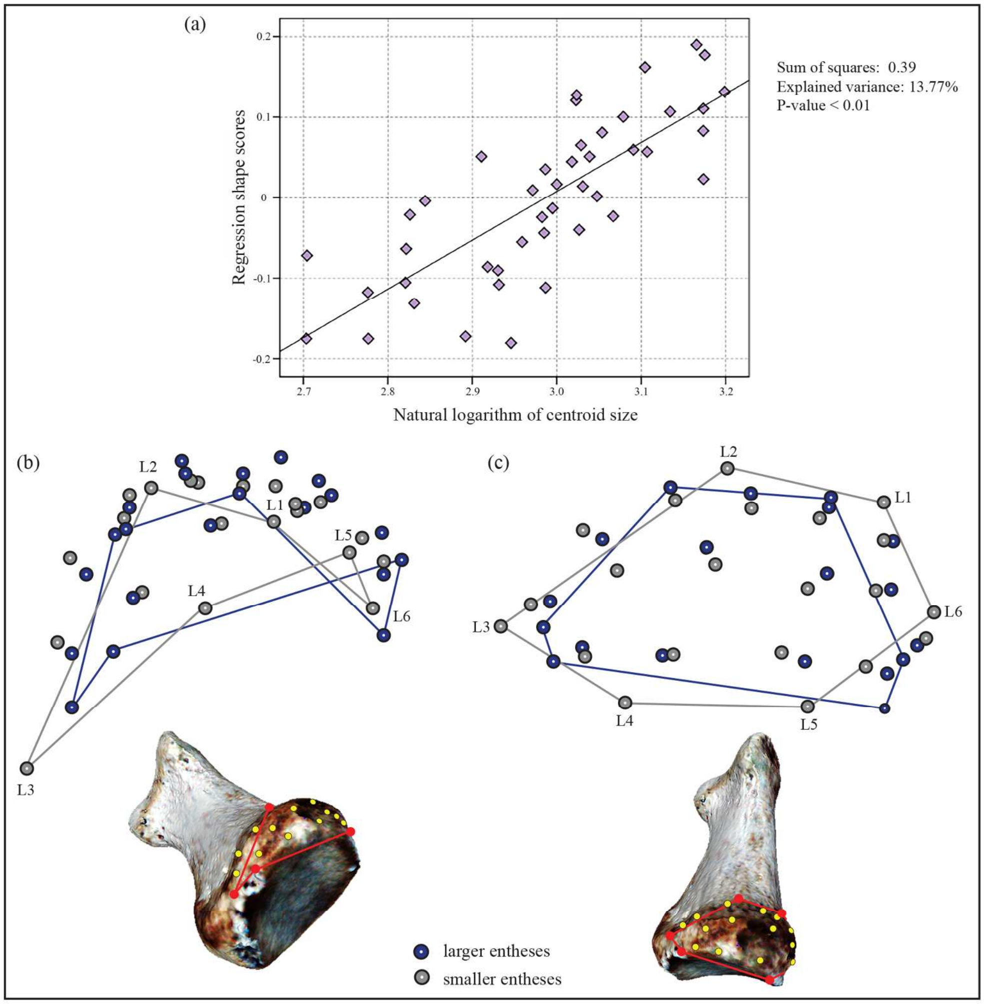

Figure 2. Plot of the shape regression scores against the logarithm of centroid size for the enthesis of abductor pollicis / flexor pollicis brevis (a) and graphs depicting allometric shape variation from a proximopalmar (b) and a lateropalmar (c) view of the bone. In this study, centroid size was highly correlated with 3D raw size (in square millimeters). The proximopalmar view indicates which landmarks are more elevated, while the lateropalmar aspect shows which surface semilandmarks are placed centrally in the enthesis. The bottom figures explain the approximate location of landmarks (both fixed and sliding surface semilandmarks) on the bone 3D models. For clearer demonstration, the color histogram of the models is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016).

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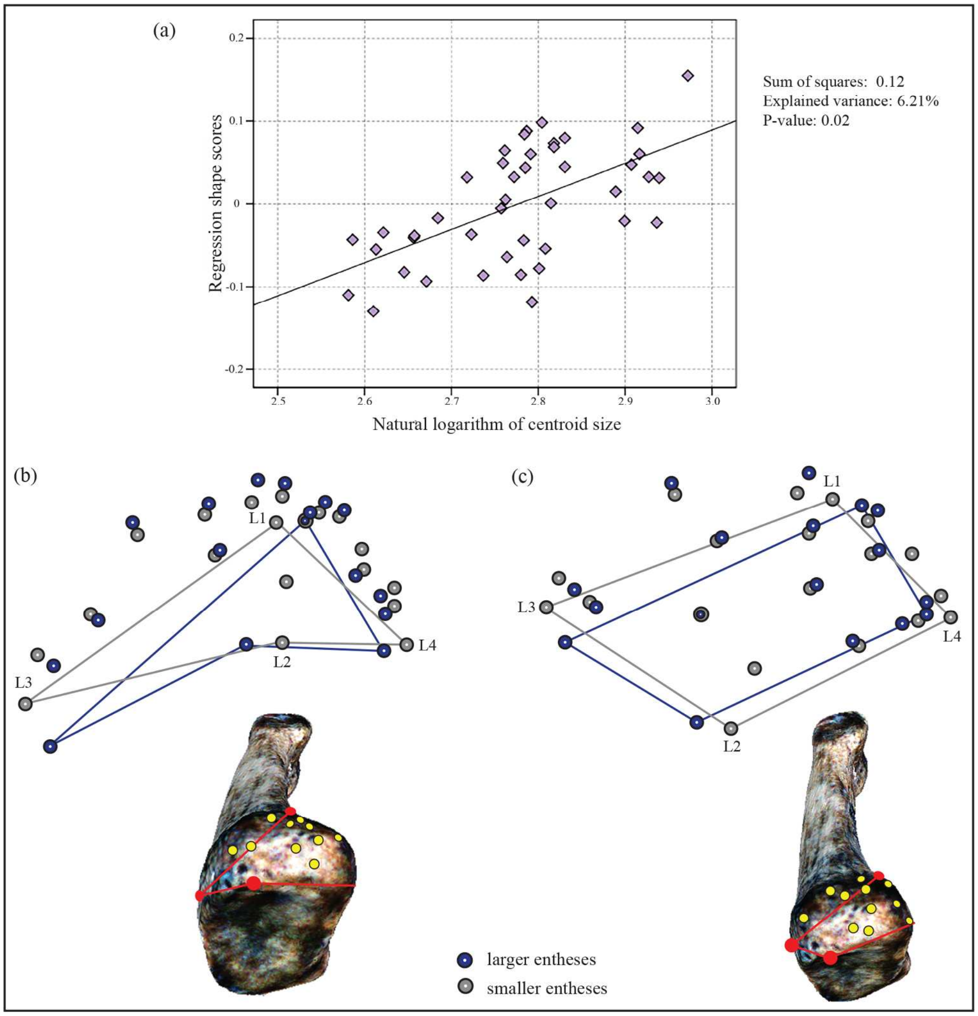

Figure 3. Plot of the shape regression scores against the logarithm of centroid size for the enthesis of abductor digiti minimi/ flexor digiti minimi (a) and graphs depicting allometric shape variation from a proximomedial (b) and a medial (c) view of the bone. In this study, centroid size was highly correlated with 3D raw size (in square millimeters). The proximomedial view indicates which landmarks are more elevated, while the medial aspect shows which surface semilandmarks are placed centrally in the enthesis. The two bottom figures explain the approximate location of landmarks (both fixed and sliding surface semilandmarks) on the bone 3D models. For clearer demonstration, the color histogram of the models is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016).

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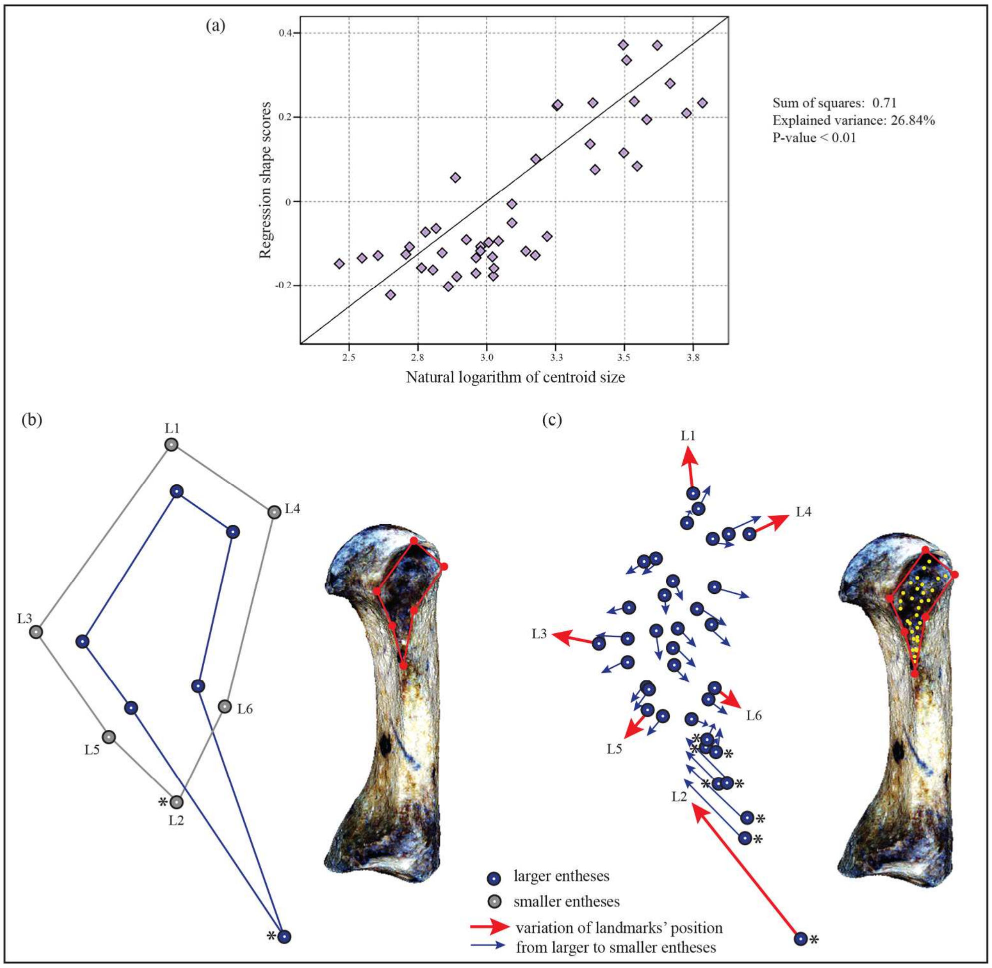

Figure 4. Plot of the shape regression scores against the logarithm of centroid size for the enthesis of opponens pollicis (a) and graphs depicting allometric variation (from a lateral view of the bone) in the position of outline fixed points (b) and surface sliding semilandmarks (c). The regression plot (a) was almost identical between the analysis with all 36 landmarks and the one focusing on the proximal ridge. In this study, centroid size was highly correlated with 3D raw size (in square millimeters). For the semilandmarks, vectors were used to demonstrate variation in surface semilandmarks (blue arrows) and fixed outline landmarks (red arrows). The two bottom figures explain the approximate location of landmarks (both fixed and sliding surface semilandmarks) on the bone 3D models. For clearer demonstration, the color histogram of the models is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016). After exclusion of the eight most proximal landmarks of the ridge (the points indicated with an asterisk), the remaining landmarks on the distal tubercle (over a hypothetical outline curve between L5 and L6) did not present significant shape variation. In all subsamples, the ridge landmarks presented approximately the same direction of shape change.

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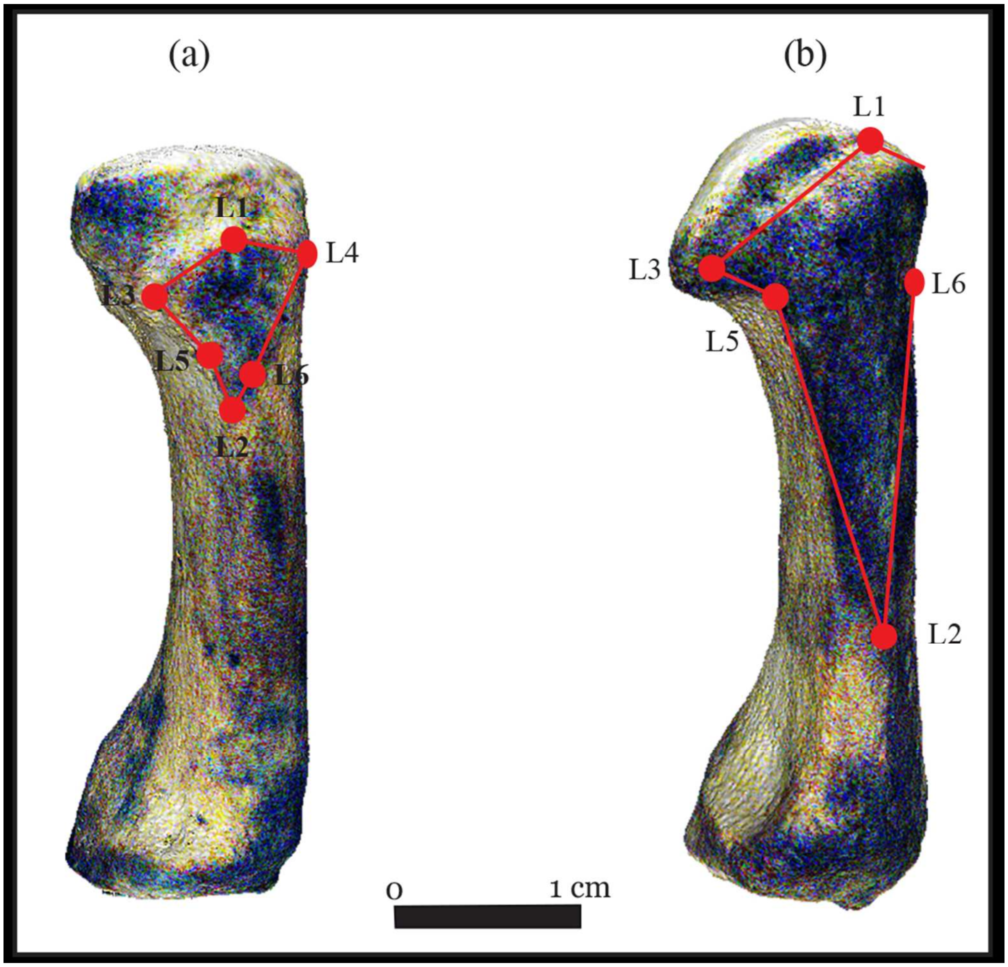

Figure 5. An example of a relatively small and a very large entheseal surface of opponens pollicis (lateral view of the bone). In the depicted 3D models, the color histogram is equalized and expanded to fit all possible colors, as described in Karakostis and Lorenzo (2016). The position of the fixed landmarks (L) is explained in Table 1 and depicted in Figure 1.

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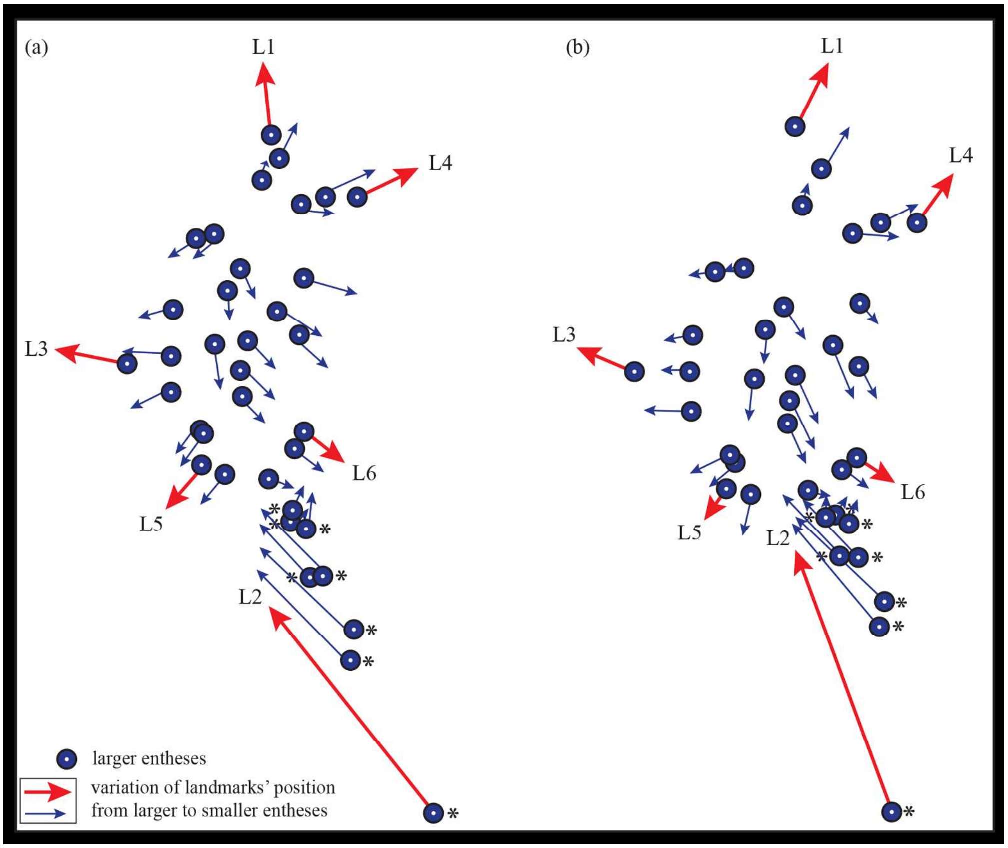

Figure 6. Shape variation in the entheseal surface of opponens pollicis (lateral view of the bone) related to its size (a) and corresponding bone length (b). The position of the fixed landmark points (L) is described in Table 1 and depicted in Figure 1. After exclusion of the eight most proximal landmarks on the ridge (indicated with an asterisk), the remaining landmarks on the distal tubercle (over a hypothetical outline curve between L5 and L6) did not present significant shape variation. In all subsamples, the ridge landmarks presented approximately the same direction of shape change.

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*"This is the pre-peer reviewed version of the following article: [***Is bone elevation in hand muscle attachments associated with biomechanical stress? A histological approach to an anthropological question*****], which has not yet been published."***

Is bone elevation in hand muscle attachments associated with biomechanical stress? A histological approach to an anthropological question

Fotios Alexandros Karakostis [a], Dimitrios Vlachodimitropoulos [b], Maria Piagkou [c], Heike Scherf [a], Katerina Harvati [a,d], and Konstantinos Moraitis [b,*]

[a] Paleoanthropology, Senckenberg Centre for Human Evolution and Paleoenvironment, University of Tübingen, Rümelinstrasse 23, 72070 Tübingen, Germany.
[b] Department of Forensic Medicine and Toxicology, School of Medicine, National and Kapodistrian University of Athens, 75 M. Asias Str., 11527 Athens, Greece
[c] Department of Anatomy, School of Medicine, National and Kapodistrian University of Athens, 75 M. Asias Str., 11527 Athens, Greece
[d] DFG Centre for Advanced Studies "Words, Bones, Genes, Tools: Tracking linguistic, cultural and biological trajectories of the human past", Rümelinstrasse 23, 72070 Tübingen, Germany.

Corresponding author
Konstantinos Moraitis, Ph.D.
Department of Forensic Medicine and Toxicology, School of Medicine,
National and Kapodistrian University of Athens,
75 M. Asias Str., 11527 Athens, Greece
E-mail: kmoraitis@med.uoa.gr (K. Moraitis)
Tel. +30-210-7462426
Fax +30-210-7462390

Abstract

In anthropological sciences, muscle attachments (entheseal bone areas) are often utilized as occupational stress markers for reconstructing the physical activities of past human populations. This approach is based on the concept that entheseal bone morphology is influenced by accumulated biomechanical stress, which is stimulated by systematic and/or intense muscle recruitment. One of the main criteria for assessing the entheseal stage of development involves the proportion of elevated surface area. However, it is not yet clear if relative bone elevation in entheses is associated with the amount of biomechanical forces exerted during physical activity, while the histology of the entheses of the human hand, which is the least-bodyweight-bearing anatomical area, is not fully investigated. Multiple previous studies on entheses have concluded that the amount of calcified fibrocartilage reflects the level of the applied biomechanical forces. On this basis, if hand entheseal surface elevation was associated with the level of biomechanical stress, then a greater concentration of calcified fibrocartilage would be expected in hand entheses' central and more projecting bone areas. More importantly, individual entheses with a greater proportion of elevated bone areas would present a higher total concentration of calcified fibrocartilage. To test these two hypotheses, a histological quantitative analysis was conducted on two thumb entheses of four body donors, who were fully documented for their biological, pathological, and occupational characteristics. The results showed higher levels of calcified fibrocartilage in the central projecting regions of entheses, while the individuals showing additional bone elevation in their marginal entheseal areas comprised substantially higher total values of calcified fibrocartilage. The observations of this pilot study support the concept that interindividual variation in entheseal bone morphology is related to different levels of accumulated biomechanical loading. Future research should employ larger sample sizes to compare individuals with distinct lifelong activities.

Keywords: Biological anthropology, Biomechanics, Manual entheses, Physical activity, Occupational stress markers, Musculoskeletal stress.

1. Introduction[1]

Previous histological research has concluded that variation among animal species in entheseal surface size is correlated with the amount of received biomechanical stress, with larger entheseal areas being adapted for greater muscle forces [1,2]. Furthermore, in human evolution, greater extension of the entheseal areas of *opponens pollicis* and first dorsal interosseous have been associated with greater muscle moment arm (e.g., [3]). In fact, recent anthropological studies found substantial variation in entheseal surface size even among modern human individuals [4,5], with some specimens presenting entheseal surfaces with a substantially greater proportion of elevated area than others (e.g., Figure 1) [5,6,7]. In this framework, identifying a consistent relationship between biomechanical forces and entheseal surface bone projection in humans would suggest that interindividual differences in the proportion of elevation coincide with varying levels of biomechanical stress. This could be induced by habitual physical activity [8] and/or perhaps affected by a genetic design [9] regulating the capacity of individuals for producing muscle force. If such a concept is verified, future interpretations of entheseal studies on human skeletal remains could provide further understanding of past habitual activities and the evolution of human biomechanics.

Fibrocartilaginous entheses typically consist of four distinct zones of tissue: pure tendon, uncalcified fibrocartilage, calcified fibrocartilage (CF), and cortical bone [10]. The zone of CF comprises the actual point of union between soft tissues and bone [11]. It performs a fundamental role in anchoring the tendon onto the bone and providing adequate resistance to shear, as tendons tend to pull on bones from oblique angles [12]. More importantly, the zone of CF -unlike its uncalcified counterpart- occasionally tends to persist in dried bone tissue [10], which makes it potentially useful for scientific fields focusing on human skeletal remains.

Even though the dimensions of the cortical bone layer have been associated with the degree of biomechanical strain, it is not yet clear if its morphology is highly regulated by genetic and/or various systemic factors [9]. By contrast, based on a plethora of studies, the thickness and distribution of CF within entheses have been directly associated with the levels of applied biomechanical stress [11-18]. For instance, recent studies have interpreted differences in the

[1] Abbreviations: CF, calcified fibrocartilage; 3D, three-dimensional.

amount of CF between specific human entheses as a result of variation in the amount of accumulated biomechanical stress [17-19]. Furthermore, older research on various muscle attachments of the lower limbs has shown that, in some entheses (e.g., on the lesser trochanter of the femur), the highest proportion of CF is found at their most central bone parts, while their marginal parts present scarcer evidence of CF [17,19,20]. Nevertheless, another study reported differences across three entheses in the distribution of calcified tissue, with one of the analyzed entheseal areas (i.e., the insertion site of the patellar ligament) not presenting a higher concentration at its most superficial point [14]. This variability of the calcified tissue's distribution among different entheses indicates that further research is required in additional anatomical regions, in order to further investigate the relationship between biomechanical stress and entheseal form in humans. Furthermore, to our knowledge, previous histological studies on entheses have not focused on interindividual variation in the pattern of CF and its potential relationship with the specimens' biological and occupational characteristics. Such information could provide an insight on whether the patterns of entheseal CF are affected by known potential factors of morphological variation in entheses [21], such as population, sex, age, various body dimensions, direct relatedness, pathology, and occupational activities.

It has been proposed that the form of entheseal bone surfaces is influenced by the combined effect of opposing biomechanical forces applied on them by body weight and muscle contraction [22,23]. As a result, the form of entheseal areas (including the proportion of elevated area) adapts for the purpose of withstanding the average of the two sets of loads applied (body weight forces versus tendon forces) [23]. Based on this concept, the relative amount of body weight applied on an enthesis performs a vital role in its surface development. Among the human skeleton's anatomical areas, the hand shares the smallest proportion of body weight. Particularly, the hand weights approximately 0.50% of total body mass in females, and 0.66% in males [24]. In fact, extrinsic hand tendons are relatively long and rise before the muscle reaches the hand [25,26]. Thus, the weight of the bulky muscles is mainly applied on the forearm and not the wrist or hand segments. This overall configuration suggests that the human hand is genetically designed to be affected by the forces of body weight as little as possible [26]. If the aforementioned concept regarding body weight is valid [23], one could assume that, in comparison to other anatomical areas, the proportion of tendon forces to body weight forces can potentially be greater for manual entheses.

However, the distribution of CF in human hand entheses has not been thoroughly investigated [26-29], while there is controversy in the anthropological literature on whether biomechanical forces have an observable effect on the morphology of entheseal hand bone surfaces [5,30]. As demonstrated in the example of Figure 1, the level and proportion of elevated bone area within human hand entheses presents great variability, even between individuals with almost identical biological profiles (population, sex, age, stature, and hand bone length). If the projecting bone parts of hand entheses were consistently associated with greater and/or more frequent biomechanical stress, then entheses with a greater proportion of elevation over the bone element's surface (see Figure 1a) would be likely linked to more intense and/or systematic biomechanical stress. For that to be the case, given that the distribution of stress within the entheseal area is reflected on the level of CF [14,17], it would be expected that the elevated bone areas of hand entheses (i.e., for this study, the lateral tubercles comprising the central portion of the enthesis) present a substantially greater concentration of CF than their marginal and flatter bone areas (i.e., the areas surrounding the tubercle, in the palmar and dorsal aspects of entheseal surfaces). Furthermore, it would be expected that individual entheses which present an additional bone projection in their marginal areas (e.g., Figure 1a) comprise a higher total quantity of CF. To test this hypothesis for hand entheses, this study conducts a histological analysis of the entheses corresponding to the left thumb's thenar muscles. The material utilized involves a small sample obtained from cadaveric donors who are fully documented for their biological, pathological, and occupational characteristics.

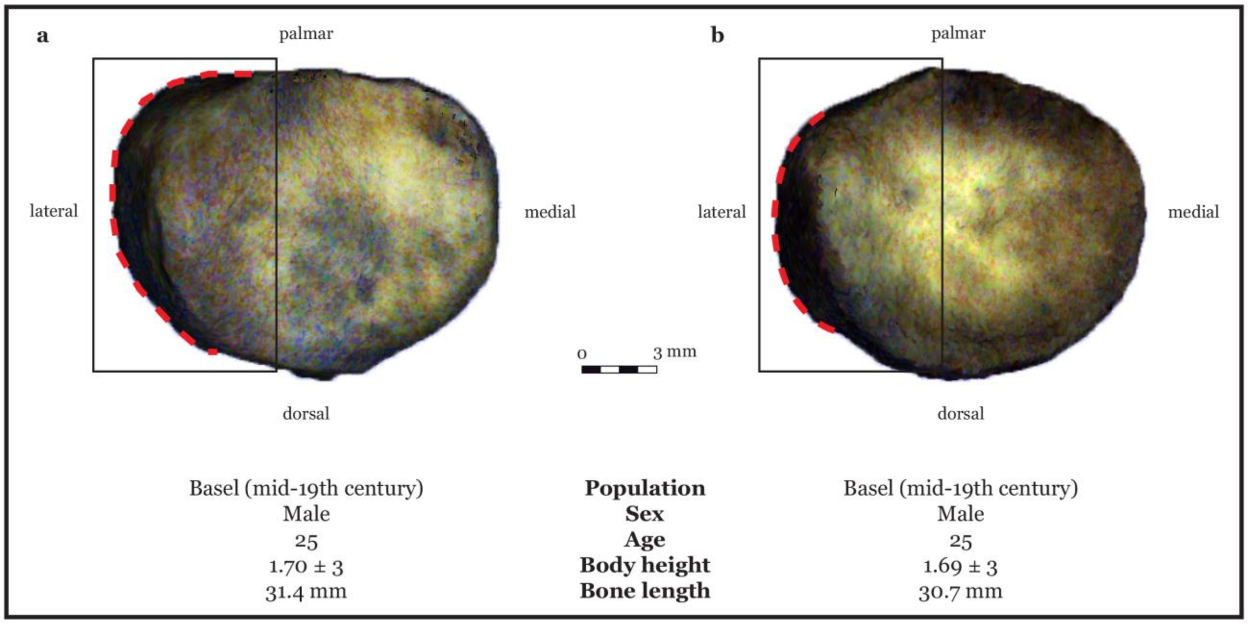

Figure 1. Proximal aspect of the phalangeal enthesis of the left *abductor pollicis brevis / flexor pollicis brevis*, in two individuals with very similar biological profiles (from the documented anthropological collection "Spitalfriedhof Saint Johann" in the Natural History Museum of Basel, Switzerland). The black rectangles indicate the area of attachment on the first proximal phalanx, while the lateral tubercles of the entheses are indicated with red dashed lines. The left specimen (a) presents elevation at its lateropalmar aspect, whereas the tubercle of the right specimen (b) is restricted to the lateral surface of the bone.

2. Materials and Methods

2.1 Sampling strategy

The sample comprises the hands of four formalin-embalmed cadavers (three males and one female) dissected in the Department of Anatomy at the Medical School of the National and Kapodistrian University of Athens (Greece). The individuals derived from a body donation program after a written informed consent. These four particular specimens were selected because they were extensively documented (population, sex, age, body weight, stature, hand length, direct relatedness, pathologies, and occupation), while three of them were of relatively young age-at-death (Table 1). Particularly, given that previous research has shown that entheses undergo substantial degenerative changes after around the age of 60 [31,32], the age-at-death of the three male specimens ranged between 45 and 55. The fourth individual was a 73-year-old female with smaller body and hand dimensions, which was included in the sample for assessing whether the results would differ for a healthy individual of distinct characteristics (Table 1). Based on their documentation, the four individuals were not directly related to each other and presented no pathology related to their hands or manual abilities. All individuals were of Greek origin and lived in the city of Athens, mainly during the second half of the 20th century.

Individual	**Sex**	**Age (years)**	**Body weight (kg)**	**Stature (cm)**	**Hand length (cm)**	**Occupation**
1	M	45	70	172	17,9	Small merchant
2	M	50	82	178	18,6	School teacher
3	M	55	87	174	19,2	Driver / carrier
4	F	73	75	167	16,5	Unemployed

Table 1. The characteristics of the four individuals analyzed, which were of Greek origin (late 20th century), non-related, and without manual pathologies.

For each individual, the material of this study involved two entheses of the left thumb (tendon, fibrocartilage zones, and bone) which comprise the insertion points for three of the four thenar muscles. The insertion area of the remaining thenar muscle (*adductor pollicis*) was not included as it presented scarce evidence of CF in the specimens of this study, possibly suggesting a different nature of attachment for this muscle. Following the recommendations of previous research on hand entheses [5], the two selected areas were located at the lateral distal aspect of the first metacarpal (within the wider attachment area of *opponens pollicis*, the small ridge near the metacarpal head) and the base of the proximal phalanx (the entire lateral tubercle accommodating the insertion points of both *abductor pollicis* and *flexor pollicis brevis*) [25,33]. These areas were selected because a previous study has presented statistical evidence that they contribute substantially to morphometric patterns reflecting habitual manual activity [5].

Furthermore, the thenar muscles play a key role in the performance of fundamental human hand grips, including those that involve thumb opposition [34].

2.2 Anatomical preparation and sectioning

Initially, all dissected hands were fixed in a formalin-phenol-alcohol solution. Skin incision was made in the wrist and palm. After removal of the skin and palmar aponeurosis, the transverse carpal ligament was divided by a sagittal incision between the thenar and hypothenar muscles. The palmar aponeurosis was detached from the flexor retinaculum and the skin of the dorsum of the hand was also removed. After the careful removal of the fascia over the thenar eminence, the recurrent branch of the median nerve was detected, as entering the thenar muscles, approximately three centimeters below the scaphoid tubercle. The next step was the cleaning of the fascia from the anterior aspect of the thumb and the exposing of the fibrous tendon sheath. Separation of the most superficial muscles' layer (*abductor pollicis brevis* and the adjacent *flexor pollicis brevis*) from the underlying *opponens pollicis* followed. The belly of the *abductor pollicis brevis* was cut off and reflected to its end to expose the *opponens pollicis*, as it is inserting along the proximal anterior surface of the first metacarpal bone (Figure 2a).

Subsequently, the four investigated thumb segments (distal portion of the metacarpal and proximal part of the proximal phalanx) were dissected. Each segment was then trimmed, using a different direction for each bone: proximodistal for the metacarpal and distaloproximal for the proximal phalanx (Figure 2b). Subsequently, the paraffin-embedded tissue blocks containing entheses were sectioned to 1.5 μm at right angles to the bone surface (Figure 2c). For both entheses, the sections were sliced in the transverse plane of the bone, in order to capture the longest axis of the entheseal bone surface (as this can be observed in the dry bone models of Figure 1). The fact that this was the longest axis of each muscle attachment in the specific specimens was visually verified before the sectioning process. It should be mentioned that human entheseal shape presents extensive variability and the same hand entheses can present different proportions in other samples [5]. For each enthesis, five sections were systematically sampled at five equal intervals of 200 μm [14], starting from the most central point of the muscle attachment. These sections were stained using the standard Masson's trichrome method.

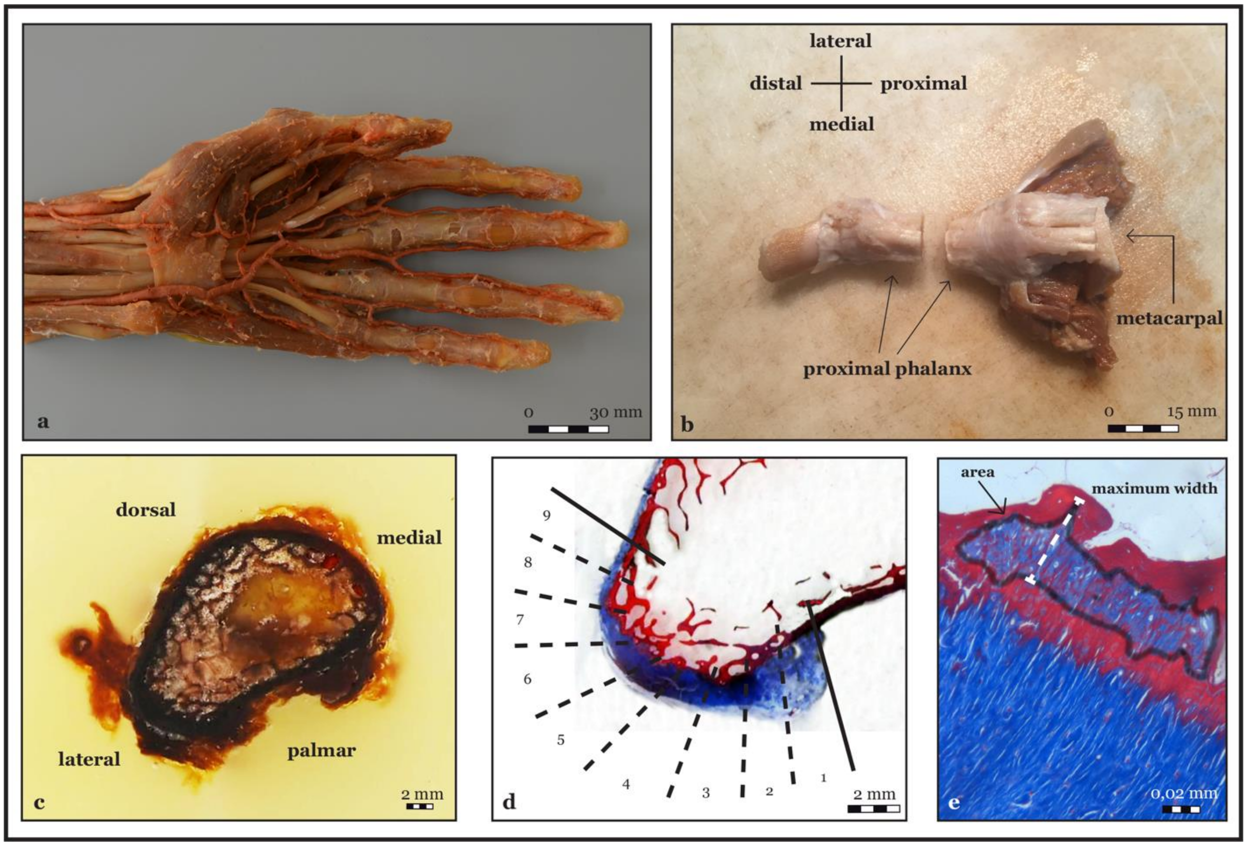

Figure 2. Steps of the analytical process: (a) anatomically prepared cadaveric hand, (b) trimming of thumb segment after formalin fixation and bone decalcification, (c) paraffin-embedded tissue block (proximal phalanx depicted), (d) stained histological section and separation of the bone area of each enthesis into nine equally-spaced regions (*abductor pollicis / flexor pollicis brevis* depicted), (e) digital quantification of the calcified fibrocartilage's maximum width in mm (white dashed lines) and area in mm^2 (black outline).

2.3 Delimitation and observation of entheseal regions

On each sampled section, the two borders of the tendon-bone attachment were determined based on systematic macroscopic and microscopic observations of the intersection between tendon and bone (Figure 2d). Then, ten equally-spaced marks (1-10) were defined from one border of the tendon-bone attachment to its opposite one using high-resolution digital photographs obtained by a Leica DFC500 camera (Leica Microsystems, Wetzlar, Germany) mounted on an optical microscope. The software package IC-Measure (The Imaging Source, Bremen, Germany) was utilized (Figure 2d). The two borders were located at the most palmaromedial (point 1) and most dorsomedial (point 10) points of the entheses, respectively. These marks separated each muscle attachment into nine equally-spaced regions (1-9). Based on systematic observations on both entheseal areas of the four analyzed specimens (before, during, and following the sectioning process), the bones' lateral aspect at this point was entirely represented by the elevated portion of the insertion area (i.e., the projecting tubercle). This lateral location and extent of the tubercles are also consistent with the form observed in both dry bone models of Figure 1 as well as in the large samples of two previous studies [4,5].

More importantly, prior to sectioning (Figure 2b), it was observed that the tubercles of specific individual entheses extended towards the palmar and/or dorsal aspect of the bone surface (as in the example of Figure 1a), without presenting traces of pathology. In the sampled sections of the same entheses, the bone outlines appeared elevated in the corresponding palmar and/or dorsal regions. In the example of Figure 2d, the lateral aspect of the bone's outline spanned from the middle of region 3 to the entire region 7. However, entheseal region 2, which lies in the palmarolateral aspect of the bone outline, presented a bone projection continuous to the lateral surface of the bone. This feature resembled the morphology of the dry bone model of Figure 1a, where the palmarolateral part of the phalangeal base is projecting. Overall, in this study's sample, both entheses of two individuals (specimens 1 and 3) showed this bone projection in at least one of their marginal areas (palmarly and/or dorsally), whereas the exactly equivalent equally-spaced bone regions were relatively flat (as in region 1 of Figure 2d) in both entheses of the other two specimens. This is in spite of the fact that these peripheral regions also comprised areas of muscle attachment in all individuals. Furthermore, the number of marginal regions with projecting bone surface also differed between the two entheses investigated (*opponens pollicis* and *abductor pollicis* / *flexor pollicis brevis*). Overall, the exact flatter bone regions for each individual enthesis in the sample can be assessed further below, in the plots of the Results section.

2.4 Measurements and comparative analysis

All sections were observed under an optical microscope using objectives of both 20 and 40 magnifications. High-resolution photographs were taken across the entheseal regions using the Leica

DFC500 camera with 12 Megapixel Power. Initially, variation in the amount of CF across the nine equally-spaced regions was assessed visually, given the profound differences observed between the entheseal periphery and the central elevated plateau. Subsequently, a quantitative approach was performed based on the total area (in mm^2) and the maximum width (in mm) of the CF zone (i.e., the distance from CF's deepest point to the tidemark) (Figure 2e). The area of CF was included in the measurements because it comprises a representation of its total concentration in each region [17].

Specifically, the photographs were imported into a computer, where measurements were taken using the software IC-Measure. On each of the five sections, the total area and maximum width of the CF zone were quantified for each equally-spaced region (Figure 2e) [14,17]. Then, using all five measurements from the five sections, the mean values were computed for each equally-spaced region in this enthesis. These mean values were compared across individuals and entheses through graphs, in order to assess whether the marginal areas (palmar and/or dorsal regions) consistently present less CF than the lateral elevated regions and whether entheses with projecting marginal regions comprise a higher concentration of CF. For the same purpose, the total CF area of each section (including all nine regions) was calculated and the mean value for each individual enthesis was computed based on the total CF areas of all five sections. This statistic was utilized as a measure of overall CF concentration in this enthesis. Additionally, the relative size of mean total CF area was included in the analysis, as a form of standardization for entheseal raw size [17]. This was computed by dividing the mean total CF area to the mean length of the corresponding enthesis (among its five sampled sections). This measurement (mean entheseal length) was defined as the length of the calcified tissue's profile (in mm) across the area of tendon-bone attachment [17] (Figure 2d). After a period of one month, the same author (FAK) repeated all measurements for all nine regions of two randomly selected sections per individual (one for each enthesis) and measurement precision was evaluated using paired t-tests. Based on the results (p-values> 0.05), there was no significant intra-observer measurement error.

3. Results

A series of systematic microscopic observations demonstrated that, within each individual enthesis of the sample, all lateral -i.e., central- bone regions presented a visually greater concentration of CF than the peripheral -dorsal and palmar- ones (Figure 3). These observations were also verified by the quantitative approach. As demonstrated in Figures 4 and 5, the zone of CF reached consistently greater values (area and width) in the lateral regions of entheses. By contrast, all marginal areas without bone projection comprised less than 0.01 mm^2 of mean CF area (Figure 5) and 0.04 mm of mean CF maximum width (Figure 4). This was also the case for specimen 4, whose characteristics were distinct in the sample (Table 1).

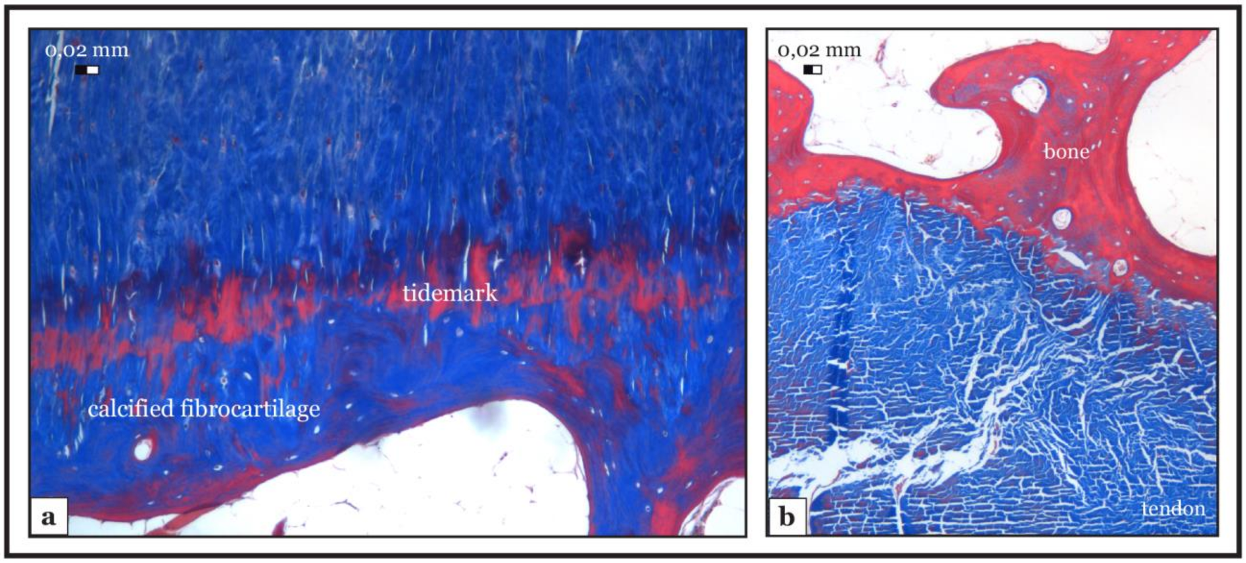

Figure 3. Example of high concentration of calcified fibrocartilage in a central (a) and a marginal (b) region of the analyzed hand entheses.

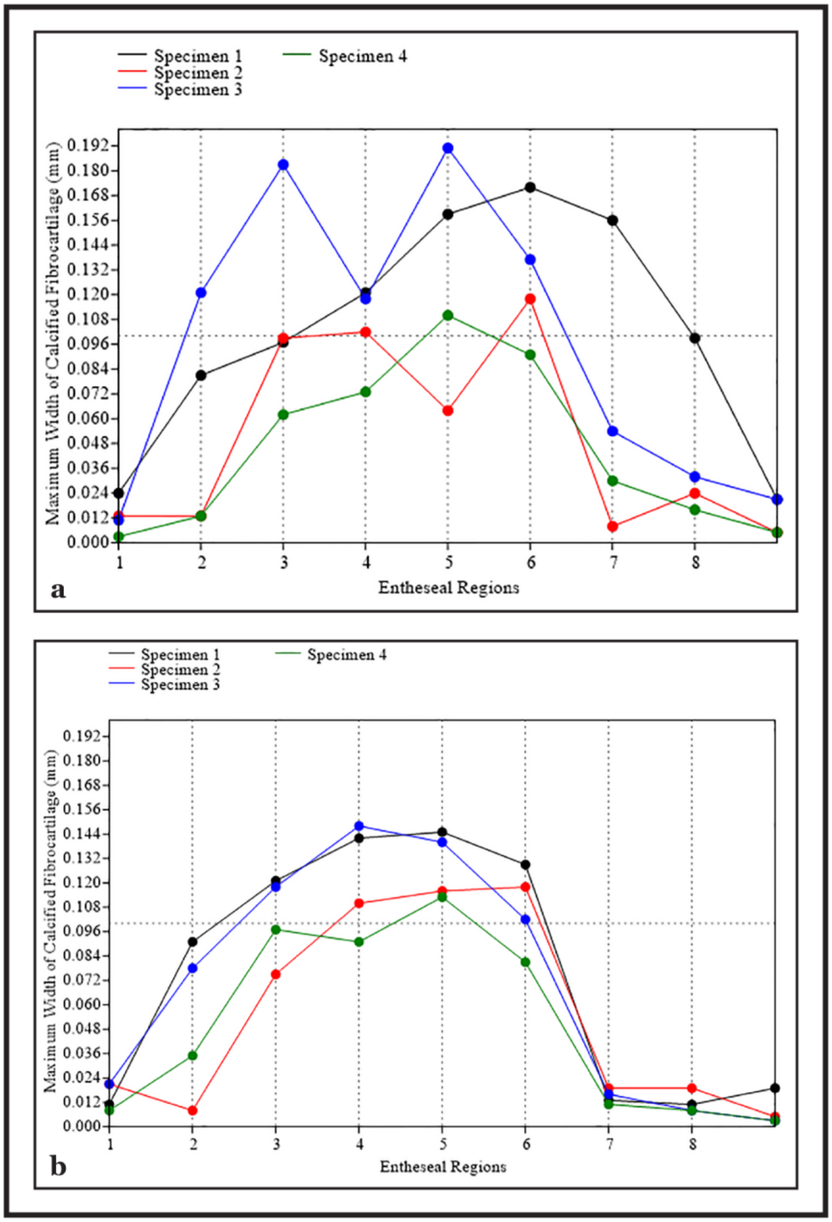

Figure 4. Plot demonstrating the mean maximum width (in mm) of calcified fibrocartilage across the nine regions of each individual enthesis for (a) the common insertion point of abductor *pollicis / flexor pollicis brevis* and (b) the insertion area of *opponens pollicis*. The values of all regions with flatter bone outline were below 0.04 mm.

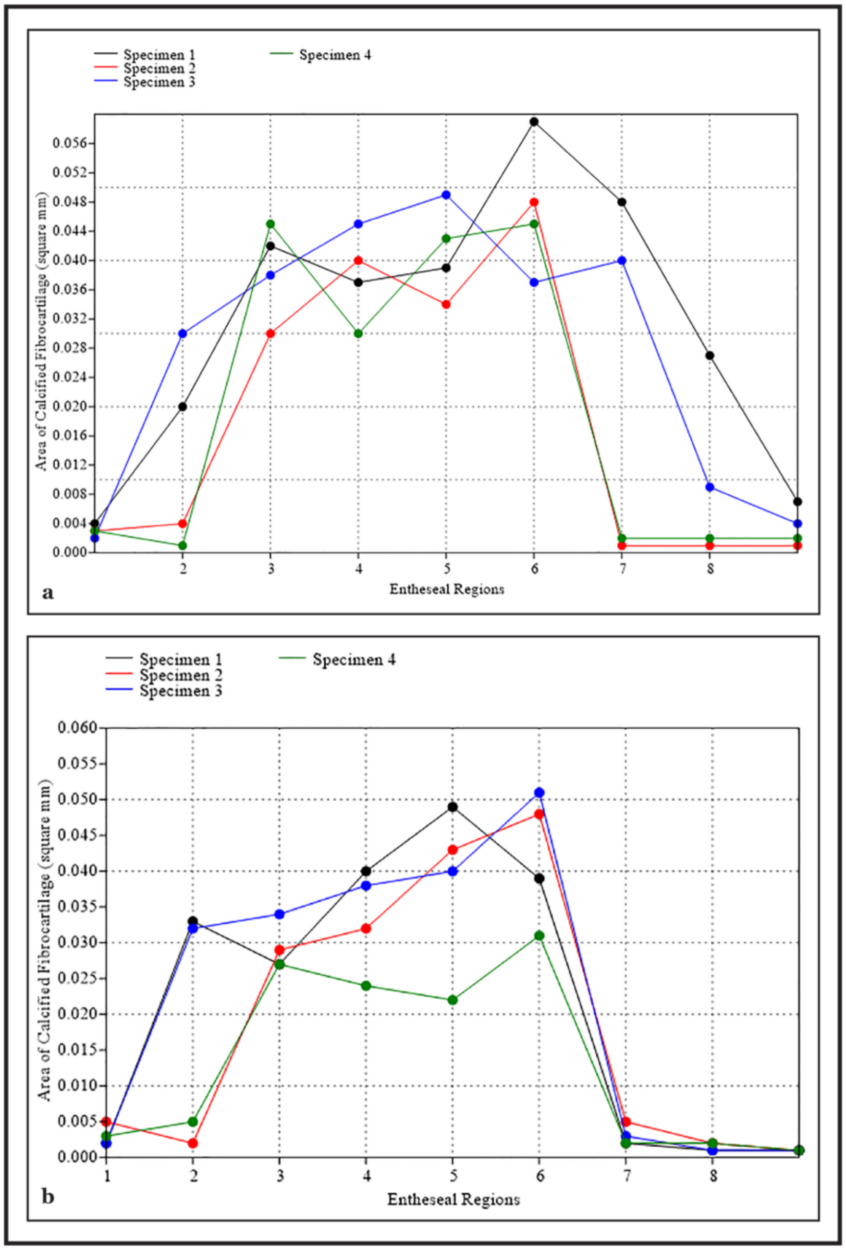

Figure 5. Plot demonstrating the mean area (in mm^2) of calcified fibrocartilage across the nine regions of each individual enthesis for (a) the common insertion point of *abductor pollicis / flexor pollicis brevis* and (b) the insertion area of *opponens pollicis*. The values of all regions with flatter bone outline were below 0.01 mm^2.

Among the five sections of each individual enthesis (located at its most central portion), there was relatively low variation of CF values for each equally-spaced region, while bone projection occurred in the same marginal regions. Across individual entheses, the statistical range (i.e., maximum value-minimum value) of CF maximum width for the same region varied from 0.003 to 0.005 mm among marginal areas and from 0.013 to 0.026 mm among central elevated regions. In CF area, this range varied from 0.002 to 0.005 mm^2 among marginal regions and 0.011 to 0.018 mm^2 for central elevated areas. All five sections of each individual enthesis showed the same pattern of CF distribution among regions, which is represented by their corresponding mean values in the plots of Figures 4 and 5. This similarity suggests a general consistency of the observed CF patterns within the most central entheseal portion.

Nevertheless, the results indicate substantial inderindividual variability, with the entheses of individuals 1 and 3 presenting greater CF values in most regions (Figures 4 and 5) as well as in mean total -raw and relative- areas (Tables 2 and 3). At the same time, both entheses of these two individuals presented bone elevation in some of their marginal palmar and/or dorsal regions (Figures 4 and 5). By contrast, specimens 2 and 4 showed lower values of CF in combination with the absence of bone projection in their peripheral entheseal regions. Between entheses, for all four specimens, the common insertion point of *abductor pollicis* and *flexor pollicis brevis* presented a substantially greater amount of CF than the muscle attachment of *opponens pollicis*, in which specific marginal bone regions were not projecting in all four individuals (Figures 4 and 5).

Both in maximum width and area of CF, each enthesis presented one region with greater mean concentration of CF than all others. In most cases, this was either region 5 or 6, in the middle of the central elevated plateau of the tubercles. Moreover, in multiple cases (e.g., the CF area of *abductor pollicis* / *flexor pollicis brevis*), there was a secondary region in each enthesis with considerably greater mean CF than its surrounding entheseal parts. This was usually either region 3 or 4 (Figures 5 and 6).

Individual	***Opponens pollicis***	***Abductor pollicis / flexor pollicis brevis***
1	0.194	0.283
2	0.167	0.162
3	0.202	0.254
4	0.117	0.173

Table 2. Mean total area (in mm^2) of calcified fibrocartilage for each individual enthesis across all nine regions.

Individual	***Opponens pollicis***	***Abductor pollicis* / *flexor pollicis brevis***
1	0.017	0.023
2	0.015	0.018
3	0.018	0.021
4	0.014	0.018

Table 3. Relative size of mean total area of calcified fibrocartilage for each individual enthesis across all nine regions.

4. Discussion

A series of previous studies have addressed the distribution of CF and/or total calcified tissue in entheseal surfaces located at high-weight-bearing anatomical regions of the human skeleton; mainly the proximal femur, the knee, and the proximal tibia (e.g., [14,17-19,20,35]). Shea et al. [20] performed a histological analysis of the lesser trochanter, reporting that its most superficial parts (numbers 3 and 4) presented a higher proportion of CF. Similarly, Evans et al. [14] performed a similar analysis on the insertion of the *quadriceps* muscle as well as the origin (on the patella) and insertion (on the tibia) sites of the patellar ligament. Even though the measurement of calcified tissue used in their analysis included the width of the bone layer (which could perhaps be highly affected by genetic factors [9]), it should be mentioned that its values were also higher at the most superficial -and most projecting- areas of the origin attachment site of the patellar ligament and the enthesis of the *quadriceps* muscle. This was not the case, however, for the insertion point of the patellar ligament, where all areas (superficial and deep) presented a similar proportion of calcified tissue [14]. Another two recent works focused on the two attachment areas of the anterior cruciate ligament in the tibia and femur [17,19], reporting a higher proportion of CF at the central region of entheses (i.e., the "middle 50%"). In fact, one of these studies [17] found substantially greater levels of CF in the femoral enthesis of this ligament, interpreting it as the result of greater tensile forces applied on this area. Similarly, another recent study [18] reported that the insertion site of quadriceps on the patella contained a greater width of CF than the patellar ligament insertion in the tibia. These authors also associated this variation in CF with the different level of exerted biomechanical forces between the two entheses.

Our study extended the investigation of CF's distribution pattern to the muscle entheses of the human hand, which is the least-bodyweight-bearing element in the human skeleton [24]. Our observations indicated a strong occurrence of the CF patterns previously reported for entheses in high-bodyweight-bearing skeletal elements: the central area of the analyzed entheses presented a substantial concentration of CF, which was scarce and frequently absent in their marginal regions (e.g., Figure 3). The fact that peripheral regions of hand entheses contained limited CF could indicate a different nature of insertion -and biomechanical forces involved- for these areas [19]. This is in line with previous research demonstrating that some entheses present fibrocartilage only in their central areas (e.g., [19]). Our findings of different CF levels within the central elevated portion of entheses-including the presence of a "peak" region for CF (Figures 4 and 5)- support the general hypothesis that some of their central areas receive greater compressive stress than others [14].

Since individual entheses with bone projection in their peripheral areas contained a greater total concentration of CF (i.e., a biomechanical stress indicator), it could be argued that interindividual variation in the extent of bone elevation (e.g., between the two examples of Figure 1) likely reflects different levels of biomechanical stress applied on entheses during muscle contraction, in combination with the effects of various potential factors, such as genes, nutrition, or hormones [9,21]. Based on this concept, the different CF levels across non-pathological individuals or entheses (*opponens pollicis* and *abductor pollicis* / *flexor pollicis brevis*) are likely due to varying amount of accumulated biomechanical loading [18]. The greater amount of CF in the phalangeal enthesis could be perhaps related to the fact that stress is transmitted to the same wider area by two different muscles. Additionally, the fundamental function of *opponens pollicis* is related to thumb opposition, a hand movement which also involves synergistic recruitment of the muscles *abductor pollicis* and *flexor pollicis brevis* [36].

Alternatively, one could argue that both CF concentration and bone elevation may be regulated by a confounding factor, such as interindividual genetic variability [9]. In that case, this relationship between bone elevation and CF would result from a different genetic design for each specimen. Nevertheless, considering the repeatedly reported association between CF and compressive load [11,17], such a hypothesis would still imply that a genetic design involving greater entheseal bone elevation in some individuals is linked to receiving a greater amount of compressive forces. It should be highlighted that previous research has reported a significant correlation between entheseal surface size and biomechanical forces among different animal species [1]. The observations of the present study imply that this may also be the case for within-species variation in entheseal morphology, suggesting that the proportion of entheseal bone elevation could be an indicator of an individual's capacity (genetic design and systemic factors) and/or behavior (habitual physical activity) for producing a certain amount of muscle forces. In any case, this apparent relationship between CF and projecting bone regions of hand entheses provides an argument for using the proportion of hand entheseal elevated area in comparative analyses (e.g., in paleoanthropology or bioarchaeology) as an indirect indicator of the level of accumulated biomechanical stress exerted on the bone during long-term muscle recruitment.

The calcified tissue of entheses is also influenced by biological age, as a consequence of degenerative changes causing a gradual decrease of cortical bone thickness which leads to a proportional increase of CF [13,37]. However, this study focused on variation in the raw dimensions of CF and not its proportional size in the enthesis. Additionally, old age has an extensive effect on the mechanisms of bone turnover [38] and entheseal change [31,32]. The oldest individual (specimen 4) presented a comparatively low total concentration of CF in both area (Tables 2 and 3) and maximum width (Figures 4 and 5). Interestingly, the entheses of this specimen were not marginally elevated (based on observations both before and after sectioning), in spite of previous research reporting greater hand entheseal size in older individuals [5]. It is not clear whether the lower CF levels (and smaller extent of bone elevation) in this specimen are related to its different age-at-death, sexual dimorphism, stature, body mass, hand length, genes, or nature of occupational activities (Table 1). It should be mentioned that specimen 2, which showed similarly low values, was a much younger male. Overall, the results demonstrated that two healthy individuals with rather distinct biological profiles (sex, age, stature, body mass, and hand length) can possibly present similar levels of CF for the same hand enthesis (e.g., specimens 2 and 4), while two biologically similar individuals (specimens 2 and 3) can vary substantially in hand entheseal CF (Tables 1, 2, and 3). In fact, it seems that an individual of relatively small body and hand dimensions (specimen 1) can present greater CF values in its manual entheses than a considerably larger individual (specimen 2). Even though the occupational documentation of the sample does not include details on the individuals' habitual activities, one could argue that specimens 2 and 4 (who showed similarly low values of CF) were involved in seemingly less strenuous jobs than individuals 1 and 3 (who presented similarly high values of CF) (Table 1). Overall, the above observations point out the value of performing further research on the effect of various factors (age, sex, stature, body mass, and occupational activities) on the interaction between entheseal bone elevation and CF levels by applying statistical analyses on an adequate number of individuals with different profiles. For instance, future research would ideally include specimens involved in distinct lifelong occupational activities, so as to re-evaluate recent anthropological hypotheses concerning the effect of habitual manual physical activity on multivariate patterns of hand bone entheses [5].

A previous study has questioned the effect of human manual muscle contraction on hand entheseal surfaces, focusing on the insertion sites of *opponens pollicis* and *opponens digiti minimi* [30]. That previous work found no direct linear correlation between certain muscle measurements and entheseal surface size in a sample of 23 specimens. However, those results were likely biased by three fundamental issues. Primarily, the age-at-death of the sample was very old (77.9±12 years), in spite of the fact that, after around the age of 60, the mechanisms of entheseal change (including the rates of bone remodeling and turnover) undergo substantial degenerative changes [31,32,38,39]. As a consequence, the three-dimensional size of entheseal areas tends to be significantly larger in older individuals [5,40], probably due to various degenerative effects in combination with lifelong accumulation of biomechanical stress [31]. If this information is considered together with the fact that old individuals typically present decreased muscle dimensions and physical activity levels [38,39], a direct linear association between their entheseal sizes and exact muscle measurements could hardly be expected. Secondly, that previous study's hypothesis and interpretations did not take into account whether human bone responds to biomechanical loading in the same way as soft tissue. Previous research has shown that new bone formation as a response to biomechanical stress and bone remodeling is typically complete after approximately four to eight months in average [41], while there are experimental cases in which the levels of bone formation (based on biochemical markers) in adult athletes remained relatively constant over 12 months of systematic training [42]. This process can be even slower for bone in fibrocartilaginous entheses, as these comprise gradients preventing stress concentration by dissipating it across the entire attachment area [13]. On the contrary, several experimental studies have shown that muscular hypertrophy and increase in physiological cross-sectional area (a measure based on muscle mass and fiber length) are observable already within two months of systematic physical exercise [43-46]. Given these differences between the tissues and considering the old age of that previous study's specimens, if the intensity of the individuals' manual activities changed for the last few months before death, the effect of these final conditions would probably be much higher on their muscle architecture compared to the underlying bone tissue. In view of the above facts, a direct linear correlation between muscle dimensions and entheseal bone surface size should rather be expected in a sample of relatively young individuals, who were involved in various long-term and consistent physical activities, and whose lifestyle did not alter for a considerable amount of time before death [5]. More importantly, all obtained measurements would have to be controlled for factors such as age, body size, and occupational activity [21]. Finally, it should be mentioned that the structure depicted in [30] as the quantified entheseal area consists of a narrow ridge located near the midshaft of the fifth metacarpal. However, a previous study led by one of us (F.A.K.) found that this type of narrow ridges along metacarpal diaphyses (within the wider entheseal area on the bone) may not be compatible for comparative analyses because they are not visible in all individuals and their exact location on the bone is frequently not homologous among different specimens [4].

Overall, one could argue that the form of hand entheseal surfaces does not necessarily reflect the muscles' dimensions at the time of death but rather the accumulated loading history of each individual. This possibility would explain why older specimens present significantly larger hand entheseal surfaces [5] in spite of age's effect on muscle mass [39], while particular hand entheses are proportionally larger in some individuals depending on their lifelong occupational activities [5].

5. Conclusions

The observations made in this small-scale pilot study suggest an association between hand entheseal morphology and levels of CF, which is closely related to biomechanical stress exerted during muscle contraction [11,13,14,17,18]. As a consequence, non-pathological individual entheses with bone projection in their peripheral areas contained a higher total amount of CF, both in raw and relative size. If manual entheseal surfaces presenting bone elevation in their marginal areas (i.e., higher total quantity of CF) are associated with more frequent and/or intense mechanical strain, then future anthropological studies on occupational stress markers could use this bone projection as an indicator of increased manual biomechanical stress. This concept is in agreement with the conclusions of a previous osteological study led by one of us (F.A.K.), which found different multivariate patterns of hand entheses between individual groups with extensive lifelong occupational differences [5]. Additionally, future anthropological research on musculoskeletal stress markers may benefit from including the quantity and distribution of CF in their entheseal analyses, given that this is occasionally preserved in dried bone remains [10].

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Disclosure

Hereby we declare no conflict of interest.

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CURRICULUM VITAE

Following Fotios Alexandros Karakostis' graduation with a degree of Archaeology (with honors) from the University of Athens, he was awarded from the European Commission with a two-year scholarship for completing the International Master on Quaternary and Prehistory (in Paris, France and in Tarragona, Spain), in which he specialized in Paleoanthropology. After graduating with distinction, he was given a scholarship by the D.A.A.D. and Leventis Foundation for pursuing a PhD in the University of Tuebingen, under the supervision of Professors Katerina Harvati and Joachim Wahl. Mr. Karakostis' area of expertise is hand biomechanics: he is mainly focusing on developing new methods for reconstructing physical activities based on areas of muscle attachments on the human hand bones. Mr. Karakostis has published several articles on hand bone morphology and biomechanics (c.f., List of Publications). Additionally, he has performed field and/or laboratory work in several countries, including Philippines, Spain, France, Israel, Greece, Germany and others.

PUBLICATION LIST

JOURNALS AND BOOK CHAPTERS (PEER-REVIEWED)

1. **Karakostis, F. A.**, Zorba, E., Moraitis, K. (2013). Sexual dimorphism of proximal hand phalanges. *International Journal of Osteoarchaeology*, 25, 733-742.

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3. **Karakostis, F. A.**, Zorba, E., Moraitis, K. (2015). Sex determination using proximal hand phalanges. *Papers of Anthropology,* 24, 37-56.

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