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### SECTION BITE MARKS

## Three-dimensional Validation of the Impact of the Quantity of Teeth or Tooth Parts on the Morphological Difference Between Twin Dentitions

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#### ABSTRACT

**BACKGROUND:** The number of teeth involved in cases of bite-mark analysis is generally fewer in comparison to the number of teeth available for cases of dental identification. This decreases the amount of information available and can hamper the distinction between bite suspects. The opposite is true in cases of dental identification and the assumption is that more teeth contribute to a higher degree of specificity and the possibility of identification in these cases. Despite being broadly accepted in forensic dentistry, this hypothesis has never been scientifically tested.

**OBJECTIVE:** The present study aims to assess the impact of the quantity of teeth or tooth parts on morphological differences in twin dentitions.

**MATERIAL AND METHODS:** A sample of 344 dental casts collected from 86 pairs of twins was used. The dental casts were digitized using an automated motion device (XCAD 3D<sup>®</sup> (XCADCAM Technology<sup>®</sup>, São Paulo, SP, Brazil) and were imported as three-dimensional dental model images (3D-DMI) in Geomagic Studio<sup>®</sup> (3D Systems<sup>®</sup>, Rock Hill, SC, USA) software package. Sub samples were established based on the quantity of teeth and tooth parts studied. Pair wise morphological comparisons between the corresponding twin siblings were established and quantified.

**RESULTS:** Increasing the quantity of teeth and tooth parts resulted in an increase of morphological difference between twin dentitions. More evident differences were observed comparing anterior vs. entire dentitions ( $p < 0.05$ ) and complete vs. partial anterior dentitions ( $p < 0.05$ ).

**CONCLUSION:** Dental identifications and bite-mark analysis must include all the possibly related dental information to reach optimal comparison outcomes.

**KEYWORDS:** forensic dentistry, bitemark, dental identification, morphology, 3D morphometric comparison

## **INTRODUCTION**

Bitemarks are patterned impressions of human<sup>1</sup> or animal<sup>2</sup> teeth on skin<sup>1</sup>, objects<sup>3</sup> or foodstuffs<sup>4</sup>. Bitemark analysis involves a comparative procedure to match dentitions of potential suspects with the associated patterned mark or injury<sup>1,3,5,6</sup>. In cases of dental identification, ante-mortem (AM) dental records of a known person are compared with post-mortem (PM) dental records of an unknown person in an attempt to identify similarities between both sets of records<sup>3</sup>. Both bitemark analysis and dental identification rely on the quality and quantity of the available dental evidence. In bitemark analysis, the quality and quantity of the evidence are dependent upon the nature of the injury itself. Information can also be extrapolated from the teeth once the injuries are shown to be dental patterned marks<sup>1</sup>.

The quality of the dental evidence is not only related to standards for the registration of images of the patterned mark but also to classification and analysis. Specifically, a higher quality of analysis is achieved using three-dimensional (3D) registration of dental evidence as opposed to the use of two dimensional (2D) imaging technology<sup>7</sup>. Moreover, evidence based on tooth morphology will be more prevalent than that based on dental treatment and pathology in the near future<sup>7</sup>, becoming more important for the identification of victims (dental identification) and suspects (bitemark analysis). Dental evidence is more useful when combining information from different teeth<sup>3,8</sup>. In this context, it has always been assumed that the quality of the evidence is directly related to the amount of teeth and tooth parts available for analysis.

Bitemark analysis is generally performed using 2D image registration<sup>1,3</sup>. However, it is also feasible in 3D, with surface scanning<sup>9</sup> and photogrammetry<sup>10</sup>.

The evidence registered is essentially based on tooth morphology, including information on dental shape, size angulation and position of the teeth<sup>10</sup>. The analysis of these evidences varied according to the contemporary technology available including the separate investigation of dental shape using transparent foils<sup>11</sup>, the separate analysis of dental shape (hollow contours), size (metric measurements) and angulation (polygons) using 2D digital overlays<sup>12</sup>, and the combination of all evidences using 3D superimpositions<sup>13,14</sup>.

In most cases of bitemark analysis the quantity of available evidence is usually limited, often consisting on the indentations of the incisal edges of the maxillary and mandibular six anterior teeth<sup>15-20</sup>. In most cases of bitemark analysis fewer teeth are available compared to dental identification cases<sup>6,8</sup>. This is one of the reasons why dental identification cases are considered to offer less legal challenge than cases of bitemark analysis in Court. However, the impact of the quantity of teeth and tooth parts affecting the differences between human dentitions has never been scientifically tested. This study, involving twin siblings, where any differences between the dentitions would be expected to be minimal<sup>21,22</sup>, is based on the pair-wise comparisons of the dental morphology following controlled and systematic modifications in the quantity of teeth and tooth parts available for comparison.

The present research aims to quantify the morphological differences between the dentitions of twin siblings using different quantities of teeth and tooth parts.

## **MATERIALS AND METHODS**

The present research was approved by the local Committee of Ethics in

Research under the protocol number: 19575613.2.0000.0020.

The studied sample consisted of 86 pairs of twins (n=172), of which 39 pairs (n=78) were monozygotic (M) and 47 pairs (n=94) were dizygotic (D). From each of the included subject (n=344) dental impressions of the maxillary (n=177) and the mandibular arch (n=177) were taken using alginate (Jeltrate Dustless<sup>®</sup>, Dentsply<sup>®</sup>, York, PA, USA) and cast in plaster (Durone<sup>®</sup>, Dentsply<sup>®</sup>, York, PA, USA). The study models obtained were digitalized as .STL files using an automated motion device with angular laser scanning (XCAD 3D<sup>®</sup> (XCADCAM Technology<sup>®</sup>, São Paulo, SP, Brazil). The .STL files were imported in a personal computer (HP Pavilion<sup>®</sup>, Hewlett-Packard<sup>®</sup>, Palo Alto, CA, USA) for duplication, using the copying and pasting command tools of the operating system (Windows 10<sup>®</sup>, Microsoft Windows, Redmond, USA). The final sample consisted of 688 .STL files. These files were imported in Geomagic Studio<sup>®</sup> (3D Systems<sup>®</sup>, Rock Hill, SC, USA) software package (GS) and stored as digital cast files (DCF).

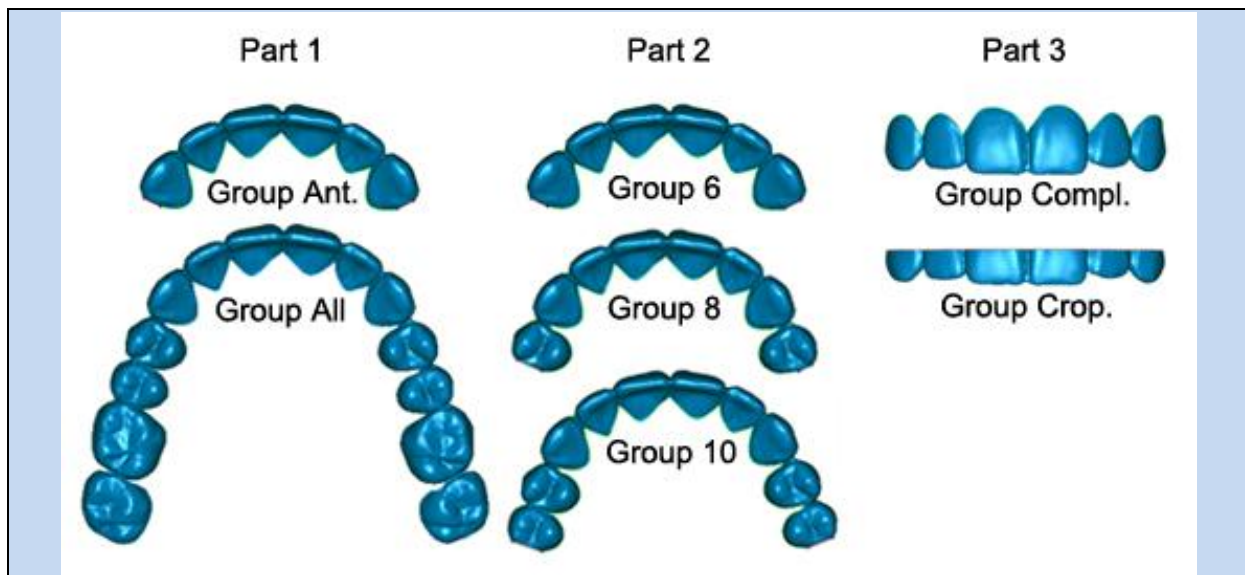
The study was divided in 3 parts (Figure 1). In Part 1, the DCF from the original 86 pairs of twins (n=172) were copied. Using GS, the original images were cropped and reduced to include the clinically visible crowns of the six anterior teeth (Group Ant.). The copied images were cropped a second time and reduced to include the clinically visible dental crowns of all of the anterior and posterior teeth (Group All). In Part 2, monozygotic twin pairs with completely erupted permanent teeth were selected (14 mandibular and 19 maxillary pairs of dentitions). The DCF of these subjects were cropped to include the crowns of 10 teeth, namely the six anterior teeth and the first and second premolars (Group 10). This group was duplicated

twice. The DCF of the first duplicate were cropped to include the crowns of 8 teeth

namely the six anterior teeth and the first premolars (Group 8), while in the second duplicate the DCF were cropped to include crowns of the 6 anterior teeth (Group 6). Part 3 used the same sample as Part 2. The DCF were cropped to include the entire morphology of the crowns of the 6 anterior teeth (Group Compl.). This group (Group Compl) was duplicated and the duplicated DCF cropped with a section parallel to the horizontal plane at the level of the highest interdental papilla (Group Crop.). All the crown cropping procedures were performed in GS, placing pre-cropping points along the cemento-enamel junction of all of the teeth including the areas of interest.

Within each group all the possible pair-wise morphological comparisons between DCF were accomplished using GS automated superimposition. The pair-wise differences were calculated in GS and expressed in millimeters as four quantification values: the maximum positive deviation (max.+); the maximum negative deviation (max.-); the average deviation (average); and the standard deviation of the average (SD). To combine the four quantification values their Euclidean distance from origin (zero) was calculated with the following formula:

$Distance = \sqrt{Max+^2 + Max-^2 + Average^2 + Standard\ deviation^2}$ . In this context, the least morphological difference between pair-wise compared DCF occurs when the distance value is equal to zero. The log-transformed distances were compared between groups using a linear mixed model with Sidak<sup>23</sup> correction for multiple hypotheses, separately for mandibular and maxillary DCF. The statistical tests were performed with significance rate of 5% using S+<sup>®</sup> 8.0 (Tibco<sup>®</sup>, Palo Alto, California, USA) software package.



**Fig.1:** Studied DCF areas of interest in each study part

DCF: digital cast files; Part 1 – Group Ant.: anterior dentition; Group All: entire dentition; Part 2 – Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Part 3 – Group Compl.: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns. DCF in Part 1 and 2 represented in 2D occlusal view and in Part 3 in 2D buccal view. Occlusal and buccal views are merely illustrative. Entire dental crowns were used and compared in a 3D environment in all study parts.

**RESULTS**

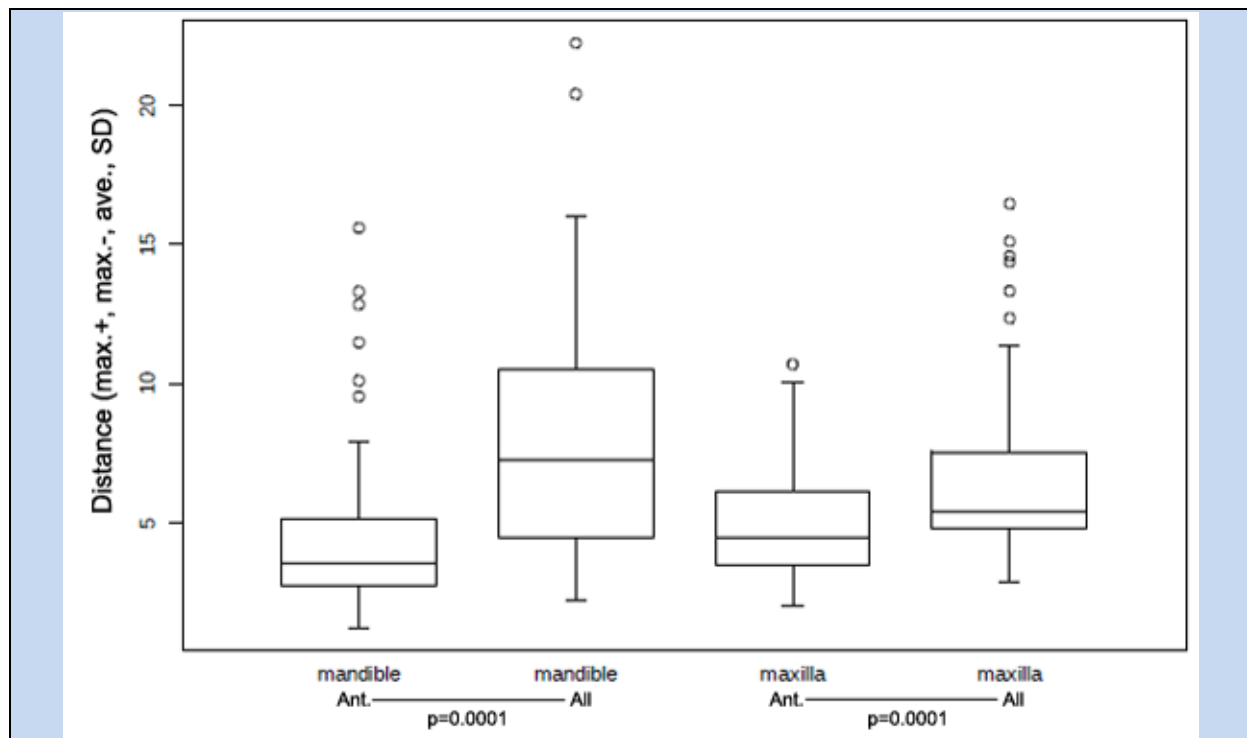
In study Part 1, the mean Euclidian distance observed comparing DCF in Group All was statistically significantly higher than the mean Euclidian distance

observed comparing DCF in Group Ant., both for the maxilla and the mandible ( $p=0.0001$ ) (Table 1; Figure 2).

Table 1 – Comparison of mean Euclidean distances, arch specific for each studied group

Dental arch	Part	Groups	Mean	p
Maxillary	1	Ant. vs. All	4.98 vs. 6.43	0.0001
		6 vs. 8	3.38 vs. 3.54	0.9088
	2	6 vs. 10	3.38 vs. 3.64	0.7843
		8 vs. 10	3.54 vs. 3.64	0.9931
		Compl. vs. Crop.	3.38 vs. 2.57	0.0027
Mandibular	1	Ant. vs. All	4.29 vs. 7.89	0.0001
		6 vs. 8	2.95 vs. 3.17	0.8858
	2	6 vs. 10	2.95 vs. 3.51	0.5145
		8 vs. 10	3.17 vs. 3.51	0.9135
		Compl. vs. Crop.	2.95 vs. 2.21	0.0122

Part 1 – Group Ant.: anterior dentition; Group All: entire dentition; Part 2 – Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Part 3 – Group Compl.: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns. *p*-values obtained with a linear mixed model using Sidak<sup>23</sup> correction for multiple hypotheses. Significance rate set at 5%.

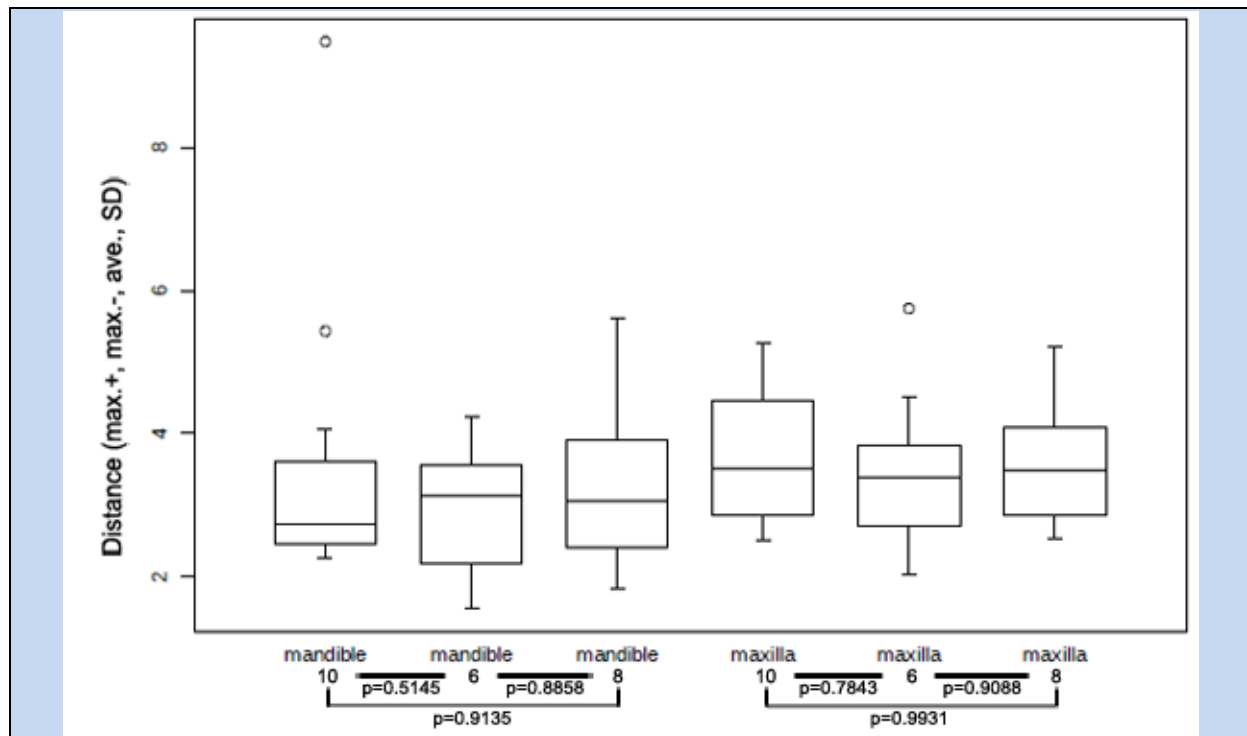


**Fig. 2:** Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups Ant. and All

DCF: Digital cast files; Group Ant.: anterior dentition; Group All: entire dentition; Mean Euclidean distance for maxillary DCF: 4.98 (Group Ant.) and 4.43 (Group All); Mean Euclidean distance for mandibular DCF: 4.29 (Group Ant.) and 7.89 (Group All); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation;  $p$ -values obtained with a linear mixed model using Sidak<sup>23</sup> correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups Ant. and All for maxillary and mandibular DCF: 0.0001 ( $p$ ).

In study Part 2, the mean Euclidean distance observed comparing DCF in Groups 6, 8 and 10 gradually increased in the maxilla as well as in the mandible. No statistically significant differences were observed between Groups ( $p > 0.05$ ) (Table 1; Figure 3).

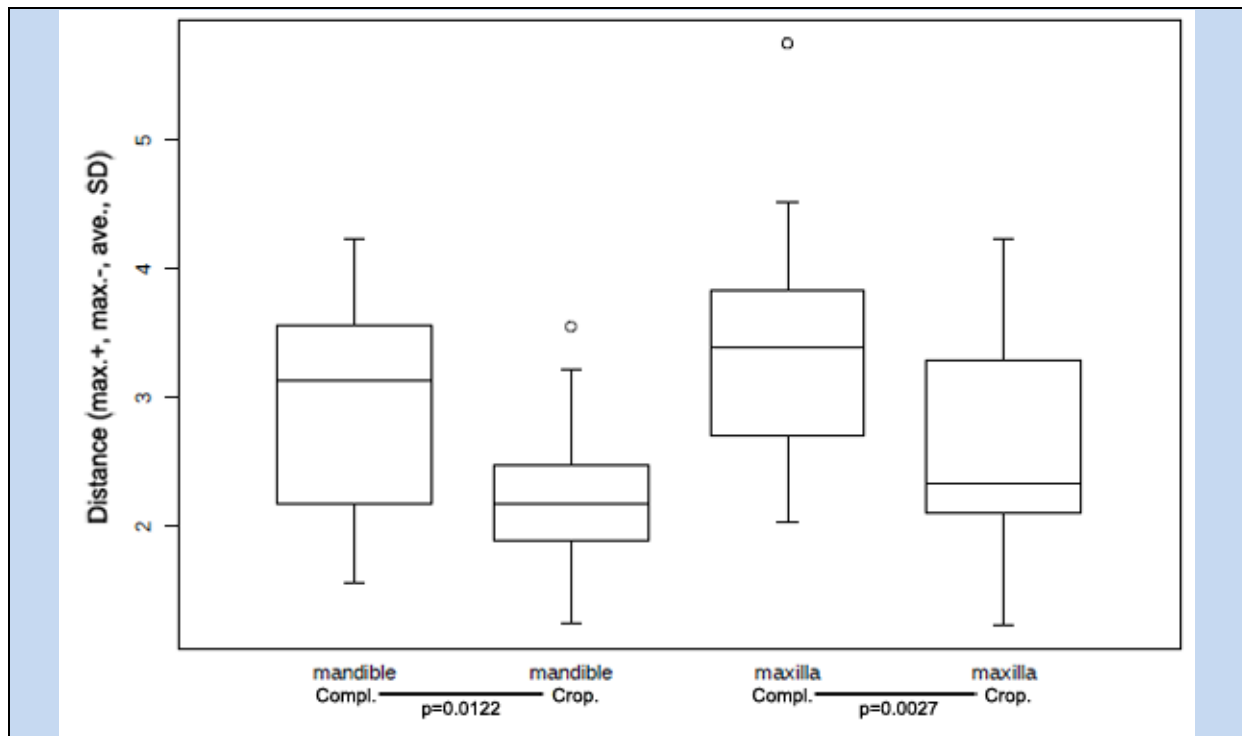
In Part 3, the mean Euclidean distance observed comparing DCF in Group Compl. was statistically significant higher than Group Crop., both for the maxilla ( $p = 0.002$ ) and the mandible ( $p = 0.012$ ) (Table 1; Figure 4).



**Fig. 3:** Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups 6, 8 and 10

DCF: Digital cast files; Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Mean Euclidean distance for maxillary DCF: 3.38 (Group 6), 3.54 (Group 8), and 3.64 (Group 10); Mean Euclidean distance for mandibular DCF: 2.95 (6), 3.17 (8), and 3.51 (10); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; *p*-values obtained with a linear mixed model using Sidak<sup>23</sup> correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups 6, 8 and 10 for maxillary and mandibular DCF: >0.05 (*p*).





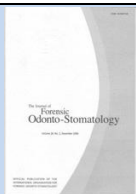
**Fig. 4:** Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups Compl. And Crop

DCF: Digital cast files; Group Compl.: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns; Mean Euclidean distance for maxillary DCF: 3.38 (Group Compl.) and 2.27 (Group Crop.); Mean Euclidean distance for mandibular DCF: 2.95 (Group Compl.) and 2.21 (Group Crop.); Max.+; maximum positive deviation; Max.-; maximum negative deviation; Ave.: average deviation; SD: standard deviation; *p*-values obtained with a linear mixed model using Sidak<sup>23</sup> correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups Compl. And Crop. for maxillary DCF: 0.0027 (*p*). Difference between the mean Euclidean distance of Groups Compl. And Crop. for mandibular DCF: 0.0122 (*p*).

## DISCUSSION

Forensic dentistry is currently using the hypothesis that an increase in the quantity of teeth and tooth parts provides an increase in the amount of dental evidence, increasing the (morphological) differences between subjects. Unlike fingerprint and DNA analysis, dental identification is not governed by the requirement of a minimum number of concordant features<sup>24,25</sup>. Quality assurance guidelines from Forensic organizations, such as the International Organization of Forensic Odonto-Stomatology (IOFOS),

recommend that all the combinations of dental evidences available must be explored<sup>8</sup>. In bitemark analysis attempts are made to take into account all of the available evidence<sup>1</sup>, but, realistically, in the majority of cases, the analysis is mainly restricted to the incisal morphology of the anterior teeth<sup>10</sup>. In forensic practice the hypothesis that is generally accepted is that increased numbers of teeth correlates to more distinctive identification potential and better comparison outcomes. The inference is that reliability factor in cases of dental identification is better than the



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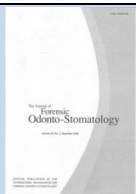
reliability factor in of cases of bitemark analysis.

Sibling pairs were sampled in order to have subjects with decreased qualitative differences in dental morphology. It justifies why randomly selected subjects or copied files were not used. Specifically, in the first the highest qualitative morphological differences are expected, while in the second, zero morphological difference will be observed between the corresponding DCF.

Uniqueness is commonly used in the forensic scientific literature to describe human dentitions with converging evidences. However, converging evidences indicate that two dentitions are at most identical but not unique. In fact, uniqueness guarantees that two dentitions in a worldwide population will not be equal. In the context of the present study, lack of uniqueness is translated as the absence of morphological difference (Euclidean distance = zero) between pairwise compared DCF. In particular, the mean Euclidean distances with highest unique power (6.43 for maxillary and 7.89 mandibular DCF) were observed comparing entire dentitions (Group All, Part 1). By contrast, anterior dentitions with partial crowns (Group Crop., Part 3) revealed the lowest unique power (2.57 for maxillary and 2.21 for mandibular DCF). Generally, this would suggest that an increase in the quantity of teeth and tooth parts increases the Euclidean distances, making dentitions potentially more unique. In Part 1, the clear statistically significant difference ( $p < 0.05$ ) between the DCF of the entire group (Group All) and the anterior group (Group Ant.) (Figure 2) demonstrates that substantial increase in the quantity of teeth relates to increasingly distinctive morphological dental evidence. Specifically, the proportion in the number

of teeth between the two groups (Group All/Group Ant.) increased by a factor of 133.33%, meaning that the proportion of mean Euclidean distances increased by 29% for maxillary and 83% for mandibular DCF (Table 1). In Part 2, morphological differences were also observed by firstly phasing in Group 8 (first premolars) and secondly by phasing in Group 10 (second premolars) but no statistically significant results were observed between these group comparisons ( $p > 0.05$ ). The proportions in the number of teeth increased by a factor of 33.33% between Groups 8 and 6; by a factor of 66% between Groups 10 and 6; and by a factor of 25% between Groups 10 and 8. This meant that the proportions of mean maxillary Euclidean distances increased by a factor of 4% (Group 8/Group 6); by a factor of 7% (Group 10/Group 6); and by a factor of 2% (Group 10/Group 8). The proportions of mean mandibular Euclidean distances increased by a factor of 7% (Group 8/Group 6); by a factor of 18% (Group 10/Group 6); and by a factor of 10% (Group 10/Group 8). In Part 3, statistically significant differences between groups ( $p < 0.05$ ) were observed (Table 1; Figure 4). Part 3, that is the analysis of the proportion in quantity of tooth material included, could be considered less accurate compared to the previous study parts, because the anterior dentition with partial crowns (Group Crop.) were horizontally cropped at the level of the highest interdental papilla, which varied discretely between twin subjects. Assuming that the anterior dentitions were cropped in half – generating a difference in tooth material of 50% between both groups (Group Crop./Group Compl.), the proportion of mean Euclidean distances increased by a factor of 31% and by a factor of 33% for





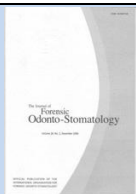
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maxillary and mandibular DCF, respectively.

Analysis of all three parts of the study revealed that higher Euclidean distance values were observed when comparing a larger quantity of tooth material or number of teeth. However, in Parts 1 and 3 statistically significant findings ( $p < 0.05$ ) were obtained, differing from Part 2 ( $p > 0.05$ ). This difference can be explained by the proportion of tooth material included for analysis. In Parts 1 and 3 the proportion of tooth material increased by at least a factor of 50% between groups, increasing the mean Euclidean distances by up to 83% (mandibular DCF of Part 1). In Part 2 the proportion of increase in tooth material varied between 25-66.66%, increasing the mean Euclidean distances less by only up to 18% (mandibular DCF between Groups G6 and G10). These results suggest that the inclusion of premolars in the anterior dentition provides little additional morphological information of negligible impact upon the mean Euclidean distances with statistical significance. The opposite is observed for the inclusion of all the available teeth in the dental arch (Part 1) and for the analysis of complete (instead of partial) anterior crowns (Part 3).

The use of anterior teeth combined with premolars and molars provides more information that can be used advantageously to differentiate between dentitions. It confirms the hypothesis that more tooth material allows for more combination of evidences<sup>8</sup> contributing to more uniqueness. In the case of bitemark analysis it also confirms the increase in reliability based on the higher quantity of tooth material considered<sup>26</sup>. Even in the absence of statistically significant findings (Part 2), the gradual increase in morphological difference observed adding

premolars, represents a clinically significant finding. It suggests that these minor morphological differences can be useful in forensic practice. They allow for positive dental identifications founded on the particular shape of premolar crowns; and for matches between a bitemark and suspect dentitions based on the comparison of the clinically detected premolar morphology. In parallel, the amount of tooth quantity is not exclusively restricted to the number of teeth, but involves also the amount of tooth parts available. In Part 3, the analysis of complete anterior crowns (Group Compl.) increased the morphological difference with 31-33% compared to partial crowns (Group Crop.). In the context of dental identification the quantity of morphological information differs if other tooth parts were considered. More specifically, the gingival half (50%) of the dental crown seems to provide more distinctive morphological information compared to the incisal half (50%). While the incisal half generated up to 33% of morphological difference between DCF, the gingival part is responsible for generating the remaining difference (up to 67%). This can be explained against the background of the inherent genetic influence on the quality of evidence that varies discretely between twin siblings. The quality of evidence may also be modified by non-genetic influence depending on which part of the tooth was included for analysis; for example a nail biting habit that would affect the incisal edge of the tooth or, for example, a periodontal disease that would affect the gingival part of the tooth. In most cases of bitemark analysis the outcomes of study Part 3 are very relevant because the analysis is commonly restricted to the incisal part of the crown<sup>1</sup> (part that impresses the bitten surface). The current



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findings suggest that the use of partial anterior crowns hampers the distinction between dentitions compared to the analysis of complete crowns. However, uniqueness of partial anterior crowns (Group Crop.) remains unproved, indicating the need for further investigations.

Regarding comparison of additional tooth material in both the maxillary and mandibular DCF, the mean Euclidean distances increased most on respect of the mandibular DCF. Specifically, in Part 1 the proportion of mean Euclidean distances between mandibular and maxillary DCF increased by a factor of 41%. In Part 2 it increased by a factor of 2% (Group 8/Group 6); 10% (Group 10/Group 6) and 7% (Group 10/Group 8). In Part 3 the increase was 1%. These findings suggest that the trend toward in the morphological differences in mandibular DCF is greater than maxillary DCF regarding of the quantity of tooth material considered. This could infer that that the mandibular DCF is a better determinant in discriminating between dentitions in cases of both human identification and bitemark analysis. Sheets et al.<sup>18</sup> (2011) also justify this finding by reason that dental crowding is more common in the mandibular arch. On the other hand, the lower unique power of maxillary DCF must be considered an important finding for potential sample stratification in studies proving the uniqueness of the human dentition, because apparently uniqueness is more hardly proved within maxillary DCF. Despite this, the morphological difference between dental arches was only prominent

in study Part 1 (41% increase). In study Part 2 (2-10% increase) and Part 3 (1% increase) the reduced Euclidean difference between arches suggests that predilection for analysis of specific dental arch in dental identifications and bitemarks must be avoided. It highlights the importance of analyzing and combining all morphological information from both dental arches in dental identification and bitemark analysis.

Further researches in the field should consider firstly using 3D scanning and performing separate comparisons of the dental crowns in each tooth position in order to systematically assess their morphological uniqueness. Secondly, root parts should be tested on their morphological information and uniqueness related to dental identifications. In both cases sibling comparisons on twin samples are recommended, enabling to study the morphological quantity with minimal variation in morphological quality of evidences.

### **CONCLUSION**

The outcome of this research provides evidence that an increase in the quantity of dental information leads to an increase in the number of morphological differences detected between dentitions. The results were based on pair-wise comparison of twin dentitions allowing quality control of the data. The research was based solely on dental morphological data.

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