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international journal of stomatology & occlusion medicine

ISSN 1867-2221

J. Stomat. Occ. Med.
DOI 10.1007/s12548-014-0105-3

Springer Medizin

01/14

international journal of
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Published by Österreichische Gesellschaft für Zahn-, Mund- und Kieferheilkunde

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A 3D computerized tomography study of changes in craniofacial morphology of Portuguese skulls from the eighteenth century to the present

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Received: 28 October 2013 / Accepted: 8 March 2014

Abstract This study examined the three-dimensional (3D) changes in craniofacial morphology between 482 identified Portuguese skulls from the eighteenth to the twentieth centuries and 150 modern Portuguese individuals randomly selected from the armed forces. The goal was to investigate the interrelationship between changes in various parts of the skull, in particular, the cranial base, the brain supporting structures, and the face. Cone beam computed tomography images from the identified skull collections belonging to the Department of Life Sciences at the University of Coimbra and Natural History National Museum of Lisbon were used. These 3D images from craniometric analyses included 19 different linear, angular, and orthogonal 3D measurements. The trend in horizontal position of the maxilla (SNA) and horizontal position of the mandible (SNB) angles showed a significant increase, while the relative position of the maxilla to mandible (ANB) and the global angle mean values decreased over time. Skulls from each subsequent century demonstrated a decrease in anterior cranial base, indicated by the mean distance between S and N landmarks. Significant negative correlations were found between SNA and anterior cranial base length (S-N). The negative correlations between SNB and anterior cranial base length (S-N) decreased from the eighteenth to nineteenth centuries. The twenty-first century skulls were characterized by a significant difference in the mean value of different craniofacial variables between males and females. The results of this study suggest changes in the 3D cephalometric measurements of craniofacial architecture. These changes are highly integrated, and show an interesting correlation between structures of the craniofacial facial complex and the anterior cranial base.

Keywords Human skull · 3D cephalometry · Cone beam computed tomography · Craniofacial measurements

Introduction

Significant morphological changes over the past 200 years in human skulls have been documented in various populations from the United States, Europe, and Japan [3, 15, 21, 22, 26, 39]. Many of the samples studied are from anatomical or forensic collections that may or may not be representative of the general population [25]. Much of the research on secular changes is from the American population; however, the American samples are complicated by a variety of factors, such as genetic changes, mixed populations, and several waves of foreign immigration. These factors make it difficult to determine the precise environmental or genetic variables that may be responsible for the observed morphological changes [45]. This study examined the changes in craniofacial morphology and the influence of the cranial base in the architecture of Portuguese skulls which represent a relatively random sample of the population from the past 312 years. This subject can be applied to several fields including anthropology, evo-

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lutionary biology, anatomy, and orthodontics. It is also a subject of considerable interest in diagnosis, as it would help to explain the functions and malfunctions of the craniofacial complex. The relationship between the cranial base and other structures of craniofacial morphology has been discussed by several different authors [2, 7, 10, 18, 19, 24, 29, 34]. The cranial base is composed of several skeletal units, supports the brain and connects it to functional systems. The location of the cranial base between the cranial vault and midface plays a key role in craniofacial morphology. Several studies have attempted to identify the relationship between cranial base and cranial facial structures, and some controversies remain. While some theories argue that the cranial base influences craniofacial morphology [2, 8], others report no significant effect [18, 34, 43]. The craniofacial architecture is the result of a very complex connection between different structures, and is constantly modified by the function of different organs [16, 36, 38]. It is important to take into account variations in craniofacial morphology over the course of time. This study used three-dimensional (3D) cephalometric landmarks to document changes in craniofacial morphology. The introduction of cone-beam computed tomography (CBCT) has allowed 3D volumetric reconstruction of the entire craniofacial complex with great precision [1, 12, 28, 32], improving our ability to understand the 3D nature of craniofacial structures with a high accuracy and precision [41]. Recent studies revealed that human cranial morphology, whether quantified using absolute linear dimensions or by using relative geometric morphometric techniques, largely reflects the population history among humans [35]. In Portugal, there is a lack of information concerning the population's craniofacial architecture measurements including a lack of 3D system values. The purpose of this comparative study was to analyze the changes in cephalometric measurements in craniofacial architecture of 3D reconstructed skulls.

Material and methods

Three-dimensional virtual models of human skulls constructed from CBCT images were used to test the hypothesis that changes have occurred in craniofacial architecture in the human skull over the course of centuries. This research protocol was approved by the National Museum of Natural History of Lisbon, by the Department of Life Sciences, University of Coimbra, and by the Kanagawa Dental College research committee. The final sample consisted of 482 Portuguese adult dry skull specimens, randomly selected from the osteological heritage of the Department of Life Sciences from the University of Coimbra and from the National Museum of Natural History of Lisbon. The Department of Life Sciences of Coimbra University houses three important identified skeletal collections for which detailed personal documentation exists. The most ancient one is the medical schools skulls collection. This collection is made up of 585 complete skulls collected between 1895 and 1903. All of the individuals were born in Portugal between 1802 and 1890 and died between 1895 and 1903. The Coimbra identified skeletal collection (CISC) consists of 505 complete skeletons. The skeletons came from the main Coimbra cemeteries and represent individuals born between 1817 and 1924 who died between 1904 and 1938. With the exception of nine individuals, all were Portuguese. The internationally exchanged collection consists of 1,075 complete skulls, all of which originate from the main cemeteries of Coimbra from the first half of the twentieth century (birth chronology 1817–1930, death dates 1904–1937; [17]). The identified skeletal collection is housed at the Museu Bocage, Department of Zoology and Anthropology of the National Museum of Natural History, Lisbon. The major portion of these large identified collections was amassed during the 1980s. Currently, 779 skeletons have biographic documentation. The years of birth range from 1805 to 1974 while the years of death date from 1880 to 1974. A sample of identified skulls ranging from the end of the nineteenth century until 100 years later was also collected. This is a unique opportunity for research. The most ancient skulls were not identified as they were recovered in an archaeological context. Although abundant it was a difficult task to find skulls fulfilling the requirements of this research. For this reason only 32 skulls were gathered from the eighteenth century. These were retrieved mainly from an archaeological excavation in Santarem, a small city in the center of Portugal. There were 2,455 skulls in the original sample, to which the following inclusion criteria were applied: skulls of Portuguese origin and nationality from individuals older than 18 years, complete cranial bone structure and the presence of teeth, providing a clinically acceptable and reproducible permanent occlusion with stable mandibular position. A total of 150 adult modern twenty-first century humans of Portuguese origin and nationality selected at random from the armed forces and the security forces were also evaluated (Tables 1 and 2). These were Portuguese adults of both sexes, 63.3 % males and 36.7 % females, with an age range of 18–58 years. The

Table 1 Distribution of the sample by sex and century

Period	Male/female	Frequency	%	Valid %	Cumulative %
Eighteenth century	Male	23	71.9	71.9	71.9
	Female	9	28.1	28.1	100.0
	Total	32	100.0	100.0	
Nineteenth century	Male	98	65.3	65.3	65.3
	Female	52	34.7	34.7	100.0
	Total	150	100.0	100.0	
Twentieth century	Male	95	63.3	63.3	63.3
	Female	55	36.7	36.7	100.0
	Total	150	100.0	100.0	
Twenty-first century	Male	99	66.0	66.0	66.0
	Female	51	34.0	34.0	100.0
	Total	150	100.0	100.0	

Table 2 Distribution of the sample by individual age and century

Age (years)	Eighteenth century		Nineteenth century		Twentieth century		Twenty-first century	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
19–24	0	0.0	18	12.0	12	8.0	27	18.0
25–29	2	6.3	28	18.7	13	8.7	26	17.3
30–34	3	9.4	26	21.3	15	10.0	32	21.3
35–39	2	6.3	15	10.0	11	7.3	28	18.7
40–44	0	0.0	29	19.3	8	5.3	14	9.3
45–49	3	9.4	16	10.7	19	12.7	16	10.7
>50	22	68.8	18	12.0	72	48.0	7	4.7

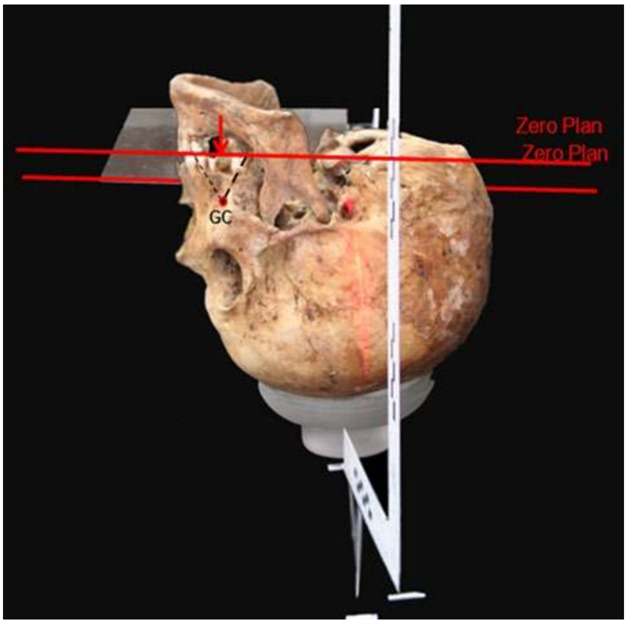

Fig. 1 Standard position of the skull of specimen nr. 485 (male) from the nineteenth century, obtained from the osteological collection at the University of Coimbra. The skull is in a fixed position, where it was placed on the occlusal plane of the upper jaw zero point, parallel to the support base, offset by a unit of hydrobalance. The mandible moves towards the center point of gravity (*GC*) in the occlusal plan without resistance, "*zero*" position

Fig. 2 Photographs of two Portuguese skulls, specimen 950 (female, *left*), from the nineteenth century obtained from the osteological collection at the department of Life Sciences University of Coimbra and specimen 11 (male, *right*), from the nineteenth century obtained from the osteological collection at the department of Life Sciences University of Coimbra. Extraoral photographs in frontal (*upper*), 45° (*middle*) and lateral view (*lower*) were positioned in a standard and stable position

following exclusion criteria were applied: individuals with previous orthodontic treatment, completely toothless individuals, and individuals less than 18 years old. The sample was submitted to a goodness of fit test to the Portuguese population, relative to the 2001 census, from which a representative sample resulted. At the time of sample collection, the idea of voluntary participation was strengthened and all individuals gave free and informed consent (Declaration of Helsinki). A complete photographic study based on extraoral and intraoral photographs was performed in a standard position (Figs. 1 and 2). A total of 12 documented photos were obtained from each skull with a digital camera (Nikon D90), to create an additional database documentation of the sample. A custom-made head holder was constructed to support the skulls during imaging and the subjects were positioned with a laser marker, according to the manufacturer's instructions (Fig. 3). Before the imaging, the dentition was rechecked for stable maximum intercuspation. To ensure a detailed 3D representation of the occlusal morphology of the maxillary dentition, the skulls were also scanned without the mandible. The CBCT scans were acquired

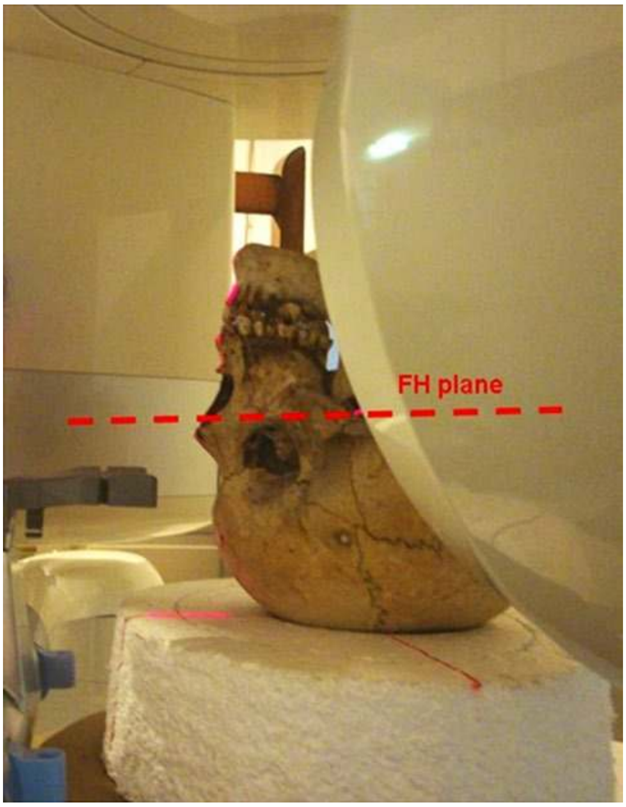

Fig. 3 Imaging of the skulls with a custom-made head holder support positioned with a laser marker (*FH* Frankfurt plane)

Table 3 Definition of anatomic 3D landmarks used in this study (illustrated in Figs. 4 and 5)

Landmark	Abbreviation	Definition on computed tomography image
Orbital	Or	Lowest point on the lower edge of the orbit
Nasion	N	Nasofrontal structure in the midline
Sella	S	Center of the pituitaria fossa
Basion	Ba	Anterior-inferior margin of the foramen magnum
A point	A	The deepest point of the midline maxillar frontal surface
B point	B	The deepest point of the mid-mandibular frontal surface
Anterior nasal spine	ANS	Central point of the anterior nasal spine
Posterior nasal spine	PNS	The most posterior point of the palate in the medial plane
Menton	Me	The lowest border of the mid-mandibular suture
Tangent gonion right	TGoR	The lowest point in the distal right position of the mandible
Tangent gonion left	TGoL	The lowest point in the distal left position of the mandible
Porion	Po	Highest point on the external ear canal
Pogonion	Pog	Most anterior point of anterior curvature of the chin symphysis
Articular point	Ar	Intersection of the images of the surface of the skull base and posterior surface of the neck of the condyle

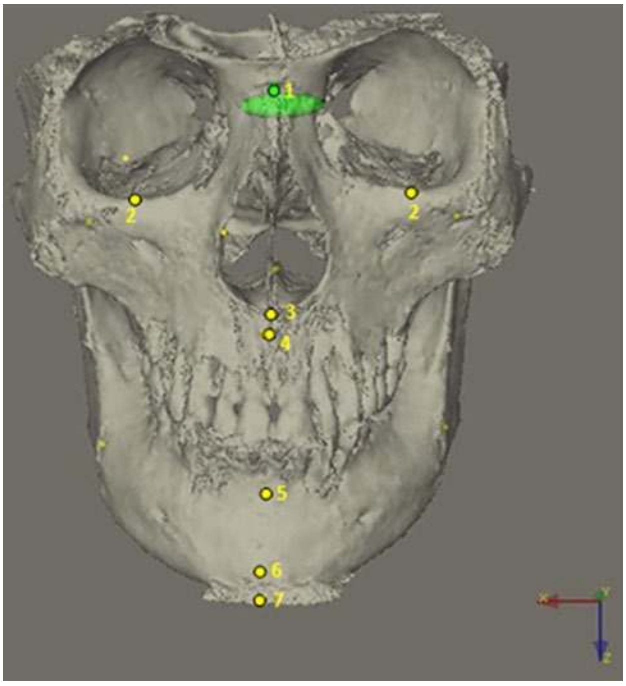

Fig. 4 Frontal view of the 3D landmarks *1* nasion, *2* orbital, *3* anterior nasal spine, *4* A point, *5* B point, *6* pogonion and *7* menton

with a Galileo compact system (Sirona Dental Systems, Bensheim, Germany). The scanning time was 14 s and 200 single exposures were performed for each skull with a 3D resolution (isotropic voxel size) of 0.3 mm and 15×15 cm field of view using SIDEXIS XG acquisition software (Sirona Dental System). Exposure parameters were controlled by automatic exposure control. To determine the measurement accuracy of the computed tomography (CT) and workstation, a 45 × 45 × 60 mm³ plaster block was placed parallel to the floor using a level vial. The CT images were taken and transferred to a workstation under the same conditions and statistical differences between the measurement and measured values were tested. The CBCT data were then exported from the SIDEXIS XG software in Dicom multifile format and imported into MAXILIM® software (version 2.3.0.3, Medicim, Mechelen, Belgium). Three-dimensional surface models were obtained using automatic thresholding software for hard tissue type models and re-evaluated by a single calibrated operator on the basis of the following criteria: quality of the 3D morphology and the presence of relevant skeletal structures. Three-dimensional craniometric analysis was performed based on 14 different landmarks (Table 3) on the skulls (Figs. 4 and 5). A CT-based reference plane was set up as defined and validated by Swennen et al. [40]: the virtual models were first positioned in a standardized way then the software produced virtual lateral and frontal cephalograms. A total of eight 3D planes were established (Table 4). The 3D cephalometric measurements, 13 angular, 5 linear, and 2 orthogonal were designed (Table 5) and exported to Excel (Microsoft ®Excel ®2007, Microsoft) with the original skull identification number. Landmarks were

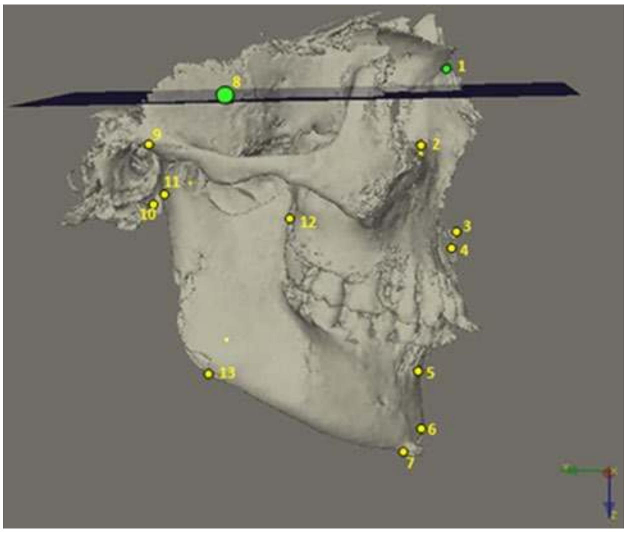

Fig. 5 Lateral view of the 3D landmarks *1* nasion, *2* orbital, *3* anterior nasal spine, *4* A point, *5* B point, *6* pogonion, *7* menton, *8* sella, *9* porion, *10* basion, *11* articular point, *12* posterior nasal spine and *13* tangent gonion

Table 4 Definition of relevant 3D planes used in this analysis

Plane	Abbreviation	Definition
Horizontal plane	HP	Plane 6° below the anterior cranial base plane (S-N) with the origin in landmark sella
Median plane	SN	Plane containing landmarks sella and nasion, perpendicular to the horizontal 3D craniometric reference plane (HP)
Vertical plane	VP	Plane with the origin in landmark sella and perpendicular to the horizontal and median 3D craniometric reference planes
Frankfurt plane	FH	Plane that passes through both orbital landmarks and the computerized 3D mean point between right and left porion landmarks
Palatal plane	PP	Plane that passes through ANS-PNS line, perpendicular to the median 3D craniometric reference plane (MRP)
Mandibular plane	MP	Plane defined by 3 landmarks: menton, right and left tangent gonion
Facial plane	FP	Plane defined by two landmarks from nasion to pogonion.
Sagittal plane	SP	Plane through sella and nasion and perpendicular to the horizontal plane

located and marked on the 3D surface-rendered volumetric image of the skull using a laser mouse on a 58.4 cm (23 inch) flat light-emitting diode (LED) screen (Samsung, Suwon, South Korea). The Maxilim models and craniometric measurements were developed by a single observer (HAP). Landmarks were chosen in order to allow the characterization and measurement of the craniofacial architecture of the Portuguese skulls (Figs. 4 and 5).

Table 5 Definition of relevant 3D measurements used in the analysis (illustrated in Figs. 6 and 7)

Measurements	Abbreviation	Definition
Angular measurements		
SNA angle	SNA	Angle between three landmarks: S, N, A
SNB angle	SNB	Angle between three landmarks: S, N, B
ANB angle	ANB	Angle between three landmarks: A, B, N
Cranial base	CBA	Angle between landmarks N-S-Ba
N-S-Me	NSMe	Angle between three landmarks: N, S, Me
Bjork	Bjork	Mathematical sum of the following measurements: saddle angle, articular angle and gonial angle
SN—MP	SN—MP	Angle between the line S-N and the plane MP
Articular angle left	S—Ar—TGo L	Angle between two lines S—ArLeft ArLeft—TGoL
Articular angle right	S—Ar—TGo R	Angle between two lines S—ArRight ArRigt—TGoLR
Gonial angle left	Ar—TGo—Me L	Angle between two lines ArLeft—TGoL TGoL-Me
Gonial angle right	Ar—TGo—Me R	Angle between two lines ArRight—TGoR TGoR-Me
Saddle angle left	N—S—Ar L	Angle between two Lines N-S S-ArLeft
Saddle angle right	N—S—Ar R	Angle between two lines N-S/ S-ArRight
Linear measurements		
Anterior cranial base	S—N	Distance between landmarks S and N
Lower anterior facial height	ANS—Me	Distance between landmarks ANS and Me
Posterior cranial base	S—Ba	Distance between landmarks S and Ba
Upper anterior facial height	N—ANS	Distance between landmarks N and ANS
Orthogonal measurements		
Orthogonal A	Ort A	Distance of the A point to the medial plane
Orthogonal B	Ort B	Distance of the B point to the medial plane

Statistical analyses

Statistical analysis was performed using the Statistical Package for Social Sciences software program, Version 17.0 (SPSS, Chicago, III). Characterization of the secular samples was performed according to sex and age; the frequency distribution of the three skeletal classes and their structural percentage are presented by century. In addition to the statistical measurements of mean and standard deviation, comparison of the means of the studied variables in two consecutive centuries was carried out using the independent sample Student's t-test; the equality of means between genders (independent samples) was also tested using Student's t-test. The limit of the confidence interval was also set to 95 % (95 % confidence interval) for SNA and SNB (gender and global) for all the centuries studied. The Pearson's correlation for some variables implicit in the study made it possible to assess the degree of association between them and their significance, for which the significance levels of 0.01 and 0.05 were used. Multivariate regression was used after checking the assumptions underlying the multivariate model—linearity, normality, multicolinearity and autocorrelation, to investigate the influence of variables SN (anterior cranial base) and orthogonal A and B on the behavior of SNA and SNB, over the centuries under study.

Results

The results of the multivariate regression of 3D craniofacial measurements showed that the craniofacial architecture of the Portuguese skulls changed significantly from the eighteenth century to the present. Descriptive data of the 3D cephalometric analyses with angular, linear, and orthogonal measurements in the final sample of 482 skulls are shown in Table 6. The results of the relationship between genders (male and female) in different craniofacial measurements are shown in Table 7. The relationship between angles and linear and orthogonal measurements is presented in Figs. 6 and 7.

Craniofacial measurements

Descriptive data of the 3D cephalometric analysis showed several alterations with significant values from the eighteenth to the twenty-first century. The SNA angle (the angle between S-N-point A), which indicates the horizontal position of the maxilla relative to the cranial base, established between the nasion and A point (the deepest point of the midline maxillary frontal surface), showed that the global mean values increased over the centuries, undergoing a slight non-significant increase in the nineteenth century, a statistically significant increase in the twentieth century ($p<0.05$), and a very marked increase in the twenty-first century ($p<0.001$; Fig. 8). The SNB (the angle between S-N-point B), which indicates the horizontal position of the mandible relative to the cranial base, established between the nasion and B point (the deepest point of the midline mandibular frontal surface), showed that there was a slight non-significant increase in the global mean value in the nineteenth century, and statistically significant marked increases in the twentieth century ($p<0.05$) and in the twenty-first century ($p<0.001$). The ANB angle (the angle between three landmarks A, B, and N) that defines the angular difference between the SNA and

Table 6 Evolution of the average scores of angular, linear and orthogonal measurements from the eighteenth century to the twenty-first century

	Eighteenth century ($n=32$)			Nineteenth century ($n=150$)			Twentieth century ($n=150$)			Twenty-first century ($n=150$)		
	Mean	Std. deviation	*p*	Mean	Std. deviation	*p*	Mean	Std. deviation	*p*	Mean	Std. deviation	*p*
Angular measurements												
SNA angle	78.7686	5.43084		78.9453	4.95287		80.2407	5.19142	*	82.5100	3.85122	**
SNB angle	72.7406	5.26552		73.3960	4.42127		74.7987	5.24553	*	77.8347	4.00629	**
ANB angle	6.8062	2.19412		6.4293	2.57326		6.0493	2.71060		4.6753	2.81758	**
CBA angle	129.7187	12.19160		126.3187	10.16344	*	127.6347	10.38273		131.7720	5.33927	**
SN-MP angle	38.9406	6.99806		40.0533	6.10564		39.7387	7.03900		33.7793	7.63500	**
Articular angle	100.2719	11.62209		105.5577	7.43167	*	105.2577	8.87267		147.3387	8.83746	**
Gonial angle	126.1953	9.02667		127.3867	6.35820		127.4167	7.48955		121.5307	7.97443	**
Saddle angle	108.5203	5.75090		109.2353	6.62901		110.0933	6.53097		124.9107	6.27306	**
Bjork angle	335.0125	18.37559		342.2067	14.33624		342.7900	15.25872		393.7793	7.63500	**
Linear measurements												
Anterior Cranial Base length	71.6250	6.26666		69.7140	5.27178		67.0473	6.40004	**	66.4033	6.93890	
Posterior Cranial Base length	44.2031	13.23891		49.3340	28.31537	*	47.0580	10.25067		34.0800	4.94333	**
Anterior facial height length	117.6563	10.08320		119.4233	7.56778		117.8833	7.63931		120.3860	14.42730	
Orthogonal measurements												
Orthogonal A	65.6250	5.51099		64.4667	5.79672		62.8933	7.35972	*	[a]	[a]	
Orthogonal B	52.9750	8.03452		51.8527	7.87844		51.0913	10.81428		[a]	[a]	

*Significant at the 0.05 level (2-tailed)
**Significant at the 0.001 level (2-tailed)
[a]Absence of values in the twenty-firstcentury

Table 7 Evolution of the average values of angular, linear and orthogonal measurements, by gender, from the eighteenth century to the twenty-first century

		Eighteenth century ($n=32$)			Nineteenth century ($n=150$)			Twentieth century ($n=150$)			Twenty-first century ($n=150$)		
		Mean	Std. Deviation	*p*	Mean	Std. Deviation	*p*	Mean	Std. Deviation	*p*	Mean	Std. Deviation	*p*
Angular measurements													
SNA angle	Male	78.5261	6.11929		79.0622	4.52620		80.1295	5.62140		82.6525	3.73107	
	Female	79.0111	3.33521		78.7250	5.71214		80.4327	4.39288		82.2333	4.09828	
SNB angle	Male	73.0000	5.98642		73.2153	4.49665		74.5484	5.65659		78.6303	4.10938	**
	Female	72.0778	2.86303		73.7365	4.29783		75.2309	4.46359		76.2902	3.32339	
ANB angle	Male	6.5348	2.39955		6.3347	2.52256		6.0674	2.86087		4.0273	2.65867	**
	Female	7.5000	1.43788		6.6077	2.68203		6.0182	2.45448		5.9333	2.71247	
CBA angle	Male	130.0478	11.85633		126.8194	9.79175		128.5811	10.10544		130.8737	5.04279	**
	Female	128.8778	13.72150		125.3750	10.86455		126.0000	10.74196		133.5157	5.51472	
Bjork angle	Male	335.7826	19.26123		342.2714	13.03009		344.1442	14.75712	*	392.9566	7.48373	
	Female	333.0444	16.79711		342.0846	16.65891		335.6564	37.15753		395.3765	7.74593	
Linear measurements													
Anterior cranial base length	Male	72.4087	6.41035		70.2592	5.13508		67.8105	6.54395		67.5162	6.38221	**
	Female	69.6222	5.73515		68.6865	5.42171		65.7291	5.97342		64.2431	7.51229	
Anterior facial height length	Male	11.23079	2.34178		122.1000	6.44568	**	120.6968	6.63573	**	123.3737	12.93463	**
	Female	6.10965	2.03655		114.3788	6.97056		113.0236	6.81162		114.5863	15.50409	
Orthogonal measurements													
Orthogonal A	Male	66.1739	5.80480		64.9398	5.41783		63.4611	7.82869		______	______	
	Female	64.2222	4.68449		63.5750	6.41105		61.9127	6.42051		______	______	
Orthogonal B	Male	54.0348	8.94003		51.6796	7.73776		51.1842	11.77020		______	______	
	Female	50.2667	4.34425		52.1788	8.20362		50.9309	9.02730		______	______	

*Significant at the 0.05 level (2-tailed)
**Significant at the 0.001 level (2-tailed)

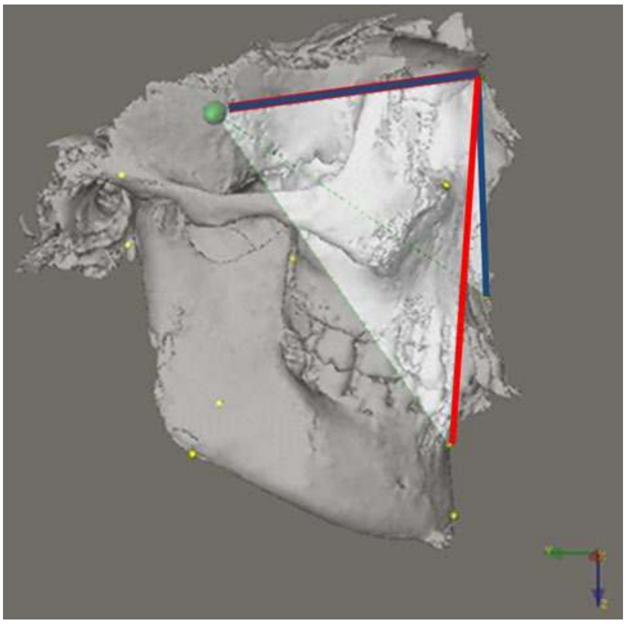

Fig. 6 Lateral image of virtual skull representing 3D angular measurements SNA angle (*blue line*) and SNB angle (*red line*)

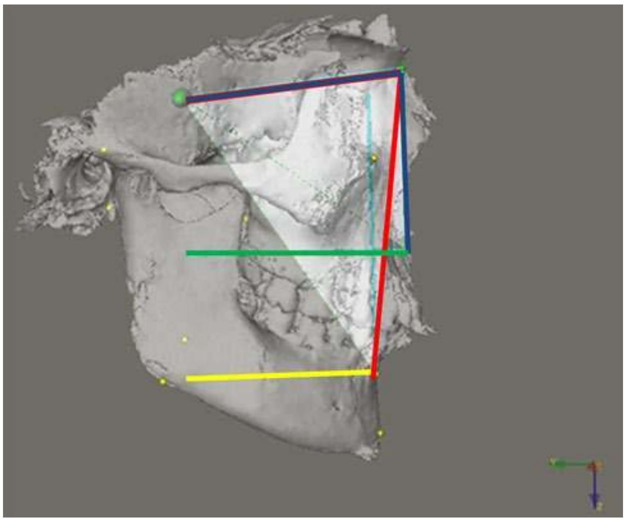

Fig. 7 3D angular measurements SNA angle (*blue line*) and SNB angle (*red line*) and the orthogonal measurements orthogonal A (*green line*) and orthogonal B (*yellow line*)

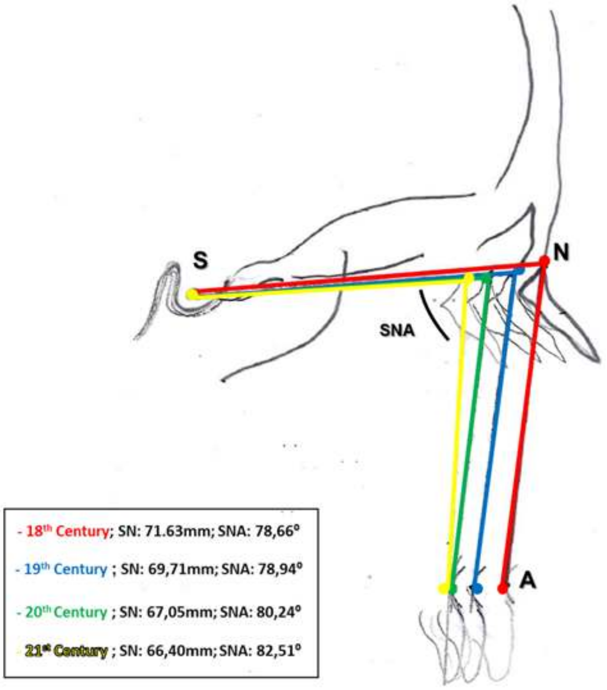

Fig. 8 Morphological characteristics of the SNA angle during the centuries studied. Definition of variable (SNA) in the mid-sagittal plane *S* sella, *N* nasion, *A* point A and *SNA* angle between maxilla and cranial base

SNB angles, presented a descending global mean value over time, especially in the twenty-first century ($p<0.001$). The result of the saddle angle (the angle between the line N-S and the line from S-Art) describes the cranial base, part of the Bjork sum angle, and showed a slight increase in the nineteenth and twentieth centuries and a marked increase in the twenty-first century. The gonial angle (the angle between Art-Go and Go-Me) describes the form of the mandible, part of Bjork sum angle, and showed a slight increase in the nineteenth century and a significant marked decrease ($p<0.001$) in the twentieth and twenty-first centuries, especially in the latter. In relation to linear measurements, there were great temporal variations with increases and decreases over the centuries in the anterior facial height (the distance from N-Gn). The anterior cranial base length (distance between S-N) used to describe the growth pattern showed a decrease in the mean values in all the centuries. When comparing the mean values using the independent sample Student's t-test, it was concluded that the difference in samples from two consecutive centuries had no statistical significance in the eighteenth and nineteenth centuries but was highly significant in the twentieth century ($p<0.001$). As for the 3D orthogonal measurements, orthogonal A showed a decrease in global mean values over the nineteenth and twentieth centuries, with a slight but non-significant decrease in the nineteenth century, and a slight but statistically significant decrease in the twentieth century ($p<0.05$). For orthogonal B, the global mean values decreased over the centuries, but without statistical significance. These findings reveal significant changes in angular, linear, and orthogonal measurements from the craniofacial architecture of the skulls over the centuries.

Sexual dimorphism

Regarding the mean differences between genders and according to the results of the independent t-test samples, the following observations were made: the observed mean values for SNB angle in males were significantly bigger than in females ($p<0.001$). In the twenty-first century, the observed mean values of the ANB angle in males were smaller than in females, with a highly significant difference ($p<0.001$) and in the twenty-first century, the observed mean values for the cranial base angle in males were significantly smaller than in females ($p<0.005$). As for the anterior facial height, the values for males were higher than for females, with a highly significant difference in the nineteenth ($p<0.001$), twentieth ($p<0.001$), and twenty-first ($p<0.001$) centuries. The presented mean values, by gender, of the variable anterior cranial base showed that those of males were higher than those of females, with a significant difference in the twenty-first century ($p<0.001$). The presented mean values, by gender, concerning the orthogonal variables A and B, showed that those of males were higher than those of females in all the centuries except for the nineteenth century. Regarding orthogonal B, however, the observed differences had no statistical significance for most levels of significance used (0.01 and 0.05). The utmost statistical difference in means between genders was observed in the twenty-first century. Thus, the mean values of the SNB, anterior cranial base, and anterior facial height variables in males were larger than those in females, with a considerably higher significance ($p<0.001$). The mean values of the ANB and cranial base angulations (CBA) variables in females were significantly bigger than those of males ($p<0.001$).

Correlation between cranial base and structures of the craniofacial architecture

Multivariate regression was used to assess the influence of the anterior cranial base (S-N) and orthogonal variables on the behavior of the SNA and SNB angles over the studied centuries. Previous data demonstrated that the regression model made up of these variables had good predictability, as all the values of total R^2 found were between 0.90 and 1 ($p<0.001$), which meant that the correlation between the dependent variable and the set of explanatory variables was nearly perfect. Therefore, regarding the SNA, in the eighteenth century 70.1 % of the variation could be explained by the orthogonal A variable, while in the nineteenth and twentieth centuries, it can be mostly explained by S-N ($R^2=0.713$ and $R^2=0.672$, respectively).

In terms of SNB, the orthogonal B variable was the one that best explained its variability in the eighteenth, nine-

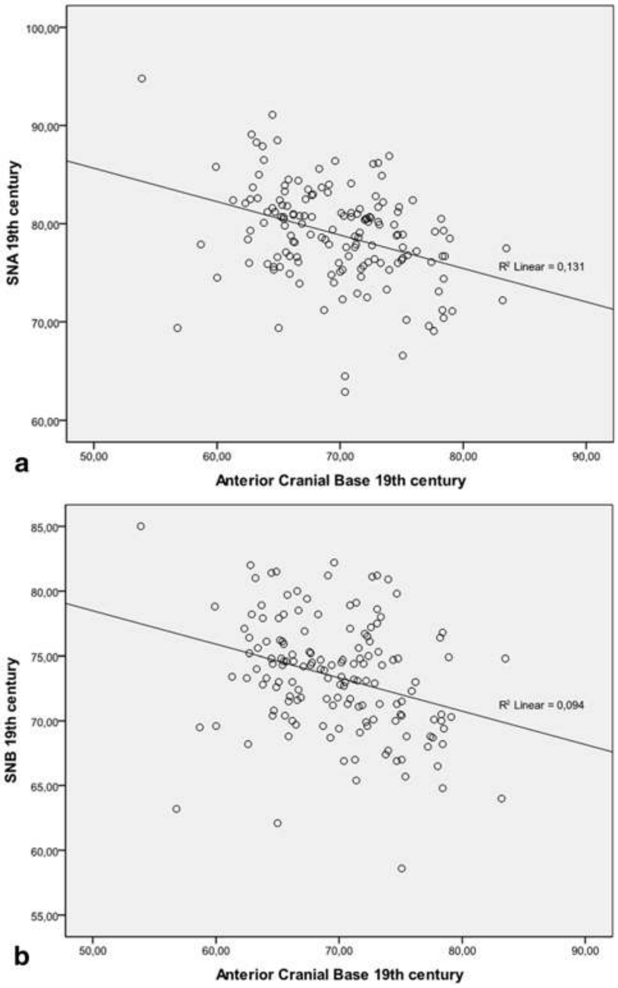

Fig. 9 Scatterplot of **a** SNA and **b** SNB scores as a function of anterior cranial base nineteenth century

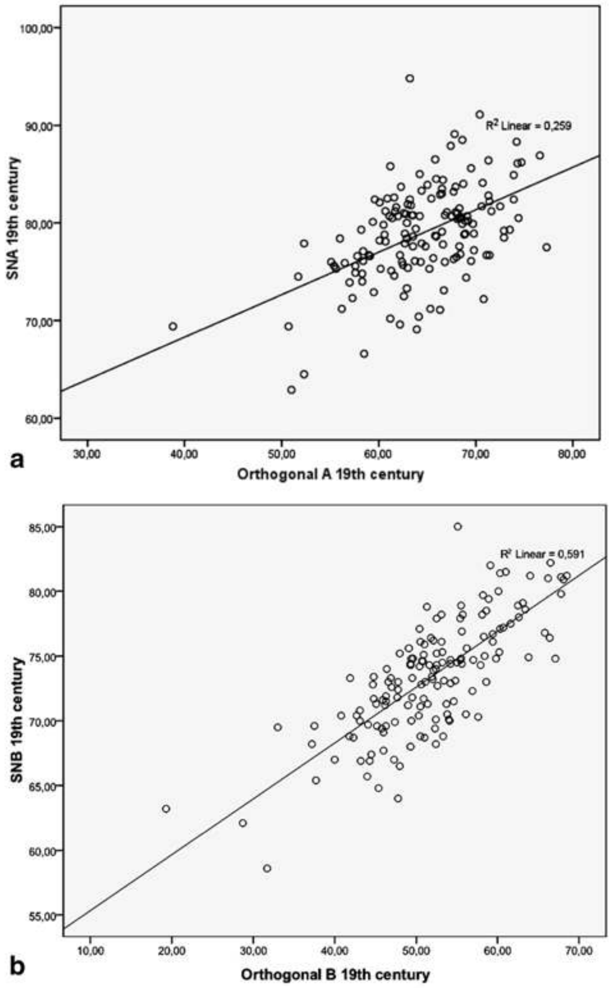

Fig. 10 Scatterplot of SNA and SNB scores as a function of **a** orthogonal A and **b** orthogonal B nineteenth century

teenth, and twentieth centuries ($R^2=0.541$, $R^2=0.591$, and $R^2=0.599$, respectively). The correlation between pairs of variables, SNA/S-N, SNA/orthogonal A, SNB/S-N, and SNB/orthogonal B, showed that the intensity of the phenomenon was linked, as shown in Figs. 9, 10, 11 and 12. The following findings can be derived from the graphs: there were decreasing negative correlations between SNA and SNB, in absolute values, which were statistically significant over the studied centuries, there were slightly positive but significant correlations between the SNA and orthogonal A ($p<0.001$), except in the eighteenth century ($p<0.059$) and there were decreasing negative correlations between SNB and S-N, in absolute values, which was statistically significant over the studied centuries, except for the twentieth century ($p=0.244$). The multivariate regression applied to the model with SNA as the dependent variable and S-N and orthogonal A as explanatory variables showed that it was significant for males ($p<0.001$), females ($p<0.001$), and for both genders ($p<0.001$) in the studied centuries. The multivariate regression applied to the model with SNB as the dependent variable and S-N and orthogonal B as explanatory variables showed that it was equally significant for males ($p<0.001$), females ($p<0.001$), and for both genders ($p<0.001$) in the studied centuries.

Discussion

This study examined the question whether significant changes occurred in craniofacial morphology measurements of Portuguese subjects from the eighteenth century to the present. The skulls from the eighteenth to the twentieth centuries from cemeteries represented a relatively random sample. Portugal's borders have remained unchanged since the thirteenth century when the Moors were driven from the southern part of the country [9]. After an analysis of the Portuguese population density, according to the χ^2-test, the twenty-first century sample included equal proportions of individuals by age, as compared to that obtained from the census of Portugal in 2011 ($p=0.011$) and therefore, represented a reference group valid for the purpose of the study. The application of 3D CT and the possibility of 3D volumetric digital skull reconstruction provided the opportunity to analyze the skulls with great accuracy [1, 5, 28, 37,

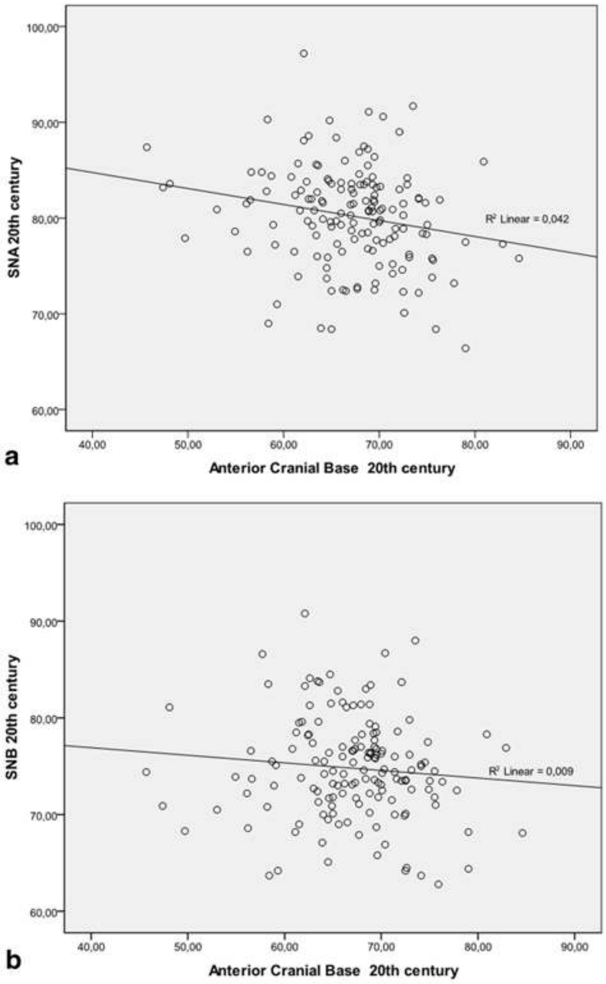

Fig. 11 Scatterplot of **a** SNA and **b** SNB scores as a function of anterior cranial base twentieth century

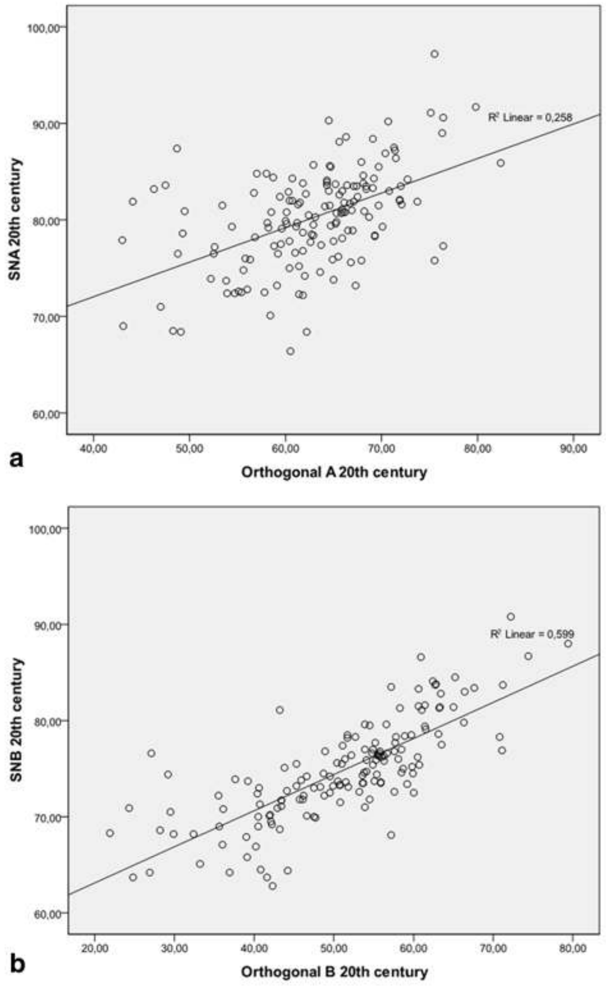

Fig. 12 Scatterplot of SNA and SNB scores as a function of **a** orthogonal A and **b** orthogonal B twentieth century

42] and reproducibility [16, 31] by applying a different computer-based software such as the one used in this study. Multiple regressions associated with the stepwise method regression analysis were used in the present study to identify the important craniofacial measurements that influenced the architecture of Portuguese skulls. On comparing the four large samples, the main finding was the consistency of the significant increase of the SNA and SNB angles. Similar results were obtained for the saddle angle and articular angle, with a slight increase in the nineteenth and twentieth centuries and a marked increase in the twenty-first century. By contrast, the ANB angle presented a global mean value decrease over time, especially in the twenty-first century. The CBA angle increased in the twentieth and twenty-first centuries. The anterior facial height presented a great temporal variability, with increases and decreases over the centuries. Significant short-term changes in cranial morphology have also been documented in several modern populations. Boas [11] was the first to report significant short-term changes in cranial morphology by comparing American-born and foreign-born offspring of immigrants. Other researchers have continued to document changes in the American population [4, 21, 39, 44]. The results of these studies suggest that the largest component of secular changes in Americans is an increase in cranial vault height, with most of that change occurring in the cranial base. Other studies documented changes in cranial morphology and interesting interrelations between structures were observed in Austrians [5, 16, 23, 37], Mexicans [30], Japanese [20, 27], Croatians [14] and Portuguese [45]. The study by Weisensee KE, Jantz RL, 2011 [45] demonstrated a significant change in craniofacial morphology of the Portuguese from 1806 to 1954, with a decrease in facial breadth and a more inferiorly placed cranial base. In order to better analyze the influence of the increases in SNA and SNB angles on the cranial morphology of the specimens, the anterior cranial base length (distance between S-N) and 3D orthogonal measurements (orthogonal A and orthogonal B) were taken. By this method the influence and relationship could be evaluated not only in the linear measurement, but also in the projection of the A point and B point to the medial plane. Surprisingly, 70.1 % of the variation of the SNA angle in the eighteenth century could be explained by the orthogonal A variable, whereas in the nineteenth and twentieth centuries, it was mostly explained by a decrease of the anterior cranial base length ($R^2=0.713$ and $R^2=0.672$, respectively). As for the SNB angle, the orthogonal B measurement best explained the variability in the eighteenth, nineteenth, and twentieth centuries ($R^2=0.541$, $R^2=0.591$, and $R^2=0.599$, respectively), and was considered to be an important determinant of the correlation between SNA/S-N, SNA/orthogonal A, SNB/S-N, and SNB/orthogonal B. These values allow the hypothesis that in a short period of time, there will be a decrease of around 2 mm per century in the linear dimension of the anterior cranial base of the skull (S-N). Simultaneously, there was a much more substantial increase of both SNA and SNB angles. These results are in line with Sato's hypothesis towards the dynamic functional anatomy of the craniofacial complex [36]. The interaction between the length of the cranial base and the complex morphology of the face seems to be particularly important in humans where the upper face is almost entirely located on the underside of the anterior cranial fossa. The relationship among the maxilla and mandible and the skull base is still an issue of permanent debate and research: there is certainly a relationship but the influence of the cranial base on the position of these structures is not well understood. This morphological alteration can be influenced by environmental factors in distinctive ways. It is interesting to note that a significant difference was found in the anterior facial height length measurements between males and females in the nineteenth century ($p<0.001$) and the twentieth century ($p<0.001$). However, in the twenty-first century sample, besides the anterior facial height length ($p<0.001$) a markedly significantly different result in the mean and standard deviations for males and females was observed in the SNB angle ($p<0.001$), ANB angle ($p<0.01$), and anterior cranial base length ($p<0.001$). This suggests a craniofacial measurement variation with gender, especially in the present sample. Sexual dimorphism in the human craniofacial system is an important feature of intraspecific variation in human fossils [6, 13, 33]; therefore, gender must be taken into consideration during anthropological, evolutionary biology, and anatomical studies, and in diagnostics and treatment planning for the individual patient.

Conclusion

This research has demonstrated a significant change in the morphology of the craniofacial complex of Portuguese skulls over the past 312 years, particularly an increase in both SNA and SNB angles and a decrease in the ANB angle. Based on the results of multivariate regression over the centuries studied, there was a stronger influence from the variable anterior cranial base length (S-N) on the behavior of the SNA angle than from orthogonal A. In addition, the influence of the variable orthogonal B on the behavior of the SNB angle was more significant than the influence of the anterior cranial base. Finally, there were significant gender differences between means, especially in the twenty-first century. Therefore, gender must be taken into consideration during orthodontic diagnosis and treatment planning for individual patients. This is one of the first times these types of changes have been documented in a European sample and identified in a large majority over such a long time period while showing obvious benefits of evolutionary changes.

Limitations of the present study

The number of skulls from the eighteenth century ($n=30$), compared with the number analyzed from the nineteenth ($n=150$) and twentieth ($n=150$) centuries, is one limitation of this study. However, the sample size for the eighteenth century ($n=30$) is considered statistically acceptable. Also, skulls of the deceased from the eighteenth to twentieth centuries were compared with a random sample of skulls of living subjects from the twenty-first century. Finally, the representation of dates by the skulls of living subjects from the twenty-first century only spanned 12 years, while representation of the eighteenth to the twentieth centuries spanned 100 years. Although only 12 years were represented by the skulls of the twenty-first century, this representation is substantial for measuring craniofacial changes.

Acknowledgments
This work was performed at the Research Institute of Occlusion Medicine and the Research Centre of Brain and Oral Science, Kanagawa Dental College, and was supported by a grant-in-aid for Open Research from SICAT GmbH & Co. Germany. The authors would like to express their gratitude to the Ludwig-Slavicek Foundation, to the Department of Life Sciences at University of Coimbra Portugal, to Professor H. Cardoso (Natural History Museum of Lisbon), to the postgraduate students (skull project) involved in the data collection process, especially M. Tanaka, O. Takeda, and S. Kishimoto, and to the members of the orthodontic department and to Professors J. Freitas and L. Lourenço.

Conflict of interest
The authors declare that there are no actual or potential conflicts of interest in relation to this article.

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