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Human Peopling and Population Dynamics in Sicily: Preliminary Analysis of the Craniofacial Morphometric Variation from the Paleolithic to the Contemporary Age

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Abstract: The geographic position, isolation, and the long and dynamic history of colonization created a human context in Sicily that allows for a particular anthropological study; information about "migratory flow" and "population influx" could be investigated in the cranial morphology of a localized geographical region. The research goals are the identification of temporal trends in facial morphology in order to assess the adaptations and the microevolutionary trends and to verify if the cranial morphology of humans was modified by the various genetic contributions and more or less related to the intense and significant migratory flows. This work includes a diachronic morphometrics study of 3D models of 95 Sicilian skulls coming from 19 populations (from the Paleolithic to the Contemporary Age), providing an overview of human biodiversity and variability in Sicily. To achieve this, a geometric morphometrics analysis of the facial features of adult human skulls was performed. The approach used allows for the identification of the main micro-anatomical and micro-evolutionary features. Considering sample size/composition, it has been possible to discriminate between prehistorical and historical populations. The results highlight a series of morphological changes related to different migratory flows that have followed one another with different intensities and effectiveness starting from the Prehistory up to the Contemporary Age. The human peopling of Sicily is a subject of continuous debate; however, this study points to the coexistence of microevolutionary patterns and population dynamics, with the latter being one of the main causes of the morphological variations.

Keywords: 3D geometric morphometric analyses; cranial morphometry; multivariate statistics; human biodiversity; Sicily

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1. Introduction

1.1. Human Biodiversity in Sicily

Due to its size and position, Sicily (located in the center of the Mediterranean Sea, it is the largest island in the basin at 25.711 km^2) allowed for the isolation and microevolutionary processes which are undetectable in continental Italy or Europe [1]. Sicily, through the geological eras, was a for species moving between the southern Apennine region of Italy and the north of the Maghreb area [2]. Its bio-geographical conditions, caused by mountain ranges, valleys, and weather, produced a vast diversification of habitats (a high rate of endemism). Because of this, it is realistic to imagine dividing Sicily into separate blocks in which substantial segregation of genes is responsible for the morphological variation which produced profound changes in the landscape and in all species involved.

The human species, since the early Peopling, in Sicily were subjected to the same phenomena of human flow and human environments' (flora and fauna) coevolution.

It has been found that these interactions have quickly and significantly influenced the island's genetic pool and phenotype [3]. Moreover, the cultural and biological contribution left by the many populations which have colonized the island since prehistory (which include Prehistoric, Greek, Carthaginian, Roman, Byzantine, Islamic, and Norman/Swabian population dynamics), combined with the environmental conditions, resulted in a peculiar human peopling context and, as a consequence, unique population variability and dynamics.

1.2. Background Studies and Sample Recognition

The excavations and the archaeological studies carried in Sicily during the last century, shed to light several finds and information about the prehistory and history of the island.

The publications related to these studies constitute an important database of references for the more recent paleontological, palaeoecological, and osteological studies which in the last four decades have increased a multidisciplinary comprehension of the island since its early colonization.

Based on these previous publications, it was possible to identify the osteological material containing skulls (Table 1). In detail, the considered references were: Bechtold et al. [4]; Belvedere et al. [5]; Brea & Cavalier [6]; Cavalier [7]; De Miro [8]; Di Stefano [9,10]; Fama' & Toti [11]; Griffo [12,13]; Kistler [14,15]; La Duca [16]; Mannino [17]; Romana [18]; Sconzo & Falsone [19], and Vassallo [20].

Moreover, recent multidisciplinary works (in the field of anthropology, paleontology and paleoecology) contributed to increasing the knowledge of ancient Sicily as reported by Becker [21–24]; Bonfiglio [25]; Borgognini et al. [26–28]; Castellana & Mallegni [29]; Costantini [30]; D'Amore et al. [31,32]; Di Salvo et al. [33–36]; Fiorentino et al. [37]; Hodos [38]; Incarbona et al. [39,40]; Garilli et al. [41]; Germana' & Di Salvo [42]; Lauria et al. [43–45]; La Rocca [46]; Mannino et al. [47]; Messina et al. [48] Micciche' et al. [49], and Sineo [50,51].These recent works were the starting point to apply new approach and techniques for the study of the population history of humanity. [31,32,52].

Table 1. Previous historical, archaeological, palaeoecological, and paleoanthropological references are available.

Place		
San Teodoro	Garilli et al. [41] Sineo et al. [50]	D'Amore et al. [31]
Uzzo	Borgognini & Repetto [27] Borgognini et al. [28]	Mannino et al. [47] Costantini [30]
Molara	Borgognini et al. [26] Becker [22]	
Marcita	Di Salvo [33,34] La Rocca [46]	Becker [22]
Polizzello	De Miro [8] Messina et al. [48]	Hodos [38]
Baucina	Castellana & Mallegni [29] Belvedere et al. [5]	Micciche' et al. [48]
Mozia	Becker [21–23] Sconzo & Falzone [19]	Lauria et al. [43–45]
Birgi	Griffo [12,13] Fama' & Toti [11]	
Tukory	Germana' & Di Salvo [42] Di Stefano [10]	

Table 1. *Cont.*

Place		
Phoenician/Punic of PA	not published	
Lilibeo	Becker [24] Bechtold et al. [22]	
Marsala	La Duca [16] Becker [24]	Di Salvo [36]
Lipari	Brea & Cavalier [6] Cavalier [7]	
Agrigento	Fiorentino et al. [37]	
C. San Pietro	Di Salvo [35] Di Stefano [9]	
M. Iato	Di Salvo [35] Kistler [14,15]	
Caltavuturo	Romana [18] Vassallo [20]	
Alia	Mannino [17]	
Rotoli	not published	

1.3. Aim of the Study

Here we report a diachronic morphometric study of a cranial sample spanning from the Paleolithic to the Contemporary Age. The aim is to assess the articulated dynamics that determined the chronological morphological diversity as a means to provide an overview of human biodiversity in Sicily. Specifically, the research goals are to study the temporal trends in facial morphology [53–55] to better understand the population influx and island-related issues; investigate the possible connections between the cultural/biological flows, the environmental changes, and the ecological pressures [52,56]. Although this is not a genetic study, the aim is to identify and describe the changes in cranial morphology associated with populations contributions (genetic variability [57]) from the end of the Upper Paleolithic to the contemporary age and to clarify population dynamics across the centuries.

2. Materials and Methods

2.1. Cranial Samples

A set of Sicilian human skeletal remains from different chronologies (Table 2) were selected to carry out a 3D craniofacial geometric morphometric (GM) analysis. A dataset of 95 human skull 3D models was built representing 19 populations (Table 2) from the Paleolithic to the Contemporary Age). Using the standards proposed by Buikstra & Ubelaker [58] and Ubeleker [59], we selected only adult crania, considering their integrity also; broken or incomplete specimens (bones not in anatomical connection and/or lacking landmarks necessary to take the anthropometric measurements mentioned below have been excluded before the analysis). The characteristics of these populations including the number of specimens and chronology can be found in Figure 1 and Table 3.

The sample composition (the number of specimens and the spatial bias in the southeast of the map) is due to the funerary rituals used on the island (incineration and inhumation in ossuaries), the lack of conservation of the osteological findings during the excavation until the end of the 70s, the modern urbanization and the excavation still in progress.

Table 2. Main Sicilian Prehistoric and Historical Periods—B.C.E. Before Common Era—C.E. Common Era.

Main Sicilian Prehistoric and Historical Periods:	
B.C.E. Before Common Era—C.E. Common Era	
Prehistory	
• Upper Palaeolithic: 38.000–8.000	
• Mesolithic: 8.000–6.000 B.C.E.	
• Neolithic: 6.000–4.000 B.C.E.	
• Eneolithic/Copper Age: 4.000–2.500 B.C.E.	
• Bronze Age: 2.500–1.100 B.C.E.	
	Early Bronze Age: 2.500–2.000 B.C.E.
	Middle Bronze Age: 2.000–1.500 B.C.E.
	Late Bronze Age: 1.500–1.100 B.C.E.
• Iron Age: 1.100–700 B.C.E.	
History	
• Antiquity: 700 B.C.E.–100 C.E.	
	Colonial Period: 700–600 B.C.E.
	Classical Period: 600–400 B.C.E.
	Hellenistic (Greek Period): 400–200 B.C.E.
	Roman Republic Period: 200 B.C.E.–100 C.E.
• Late Antiquity (Roman Empire Period): 100–476 C.E.	
• Middle Ages: 476–1492 C.E.	
	Byzantine Period: 476–1.000 C.E.
	Islamic Period: 1.000–1.300 C.E.
	Norman/Swabian Period. 1.300–1.492 C.E.
• Modern Ages: 1.492–1.789 C.E.	
• Contemporary: 1.789 C.E. to Nowadays	

Table 3. Sample Specimens, Dating and Periods.

Site	Specimens	Dating	Periods
San Teodoro	2	14.500 B.C.E. -^{14}C	Upper Paleolithic
Uzzo	2	9.000 B.C.E.	Mesolithic
Molara	1	9.000 B.C.E.	Mesolithic
Marcita	4	2.300–1.100 B.C.E.	Bronze
Polizzello	2	900–800 B.C.E.	Iron
Baucina	2	755–700 B.C.E. -^{14}C	Antiquity
Mozia	2	800–400 B.C.E.	Antiquity
Birgi	5	700–100 B.C.E.	Antiquity
Tukory	2	600–300 B.C.E.	Antiquity
Phoenician/Punic of PA	4	600–300 B.C.E.	Antiquity
Lilibeo	4	400.100 B.C.E.	Antiquity
Marsala	6	200 C.E.	Antiquity
Lipari	1	300–400 C.E.	Late Antiquity
Agrigento	1	400–500 C.E.	Late Antiquity
C. San Pietro	2	1.000–1.300 C.E.	Middle Ages
M. Iato	3	1.000–1.300 C.E.	Middle Ages
Caltavuturo	5	1.000–1.500 C.E.	Middle Ages
Alia	45	1.800 C.E.	Contemporary
Rotoli	3	2.000 C.E.	Contemporary

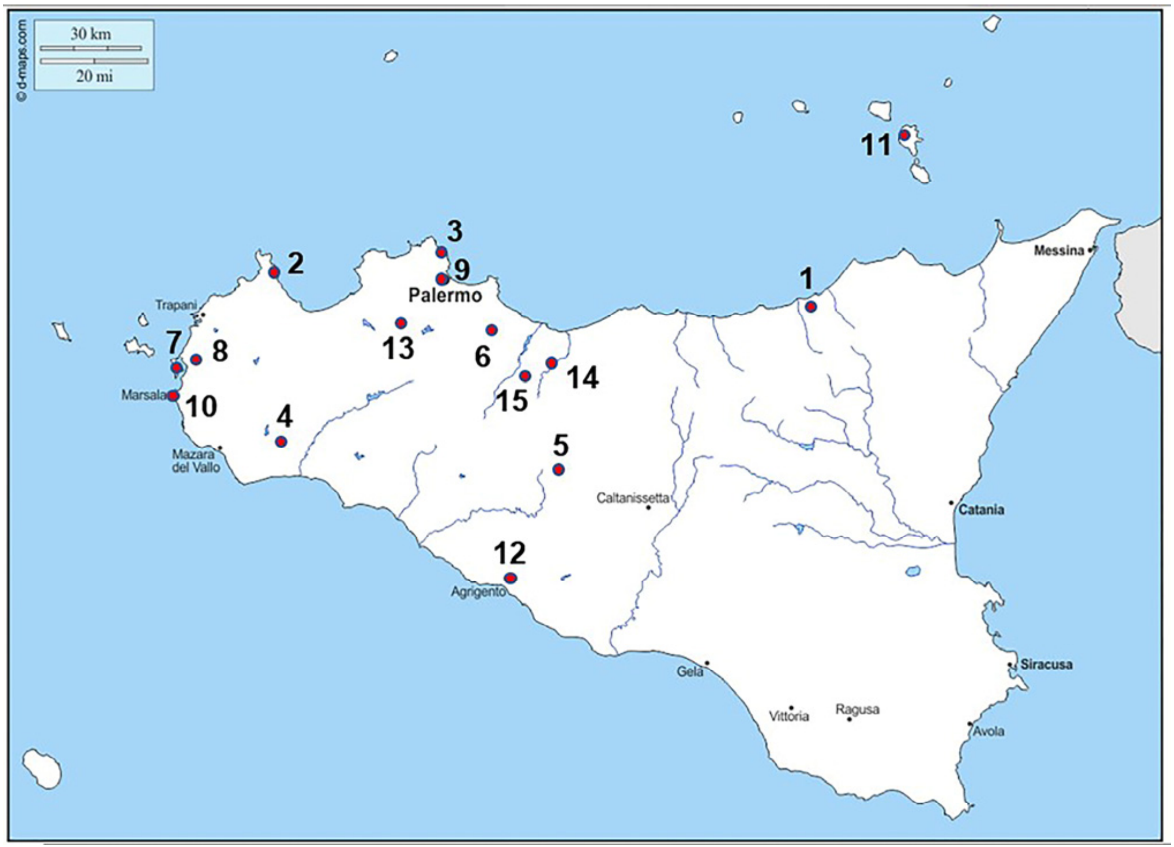

Figure 1. Sample Site Map. —Key: 1-Grotta di SanTeodoro; 2-Grotta dell'Uzzo; 3-Grotta della Molara; 4-Marcita; 5-Polizzello; 6-Baucina; 7-Motya; 8-Birgi; 9-Caserma Tukory (PA); 9-Phoenician/Punic of Palermo; 9-Castel San Pietro (PA); 9-Rotoli (PA); 10-Lilibeo; 10-SanGiovanni Marsala; 11-Lipari; 12-Agrigento; 13-Monte Iato-Position(B); 14-Caltavuturo; 15-Alia.

2.2. Methods

Three-dimensional models were, almost exclusively, built through photogrammetric reconstruction following the method proposed by Lauria et al. [60]. Only the specimens coming from Grotta di San Teodoro and Grotta della Molara were acquired by computed tomography (CT) (Photogrammetric and CT models were scaled and exported in .PLY format).

To achieve our goals, a geometric morphometrics (GM) analysis of facial features (upper face and part of the calvarium) of adult human skulls was performed to explore the morphological variation [30–32,61,62]. All data have been collected and analyzed taking into consideration all historical, archaeological, geological, palaeoecological, and paleoanthropological information available.

GM analysis [63,64] was carried out using a configuration of 26 Landmarks (78 coordinates for each of the 95 skulls) (Table 4 and Figure S1) marked by the software "Landmark3.6" (Institute for Data Analysis and Visualization group at the University of California, Davis) [65]. Anatomical Landmarks [58] were placed on the junctions of cranial sutures (Landmarks Type1) and on anthropometric points (Landmarks Type2) [66]. "MorphoJ 2.0" (University of Manchester, UK) [67] and "PAST 2.0" (Natural History Museum of Oslo—University of Oslo, Norway) [68] software programs were used to perform the morphometric and statistical analysis.

Running MoprhoJ, the Raw coordinates were initially subjected to a generalized Procrustes analysis (GPA) [69]. GPA removes the effects of translation and rotation in the raw coordinates data and standardizes each specimen to unit centroid size [70–72].

Using MoprhoJ, the Procrustes fitted coordinates were visualized by performing the Shape Changes Graphs displaying the Lollipop Graph and Wireframe Graphs that illustrates the shape changes from a starting shape (the mean shape in the sample) [73]. These graphs make the resulting three-dimensional forms easier to be visualized.

The Lollipop graph shows the shifts of the landmarks with straight lines. Each line starts with a dot on the landmark (in the starting shape) and the related lengths and direction indicates the movement and the magnitude of the respective landmark from the starting shape to the target shape [74].

Wireframe Graphs (connects the landmarks with straight lines) display the starting shape as a light blue outline and the target shape shown (the most extreme of the specimens) as a dark blue outline showing the direction of the straight lines' changes [73].

Table 4. Anatomical landmarks (Buikstra & Ubelaker 1994) configuration and numeration according to the software MorphoJ and "Landmark3.6".

MoprhpoJ	Landmark3.6	Landmarks Configuration	Type
1	0	Prostion	1
2	1	Nasospinale	1
3	2	Nasion	1
4	3	Glabella	2
5	4	Bregma	1
6	5	Lambda	1
6–8	6–7	Point between the dental alveoli I2/C	1
9–10	8–9	Alare	2
11–12	10–11	Zygomatic-Maxillary suture—lower margin	1
13–14	12–13	Zygomatic-Maxillary suture–upper margin	1
15–16	14–15	Maxillary-Frontal suture	1
17–18	16–17	Ectoconchion	1
19–20	18–19	Fronto-Temporal-Malar	1
21–22	20–21	Frontotemporal	1
23–24	22–23	Occipital—Temporal—Parietal intersection	1
25–26	24–25	Stephanion	1

The Raw coordinates (exported from MorphoJ) were subsequently uploaded on PAST and again procrustized before Principal Component Analysis (PCA) based on a Covariance Matrix. A PCA was performed on the Procrustes fitted coordinates in order to study the landmark positions in the collection of the specimens [74].

Since PCA is an exploratory analysis [75] based on the visualization, we conducted also a MANOCA/CVA always carried out with PAST on the Procrustes-fitted coordinates.

Canonical variate analysis (CVA) is a type of discriminant analysis, used with more than two groups, that maximizes separation between the given groups [76]. CVA is mathematically closely tied to the Multivariate ANalysis Of VAriance (MANOVA), which is a multivariate procedure to test the equality of the multivariate means of several multivariate samples [74]. PAST (as others' software) presents the MANOVA as part of the CVA output. Moreover, PAST directly performs two other tests used as part of MANOVA, the Wilks' lambda Test [77], and the more robust Pillai trace test [78]. Both assume as a null hypothesis (H0) that all samples are taken from populations with equal multivariate means [74].

To itemize the distances between pairs of groups, PAST was employed to create a Neighbor-joining (NJ) tree (UPGMA cluster procedure and Euclidean distance matrix) [79] leaving the software to automatically recognize the outgroup. NJ reflects the distances as faithfully as possible, and it was based on the averages of the Procrustes fitted coordinates for each period.

To better understand the Sicilian heterogeneity the same sample was divided into two groups to analyze in detail the human peopling and population influx during Prehistory and History always performing a PCA and NJ. (MANOVA/CVA is possible only when the variables are less than the specimens).

3. Results

3.1. Shape Variation and Changes in Direction

The lollipop graph (Figure 2a) shows the direction and magnitude of the shape variations highlighting that the changes are located mainly on the landmarks placed on the superior jaw (1, 2, 7, 8, 9 and 10), on the left and right sides of the face (landmarks 19, 20, 21 and 22), and on the cranial vault (landmarks 5, 23, 24, 25 and 26). Specifically, the first group of landmarks moves inwards while the other two move outward. With regards to the cranial vault, the parietals and the occipital all change in the direction of the mesocephalization with the parietal bones presenting the wider variation.

Comparing the lollipop graph with the wireframe graph-superior view (Figure 2a), it is possible identify a slight decrease of maxillary prognathism and a mesocephalization of the cranial vault as the skull becomes tighter and slightly less elongated.

Focusing on the wireframe frontal view (Figure 2c), the graphs display a general widening and shortening of the face mainly due to the nasal bones and the maxilla, which all shorted vertically. The wireframe lateral view (Figure 2d) shows that the frontal and occipital bones remained essentially the same size, but they both became slightly wider. A remarkable increase in shape is contrarywise detectable in the parietals bones that are characterized by an increase in size and shape. The whole structure gets shorter in the anterior section (nasal aperture and maxilla) and taller on the superior one. To sum up, the face length decreases, and the skull becomes taller and wider. Maxillary prognathism (never extremely severe) corresponds to Mesolithic specimens that had a lengthy and more robust skull compared with their wider and smaller modern counterparts.

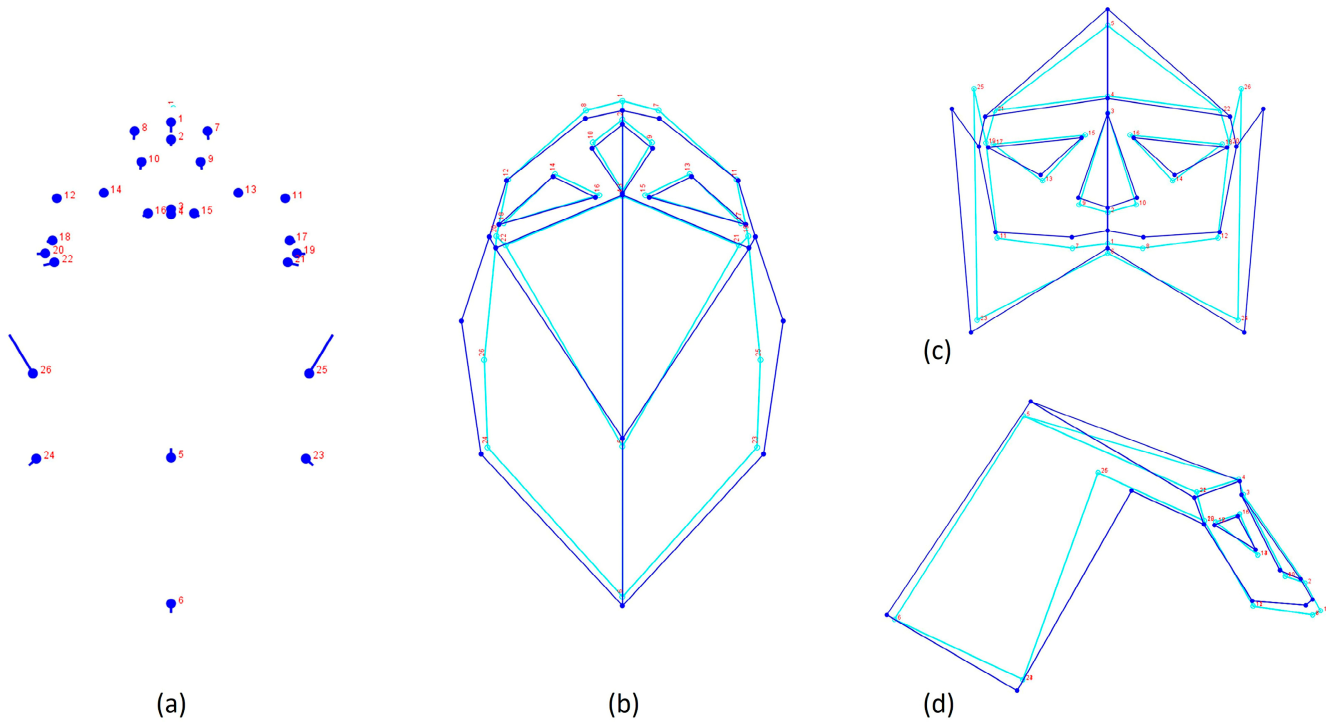

Figure 2. Cranial Shape Variation (light blue-dark blue): PC1 Lollipop Graphs (**a**); PC1 Wireframe of Superior View (**b**); PC1 Wireframe of Anterior View (**c**); PC1 Wireframe of Lateral View (**d**).

3.2. PCA and MANOVA/CVA Procrustes Coordinates in Shape Space

PCA and MANOVA/CVA plots are always influenced by the number of specimens and the sexual dimorphism hence their interpretation were considered in relation to the sample size and sample composition.PCA and MANOVA/CVA have always been interpreted considering that sample size and sample composition (number of specimens and sexual dimorphism) can influence the analysis. Nevertheless, the obtained plots produced plausible results.

The eigenvalue distributed among all the components decreases gradually over the PC axes (Figure S2a) until the PC4. After the PC4, between the components of the prehistoric specimens, the eigenvalues halved in each component, denoting notable variations only between the components of the prehistoric specimens.

The pattern obtained for the PCA (Figure 3a,b) and the MANOVA/CVA (Figure 3c,d) highlights a sudden clear separation between the Würm-Settlers of San Teodoro and the hunter-gatherers of the Mesolithic, themselves separated from the other prehistorical populations.

The Mesolithic, still characterized by robusticity and less variability, presents no negligible differences in shape compared to the Bronze Age and Iron Age specimens.

In turn, Bronze Age and Iron Age groups already share some features with the first historical groups with their specimens that however lie on the right side of the PC1 axis close to the PC2 axis, staying well apart from the Contemporary.

In this intricate scenario, it is not negligible that the two late antique specimens were once separated by Antiquity and the Middle Ages Finally, contemporary specimens, as expected, present a wide heterogeneity, mainly placed on the right side of the PC1 axe.

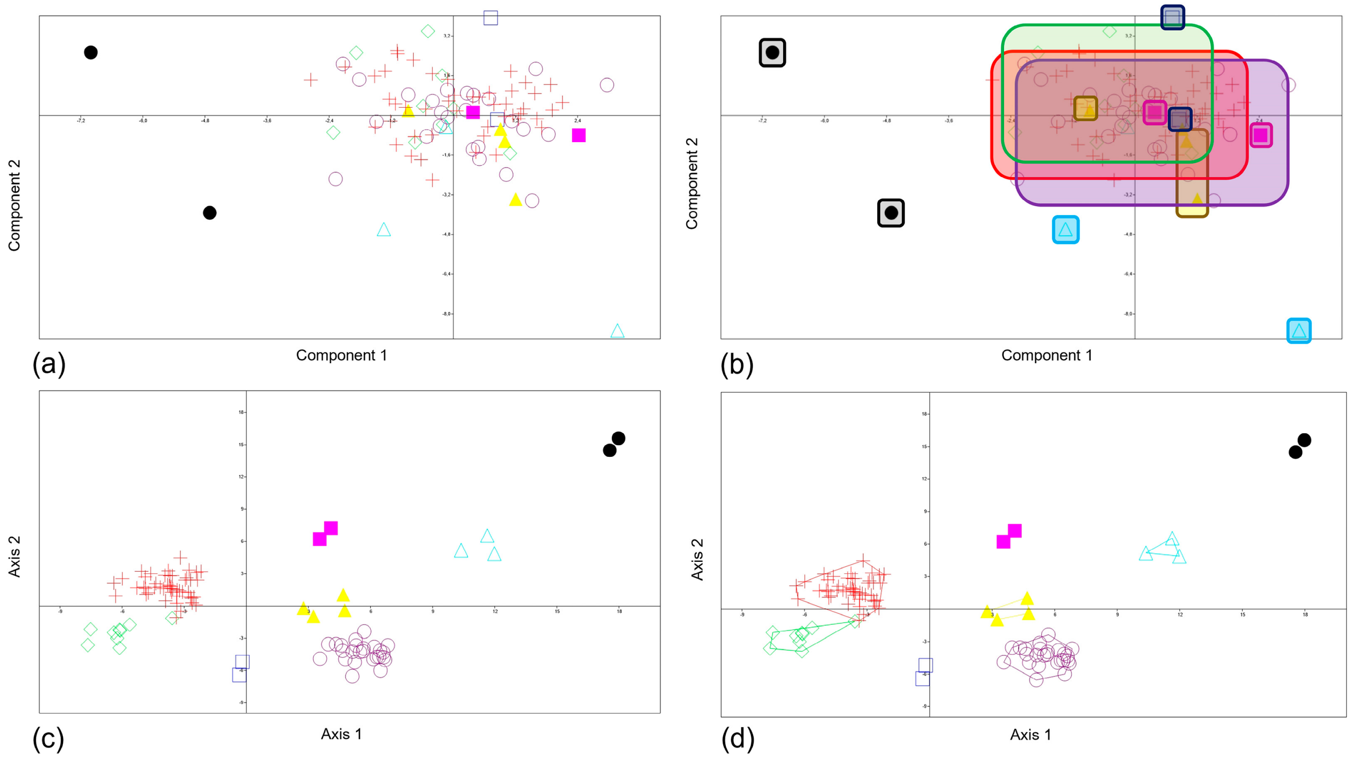

Figure 3. PC1vsPC2 of Procrustes fitted coordinates (**a**); PC1vsPC2 Box Colour of Procrustes fitted Coordinates; MANOVA/CVA of Procrustes fitted Coordinates (**b**); MANOVA/CVA of Procrustes fitted Coordinates (**c**), MANOVA/CVA Convex of Procrustes fitted Coordinates (**d**). (●) Upper Paleolithic, (Δ) Mesolithic, (Δ) Bronze, (□) Iron, (○) Antiquity, (■) Late Antiquity, (◊) Middle Ages, (+) Contemporary.

To summarize a not homogeneous layout of the morphospace hold the prehistoric specimens while the historical ones lies close to each other in a morphospace that assumes a concentric layout.

A multivariate analysis was completed by calculating the Procrustes averages for each site (Molara, Agrigento and San Giovanni Marsala were always considered evaluating that were represented by a single specimen).

The eigenvalue and percent variance gradually decrease on the PC axes only after PC4 (Figure S2b). This trend denotes an important variation included in the first components with the scree plot indicating how only the first two PCs could be considered significant.

The scatterplot (Figure 4) marks the separations between the Paleolithic hunter-gatherers of San Teodoro and the Mesolithic ones of Uzzo and Molara.

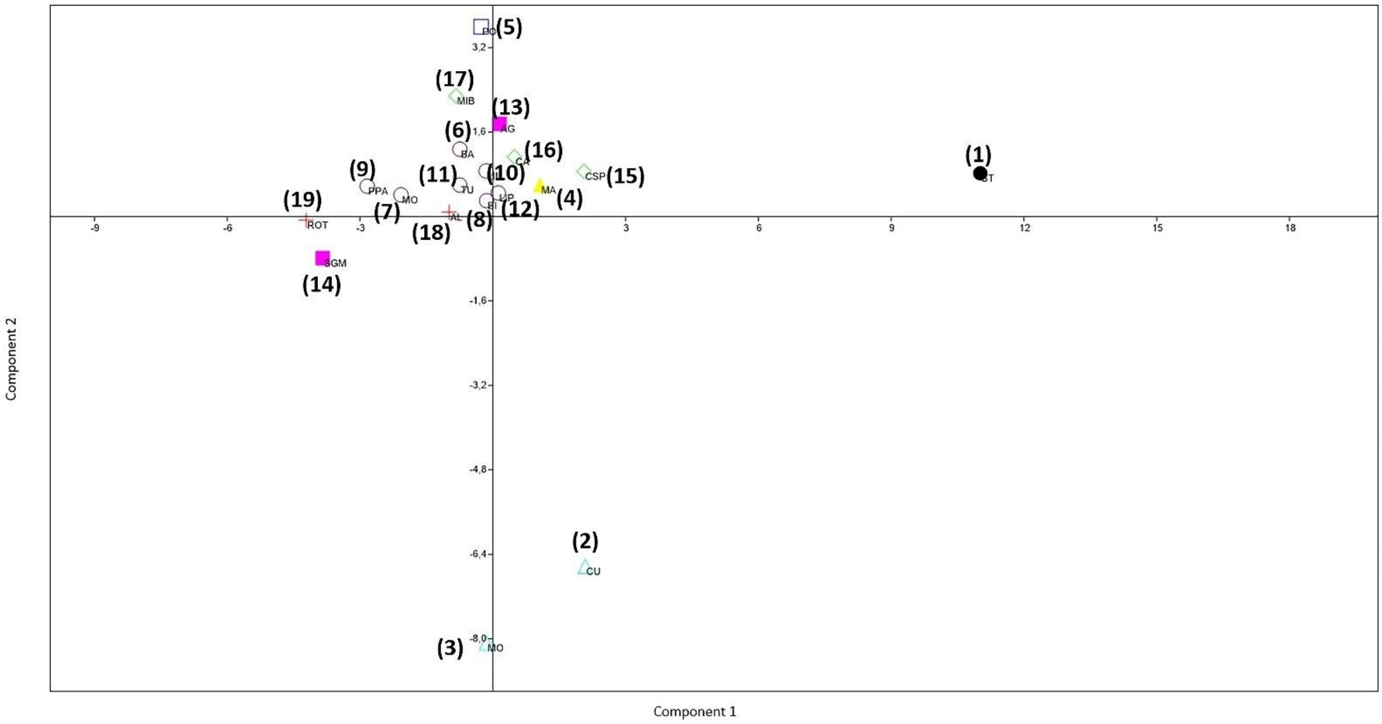

Figure 4. PC1vs PC2 of the average of the Procrustes Coordinates for each site: 1-SanTeodoto; 2-Grotta dell'Uzzo; 3-Molara; 4-Marcita 5-Polizzello; 6-Baucina; 7-Mozia; 8-Birgi; 9-CasermaTukory; 10-Phoenician/Punic of Palermo; 11-Lilibeo; 12-Lipari; 13-SanGiovanniMarsala; 14-Agirigento; 15-CastelSanPietro, 16-MonteIatoB; 17-Caltavuturo; 18-Alia; 19-PalermoRotoli.

Among the historical groups, moreover, it stands out that the Phoenicians/Punic that settled in Birgi and Lilibeo are close to each other and separated from the other Phoenician/Punic settlements of Mozia.

The Middle Ages of Caltavuturo, Castel San Pietro and Monte Iato B (Indigenous, Byzantine and Islamic) present much variability (quite comparable with the contemporary people of Alia (18th century) and Rotoli (21st century).

Finally, the multivariate comparison tests of variance (Wilk's lambda and Pillai trace) both returned p (same) values < 0.001 at a significance level of $\alpha = 0.05$.

3.3. Neighbour-Joining of Procrustes Coordinates

The NJ tree obtained with the Procrustes coordinates of the entire Sicilian sample (Figure 5) shows the Outgroup of San Teodoro (automatically recognized by PAST) separated into two different main clusters, one that has Mesolithic roots and the second that generates all the other groups. In these splits, the Bronze and Iron roots precede the historical one. Finally, the more recent groups (Middle Ages and Contemporary) cluster relatively closer to each other, showing a certain similarity.

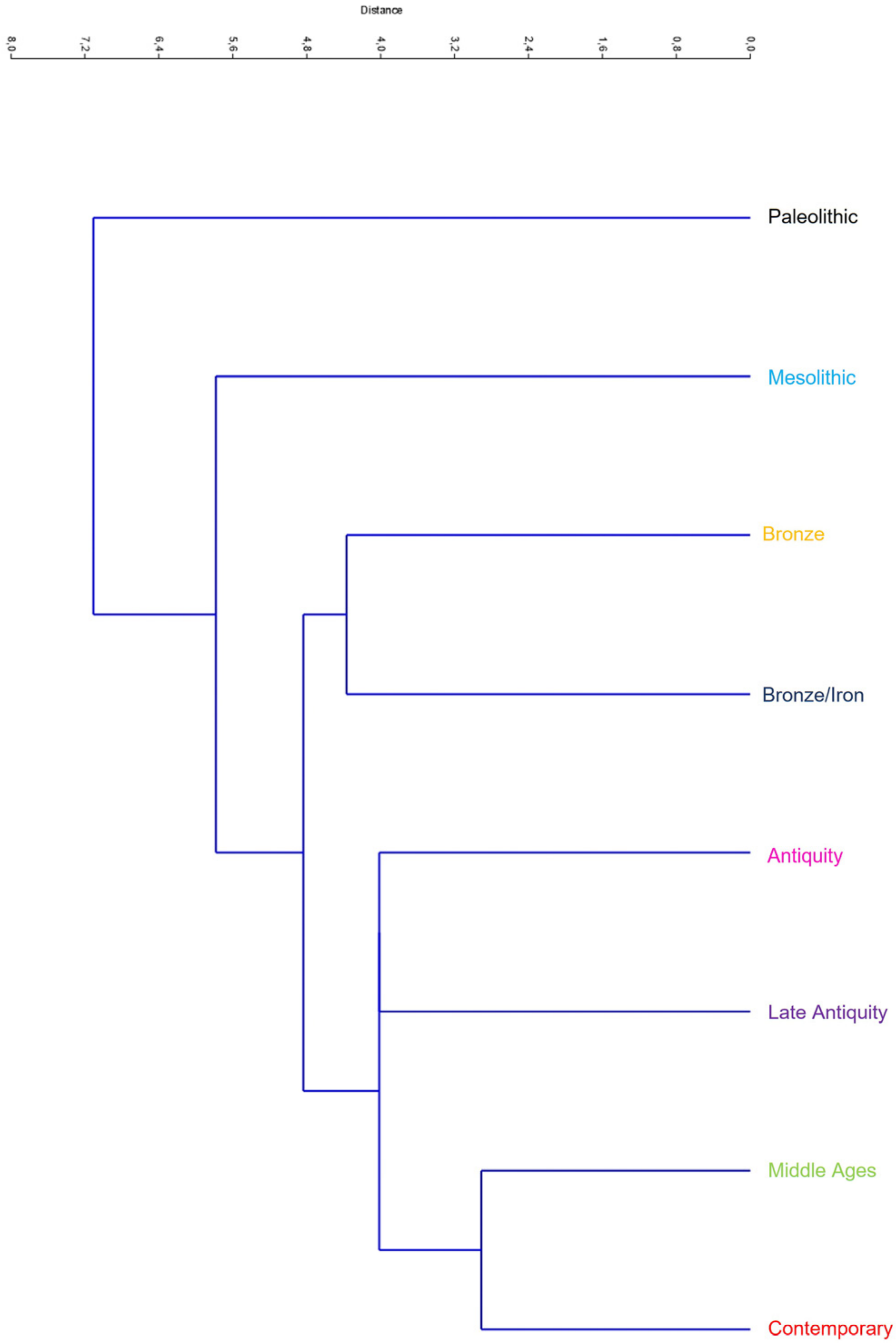

Figure 5. Procrustes Coordinates Neighbor-Joining hierarchical tree (UPGMA cluster procedure and Euclidean distance matrix) representing the divergences in Sicily from Paleolithic to the Contemporary Age.

4. Prehistory and History: Geometric Morphometrics

4.1. Prehistory and History: Shape Variation and Comparison

1. Group 1-Prehistory: Upper-Palaeolithic, Mesolithic, Bronze, Iron.
2. Group 2-History: Antiquity, Late Antiquity, Middle Ages, Contemporary.

The changes in the shape shown by the Lollipop and Wireframe graphs highlight how among the prehistoric the landmarks placed on the superior jaw (landmarks 1, 7, 8, 13 and 14) (Figure 6a,b), slightly moved forward and inwards. At the same time, the facial and frontal bones (Figure 6c) do not undergo any change. In that period, the main changes occur in the cranial vault, with the parietals (landmarks 23, 24, 25, 26 and 5) (Figure 6d) moving forward and the occipital moving slightly inwards, keeping an elongated and narrow shape on the posterior part.

Contrarywise during historical periods the changes in shape do not affects the landmarks of the superior jaws (Figure 6e,f) but instead changes regards the Front-Temporal bones (landmarks 15, 16, 17, 18, 19 and 20), which become wider and shorter (Figure 6g).

Moreover, the parietals (landmarks 25, 26 and 5) and occipital (landmarks 23 and 24) bones become wider and taller (Figure 6h).

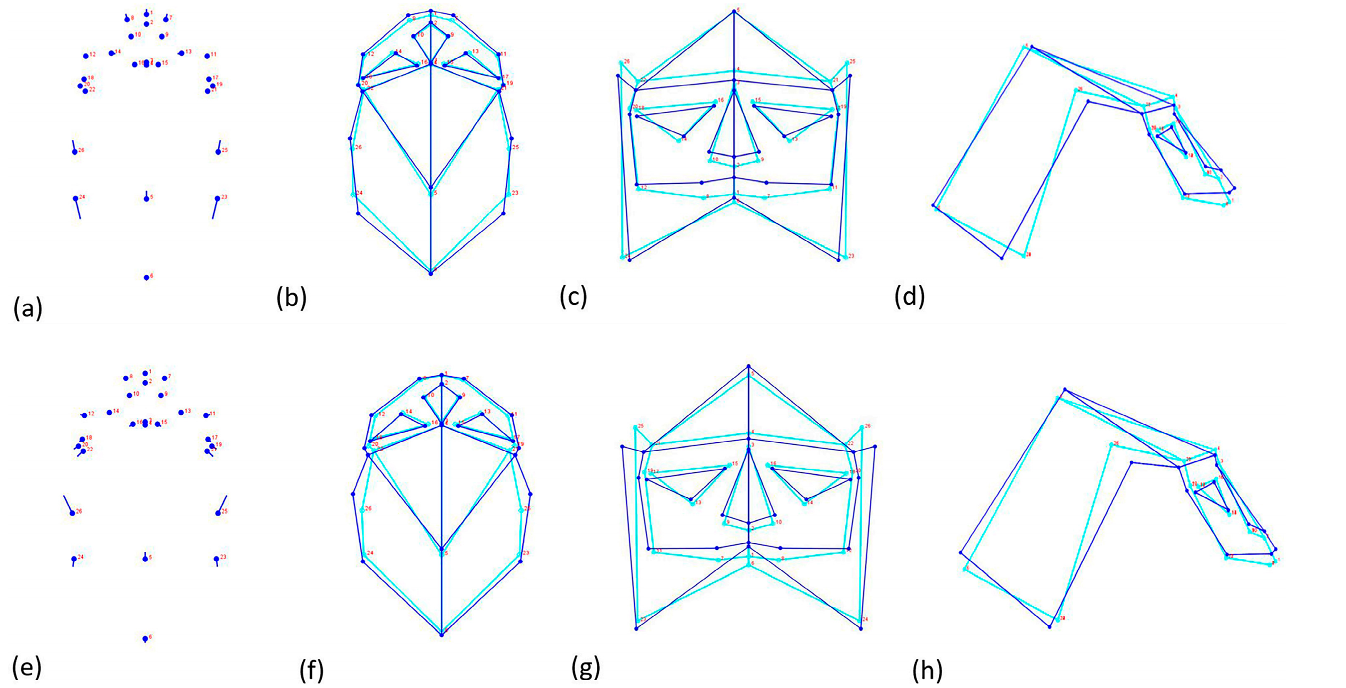

Figure 6. Groups Cranial Shape Variation (light blue-dark blue): Group1-PC1 Lollipop Graphs (**a**); Group1-PC1 Wireframe of Superior View (**b**); Group1-PC1 Wireframe of Anterior View (**c**); Group1-PC1 Wireframe of Lateral View (**d**); Group2-PC1 Lollipop Graphs (**e**); Group2-PC1 Wireframe of Superior View (**f**); Group2-PC1 Wireframe of Anterior View (**g**); Group2-PC1 Wireframe of Lateral View (**h**).

4.2. Prehistory and History Shape Changes

PC1 and PC2 of both the groups cover around 90% of the variance while, in terms of eigenvalues, those of Group1 decreased constantly and generally without significant variations among all the components (Figure S2c), while Group2 was characterized by relevant steps across each component (Figure S2d).

Regarding Group1 (Prehistory), the PCA (Figure 7a,b) shows a clear separation between Palaeolithic Würm-Settlers and the Mesolithic specimens with Bronze and Iron groups clustered close to each other but separated from the Mesolithic one (inhomogeneous morphospace).

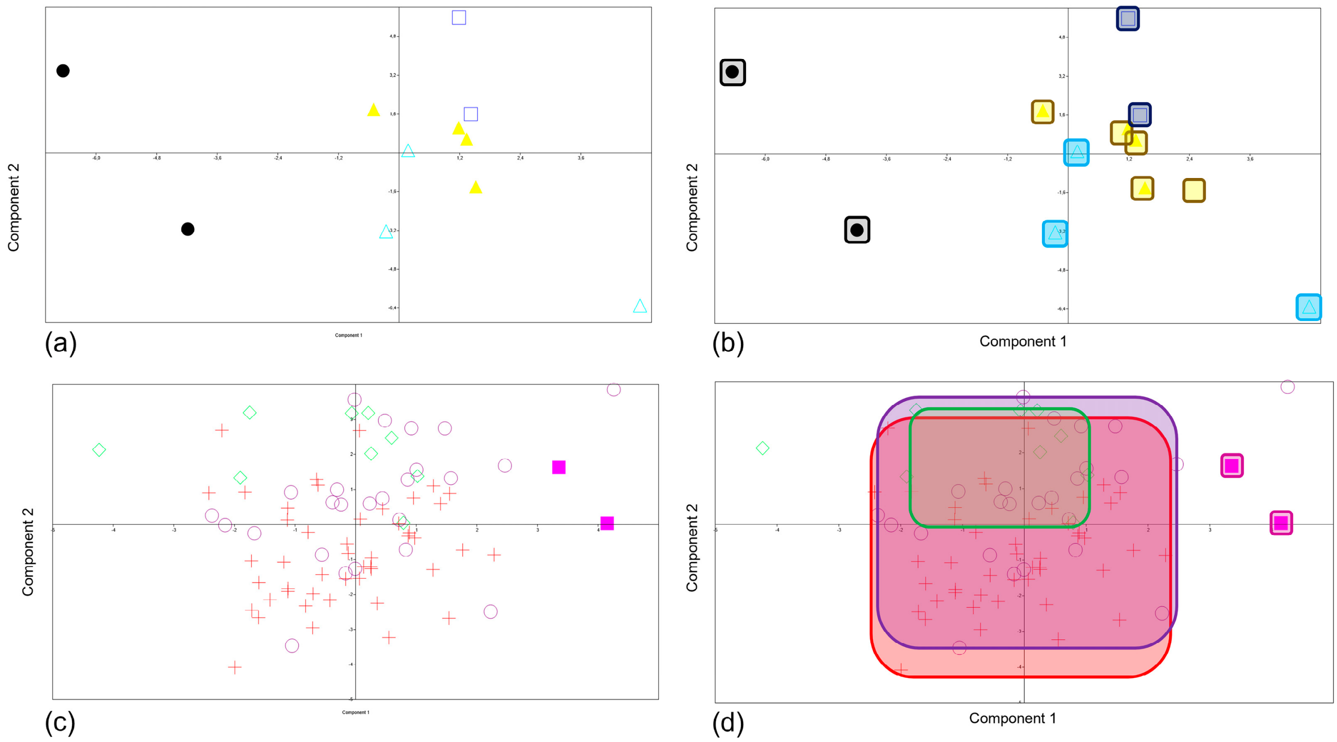

Figure 7. Group1-PC1vsPC2 of Procrustes Coordinates (**a**); Group1-PC1vsPC2 Box Color of Procrustes Coordinates (**b**); Group2-PC1vsPC2 of Procrustes Coordinates (**c**); Group2-PC1vsPC2 Box Color of Procrustes Coordinates (**d**). (●) Upper Paleolithic, (Δ) Mesolithic, (Δ) Bronze, (□) Iron, (○) Antiquity, (■) Late Antiquity, (◊) Middle Ages, (+) Contemporary.

Group 2 (History) presents another significant situation in which the morphospace assumes a more concentrical organization with a homogeneous distribution of the specimens along the four axes (Figure 7c,d).

Figure 8 shows a simple and quick way to represent the Sicilian scenario. Figure 8a displays the sporadic and discontinuous "Human Flow" that occurred during prehistory with Paleolithic and Mesolithic human groups (separated by the two PC2 axes) still separated into different populations. The Bronze and Iron populations appear as separate groups but are nevertheless found close to each other and to the positive PC1 axis and negative PC2 axis. Figure 8b places the antiquity groups widely distributed along all PCs axes (positive and negative sides), highlighting the relevant increase in variability of the genetic pool and morphological changes. This correlation is further underlined by the position of the two late antiquity specimens (from the Roman Empire) that are located far away from the other groups. Among the Medieval, we note the difference between the Norman/Swabian of Monte Iato and the mixed populations (Islamic and Indigenous/Islamic) of Castel San Pietro and Caltavuturo which lie close to each other and are separated from the former by a PC1 axis. Finally, note the positions of the Contemporary samples from Alia (18th century) and Rotoli (21st century) that lie on the same sides of the PC axes but are also separated.

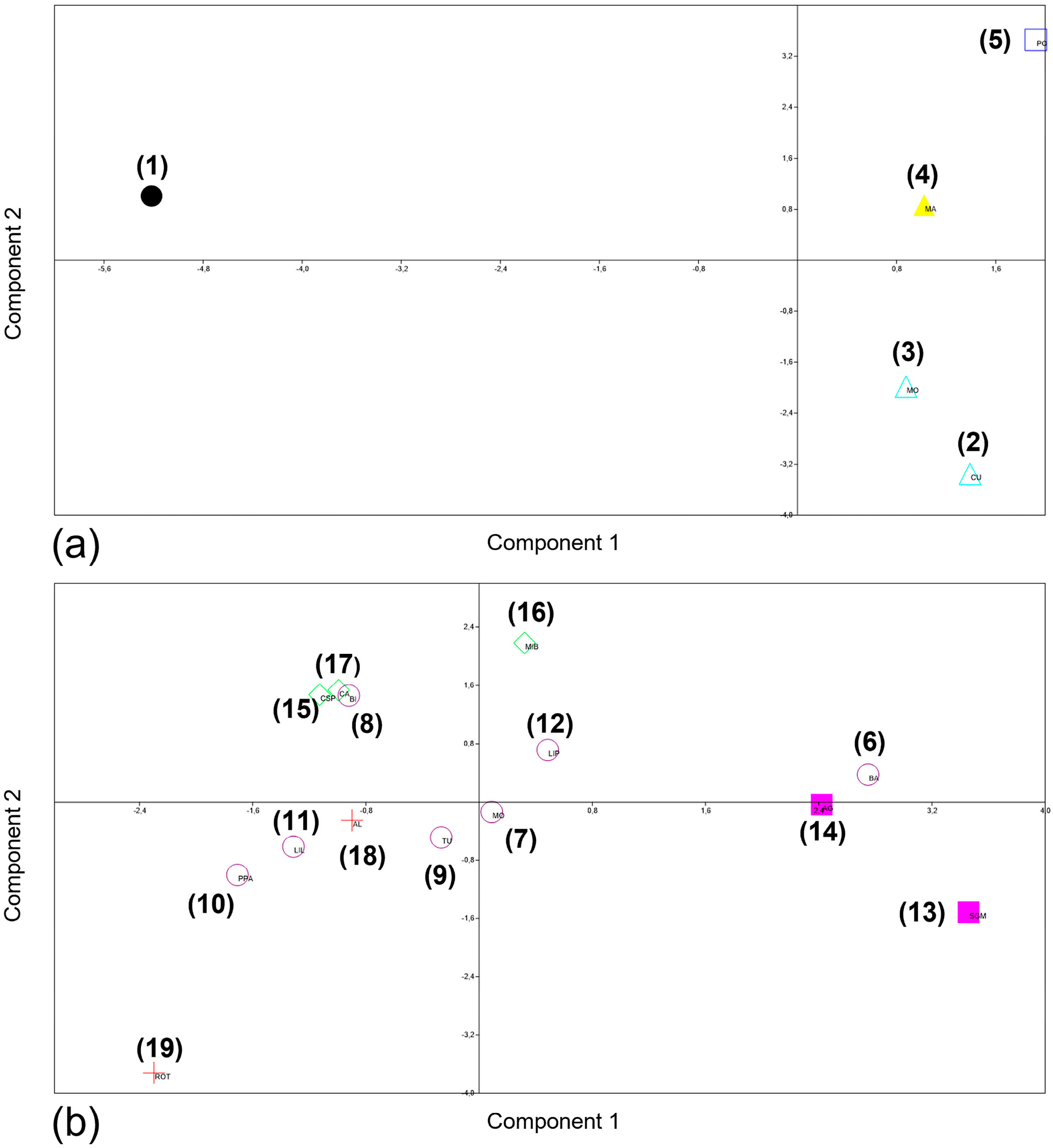

Figure 8. Group 1-PC1vsPC2 of the average of the Procrustes Coordinates (**a**); Group 2-PC1 vs PC2 of the average of the Procrustes Coordinates (**b**). 1-SanTeodoto; 2-Grotta dell'Uzzo; 3-Molara; 4-Marcita 5-Polizzello; 6-Baucina; 7-Mozia; 8-Birgi; 9-CasermaTukory; 10-Phoenician/Punic of Palermo; 11-Lilibeo; 12-Lipari; 13-SanGiovanniMarsala; 14-Agirigento; 15-CastelSanPietro, 16-MonteIatoB; 17-Caltavuturo; 18-Alia; 19-PalermoRotoli.

4.3. Neighbor-Joining of Procrustes Coordinates

Group1 and Group2 NJ trees (Figure 9a,b) displays the divergences starting from the Paleolithic and Antiquity groups that were automatically recognized as outgroups.

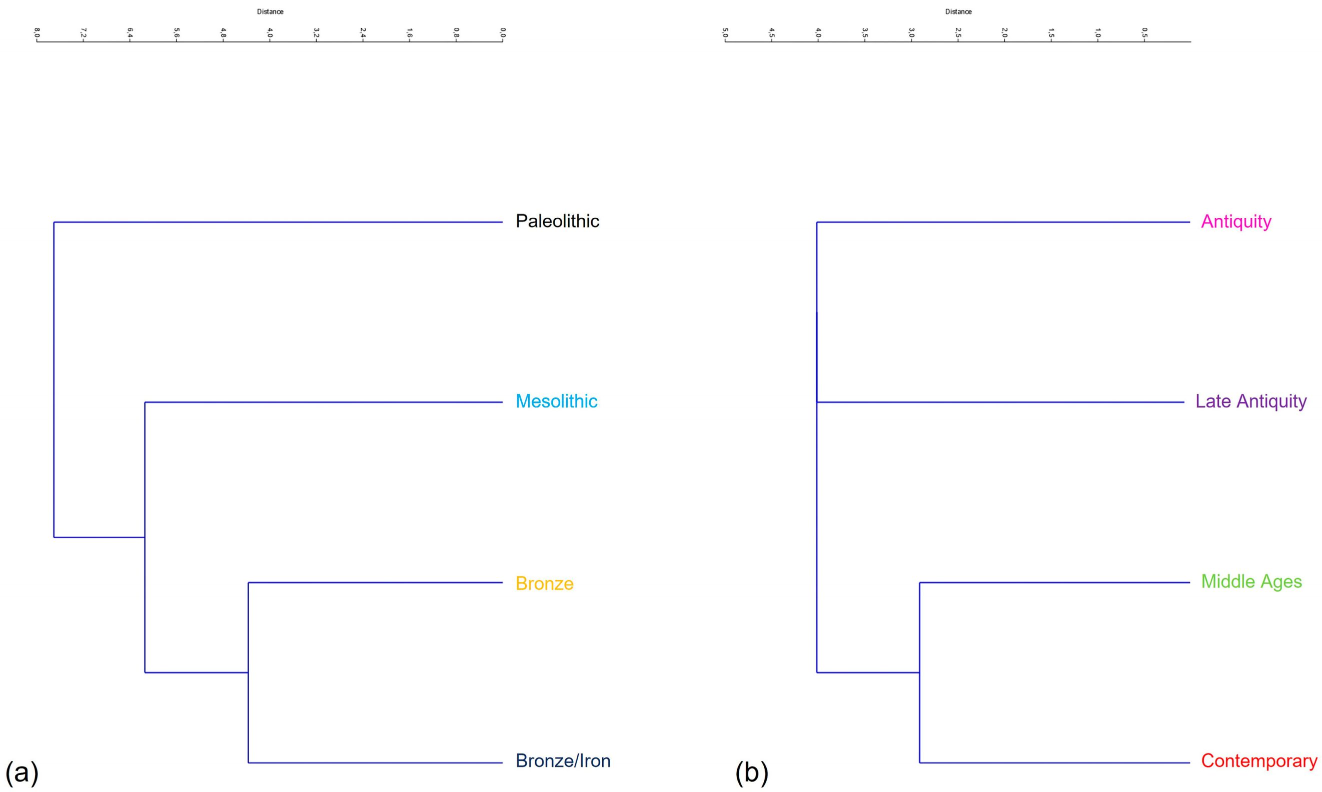

Figure 9. Procrustes Coordinates Neighbor-Joining hierarchical tree (UPGMA cluster procedure and Euclidean distance matrix) representing the divergences in Sicily into Prehistory (**a**) and into Historical Periods (**b**).

5. Discussion

This study aims to identify and describe the trends in facial morphology (micro-anatomical and micro-evolutionary features) during the ages and highlight the chronological diversity in morphology to evaluate the population influx and the related populations' dynamics [53–55,80] in Sicily.

Nevertheless, this is not a genetic study, and we also investigated the possible connections between morphological changes and the cultural/biological flows [52,56], the environmental changes, and the ecological pressures [57].

To achieve our goals, we performed a diachronic morphometric study on 95 skulls' 3D models related to a period spanning from the Paleolithic to the Contemporary Age [81].

The study includes a shape changes visual analysis (lollipop and wireframe graphs) followed by an exploratory analysis carried out by PCA, both supported by MANOVA/CVA and NJ analyses. Furthermore, MANOVA/CVA was associated with Wilk's lambda and the Pillai trace test to evaluate the H0 of equal multivariate means between the groups. The main result focused on the correlation between facial trends, variability and population dynamics, indicating that a morphometric approach is extremely suitable to reconstruct past scenarios with a high degree of effectiveness [82].

The shape changes graphs displayed the Prehistoric skulls still characterized by elongated occipital bones and more prognathic narrower faces. Despite these features, the skulls already presented the beginning of the mesocephalization with a slight widening of the cranial vault due to the increase of parietal roundness. The mesocephalization trend was more intense in the facial and occipital bones during antiquity and the following period.

In general, the slight mesocephalization is detectable in the general trend of decreasing maxillary prognathism with the skull becoming more narrow and slightly less elongated as the face becomes wider and shorter.

All of the multivariate statistical analyses highlight a correlation between population influx and cranial variability [57], contributing to clarify the population dynamics of the island.

Our results show a series of demic migration (low density) and population discontinuity that characterized Sicilian Stone Ages, and persisted during the Bronze Age until the Iron Age (underlined by the inhomogeneous morphospace shown in Figure 7a,b). Population influx and variability correlations are well shown in the homogenous morphospace that characterizes the historical groups denoting as these results in no well-defined populations.

With regard to the early colonization of the island and the Prehistoric human flow, the plots obtained (PCA, MANOVA/CVA and NJ) display a separated clustering for the Upper-Paleolithic Würm-Settlers and the Mesolithic hunter-gatherer specimens, with the first one possibly representing the first evidence of human colonization in Sicily [30–32,52].

The obtained data implement and enhance the preliminary morphometrics study that associated the morphology of the Sicilian Paleolithic specimens with the other Upper-Paleolithic groups from Western Europe and south-central Italy [52], that in turn and still kept some archaic characters [83].

Furthermore, the data also agree with palaeoecological studies that hypothesize that during the last glacial peak, the climatic conditions (steppe or semi-steppe environment and extremely low rainfall values) [39] did not allow a consistent occupation by *Homo* [84]. Only the transition between the last glacial period and the Holocene (Deglaciation Period) brought progressive climatic mildness to the island. The gradual development of the steppes to wooded steppes and consequently to temperate forests favored the substantial population (density and genetic) of the island [40].

Therefore, only the final part of the Paleolithic period presented stable climatic conditions that allowed hunter-gatherers to move on from a cyclical occupation of the island and adopt mobile-forager/semi-sedentary ecology that lasted during the Mesolithic, Bronze Age, and Iron Ages [51].

In addition to the Paleolithic era, the Mesolithic craniofacial morphometrics seem to favor a series of low-density migration during the early colonization of Sicily.

Even considering that Molara is represented by a single specimen, the Upper Paleolithic-Mesolithic divergence could reasonably be the result of punctual migrations and the isolation of human groups during the Sicilian Stone age. Furthermore, a slow but progressive increase of the migratory flows (frequency and density) during the island's Metal Ages (still characterized by punctual migrations), is displayed by the settlements of Marcita (Bronze Age), Baucina and Polizzello (Iron Age) that lye close in terms of history but separated from each other.

Notable is that the Mesolithic specimens do not present remarkable differences in shape compared to the Bronze Age and Iron Age specimens and that they are still characterized by robusticity and low variability [32].

Despite the lack of samples from the Neolithic and the Eneolithic periods, evaluating the results is reasonable to suppose that Bronze Age and Iron Age were still subjected to demic migration (low density) and population discontinuity.

As concerns the historical specimens, some populations kept some archaic characters after the Iron Age (historical era); nevertheless, population continuity (a consequence of the coexistence of several Mediterranean populations) from antiquity to the Middle Ages produced a progressive increase in variability underlined by the absence of substantial variation between the eigenvalue and the percent variance in the PC. The absence of an internal relationship caused by the intricate colonization period is instead present in the prehistoric sample in which we can find a clear variation between the PC.

Among the historical groups, it stands out that the Phoenician/Punic that settled in Birgi and Lilibeo are close to each other and separated from the other Phoenician/Punic settlements of Mozia. This is evidently related to the strong influence of Greek and Roman colonization, and that a certain degree of variability had already been reached before the Middle Ages. The similarities among the Antiquity and Late Antiquity (Roman Empire) groups were the result of complex and varied migration patterns during the Roman Age in those centuries.

Correlations between population influx and variability can also be observed in the influence of Islamic settlers on the Indigenous peoples during the Middle Ages.

The homogenous morphospace of all these groups (concentric organization—Figure 7c,d) highlights how the correlation between population influx and variability resulted in no well-defined populations when compared with the contemporaneous period.

Although a wide variability was expected for the Contemporary specimens, the same distribution displayed during the Antiquity, the Late antiquity and the Middle Ages suggests an increase in variability and a strong heterogeneity that could be compared with the one observed in the Contemporary Age.

Apart from the increase in specimens available for the historical groups, the periods mentioned had contributions from the Phoenician/Punic, Greek, Roman, Byzantine, Islamic and Norman/Swabian dominations, which had already produced a huge heterogeneity without modern dispersal patterns. In fact, the geography and history of the island are not negligible. Located in the center of the Mediterranean Sea, for millennia Sicily was a crossroads and a meeting place (maritime hub) for commercial exchanges between many people coming from different geographical areas (different cultures and several empires) each with their own genetic pool.

Finally, these dynamics produced a not negligible allometry supported by Wilk's lambda and Pillai trace tests, performed in association with the MANOVA/CVA, that both returned a p (same) value < 0.001 in a significance level of $\alpha = 0.05$ that rejects the H0.

To summarize, our results do not suggest a dramatic shift in cranial morphology. Rather, some steps of remarkable variation during Prehistory (the inhomogeneous layout suggests a discontinuous contribution of populations influx during Prehistory) were juxtaposed with a continuous slow degree of morphological differentiations from the other historical ages produced by the interaction between the indigenous people and settlers.

When evaluating the variation between human groups arriving to a localized geographical region (like an island), it is important to consider that the genetic pool is often stressed by genetic drift phenomena such as the bottleneck and founder effect [85]. In addition to these stochastic forces, adaptive changes (such as the masticatory-inducted phenotype) are in parallel impacted by cultural variations with the same plasticity but with a slow degree of diversification [86].

In particular, patterns of the cranial vault and the upper face are evolving largely neutrally [87]. Nevertheless, the large differentiation of facial shapes during the centuries could not only be explained by adaptive changes, but also by the arrival of new genetic components [88].

6. Conclusions

The timing and dynamics of the human peopling of Sicily and the island-related issues are subjects of continuous debate and are currently of great interest.

The proposed approach based on the GM of the skulls highlights the coexistence of microevolutionary patterns, population dynamics, and migrations (with the latter being one of the main causes of morphological variations) allowing for the reconstruction of past scenarios.

The multivariate plots highlight the fact that the Upper Paleolithic specimens of San Teodoro could be formerly considered as the first human group to establish a permanent settlement in Sicily, followed by a series of punctual migrations during Prehistory.

The variation among the Eigenvalues and the Percentage of Variance denotes that only the Bronze Age and the Iron Age migrations carry the prime plainness of morphological changes.

After the Iron Age (historical era), the population continuity (consequence of cohabitation and alternations of several populations) produced a progressive increase of variability that is denoted by the absence of significant variation between the Eigenvalue and the Percentage of Variance.

The correlation between population influx and variability is observable in both the Prehistorical and Historical Groups. The Prehistorical specimens lying on an inhomogeneous morphospace allows for the highlighting of the primeless migration from the Italian Peninsula. The historical specimens displayed in a homogenous morphospace resulted in no well-defined "populations" due to the alternation and cohabitation of several populations. Further analysis increased by new specimens (materials not already excavated), joined with further isotopic and genetic analysis will clarify some of the issues discussed earlier.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/heritage6020066/s1, Figure S1: S1-Anatomical Landmark's Positions: Draw from Buikstra & Ubelaker (1994) and Captured Screenshot from "Landmark3.6"; Figure S2: S2-Eigenvalue, % of Variance and Scree Plot with Broken Stick (in red) of the PC covered by: Sicilian Sample (a); Average of each site (b); Group1 Sample (c); Group 2 Sample (d); Group1 Average of each site €; Group1 Average of each site (f).

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