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ANALISIS *GEOMETRIC MORPHOMETRIC* UNTUK MENGKUANTIFIKASI DIMORFISME SEKSUAL PADA MANDIBULA DEWASA PADA POPULASI MALAYSIA DAN AUSTRALIA SELATAN MODERN

GEOMETRIC MORPHOMETRIC ANALYSES TO QUANTIFY SEXUAL DIMORPHISM IN THE ADULT MANDIBLES IN MODERN MALAYSIAN AND WESTERN AUSTRALIAN POPULATIONS

Tjiang SL[1]*, Obertova Z[1], Flavel A[1], Franklin D[1]
[1]*Centre for Forensic Anthropology, The University of Western Australia*
35 Stirling Highway, Perth WA 6009 Australia

ABSTRAK

Mandibula dewasa dapat digunakan untuk memperkirakan jenis kelamin dan asal/keturunan (*ancestry*). Namun, penelitian mengenai derajat dimorfisme seksual dan afinitas populasi mandibula masih sangat terbatas, sedangkan standar antropologi yang spesifik untuk populasi tertentu sangatlah penting untuk identifikasi forensik. Penelitian ini bertujuan untuk mengkuantifikasi dimorfisme seksual dan afinitias populasi mandibula dewasa pada populasi Malaysia dan Australia Selatan modern dengan menggunakan 101 CT scan mandibula laki-laki dan perempuan dari populasi Malaysia dan Australia Selatan. Sebanyak 32 penunjuk anatomi dikumpulkan dari CT scan, dengan menggunakan ukuran *centroid* dan analisis PCA untuk mengkuantifikasi variasi ukuran dan bentuk berdasarkan jenis kelamin dan populasi. Ketepatan klasifikasi diuji menggunakan DFA. Hasilnya, terdapat perbedaan signifikan berdasarkan jenis kelamin ($p < 0.05$) dan populasi ($p < 0.01$) pada semua kelompok untuk ukuran *centroid* dan PCA. Ketepatan klasifikasi didapatkan lebih tinggi pada estimasi jenis kelamin berdasarkan ukuran *centroid* (91-93%) dan estimasi asal/keturunan (*ancestry*) berdasarkan PCA (89-94%). Mandibula laki-laki lebih besar daripada mandibula perempuan, di mana *condylus mandibularis*, lebar *ramus*, dan *angulus mandibularis* merupakan fitur anatomis yang paling mencerminkan dimorfisme seksual. Ukuran *centroid* tidak berbeda secara signifikan untuk penentuan afinitas populasi, namun mandibula Malaysia cenderung menunjukkan ciri yang lebih "feminin" sedangkan mandibula Australia Selatan cenderung menunjukkan ciri yang lebih "maskulin". Hal ini mengindikasikan bahwa tiap populasi memiliki gradasi dimorfisme seksual yang berbeda. Analisis *geometric morphometric* dapat digunakan untuk memperkirakan jenis kelamin dan asal/keturunan (*ancestry*) dari mandibula dewasa. Estimasi jenis kelamin dan asal/keturunan (*ancestry*) lebih akurat jika dilakukan secara terpisah (tidak simultan), di mana ukuran mandibula lebih akurat untuk penentuan jenis kelamin, sedangkan variasi bentuk mandibula lebih akurat untuk penentuan asal populasi.

Kata kunci*: dimorfisme seksual;* geometric morphometric*; mandibula; perkiraan asal/keturunan (*ancestry*); perkiraan jenis kelamin; variasi populasi.*

ABSTRACT

The adult mandible can be used to estimate sex and ancestry. However, there are limited studies on the degree of sexual dimorphism and population affinity of the mandible, while population-specific anthropological standards are essential for forensic human identification. This study aimed to quantify sexual dimorphism and population affinity in adult mandibles in modern Malaysian and Western Australian populations. A total of 101 CT scans of the mandible were used in this study, consisting of male and female of Malaysian and Western Australian origins. Thirty-two anatomical landmarks were collected, where the centroid size and PCA analysis were used to quantify the size and shape variations between sexes and between populations. Classification accuracy rates were calculated using DFA. There were significant differences between sexes in all groups for centroid size and PCA ($p < 0.05$), and between populations for PCA ($p < 0.001$). Classification accuracy rates were higher for sex estimation from centroid size (91-93%) and ancestry estimation from PCA (89-94%). Male mandibles were larger than females, and the mandibular condyles, ramus breadth, and gonial angle were the most sexually dimorphic features. For population affinity, size did not contribute significantly, but Malaysian mandibles tended to display more "feminine" features while Western Australians appeared to be more "masculine", indicating that different populations have different gradations of sexual dimorphism. Geometric morphometric analyses can be used to estimate sex and ancestry from adult mandibles. The estimations of sex and ancestry are more accurate when performed separately rather than simultaneously, where mandibular size is more accurate for sex estimation, while the shape variations are more accurate for distinguishing between populations.

Keywords: *ancestry estimation; geometric morphometric; mandible; population variation; sex estimation; sexual dimorphism.*

****Corresponding author:***
Tjiang SL
Centre for Forensic Anthropology, The University of Western Australia
35 Stirling Highway, Perth WA 6009 Australia
e-mail: tjiang.sarilestari@gmail.com

INTRODUCTION

It has been well established that the human skull varies between sexes[1] and populations[2,3], which means it can be used to estimate both sex and ancestry. The mandible is a part of the skull which has also been proven to be sexually dimorphic[4,5,6] and population-specific[6,7,8,9]. Currently, there is only a limited number of research on sexual dimorphism and population specificity in the human mandible[10]. Meanwhile, in some instances such as commingled remains or incomplete/fragmented remains, the mandible or parts of mandible are a robust sample that could withstand the environmental factors[11] and could be assessed individually to estimate the biological sex and/or population affinity[12]. Therefore, it is important to conduct a study to explore parameters of sexual dimorphism and population affinity of the mandible, as it may be a useful alternative to identify the remains of an unknown individual.

As forensic anthropology has legal and human rights implications, it is also essential to develop population-specific standards with reliable methods. To achieve this goal, it is crucial to understand the extent and expression of sexual dimorphism in the adult mandible, and also the mandibular morphological variations that can differentiate between populations. These variations should be quantified to provide statistical accuracy and error rate—thus, the results can be applied in the forensic practice. Moreover, providing statistical rigour for scientific methods is a part of the criteria for scientific evidence admissibility in the court.

Conventional methods of estimating sex and ancestry from human mandible include Giles' morphometric method to estimate sex with a claimed accuracy of over 80%[4] and Rhine, Brues, and Krogman's morphoscopic criteria for estimating ancestry[13,14,15]. However, the samples used in these studies are not of the contemporary population (the samples were from the early 20th century) and only include several populations groups in the U. S. ("White", "Black", and "Asian"); therefore, they may not be representative of other, more current populations. Moreover, morphometric methods are less sensitive in visualising the shape of the object and morphoscopic methods are prone to subjectivity. This means that morphometric methods do not significantly take shape variations into account when assessing an object, while morphoscopic methods mostly rely on the examiner's judgment and interpretation instead of the "true value" provided by linear measurements.

Franklin et al. (2007) conducted geometric morphometric analyses of the mandible and found that the adult mandible can be used to "*identify both sex and population affinity with increased sensitivity and objectivity compared to standard analytical techniques*". Furthermore, by using "*known skeletal collections or appropriate clinical data*", geometric morphometric analyses can be used to develop local forensic standards for both sex and ancestry estimation for the purpose of forensic human identification[6]. The study itself demonstrated that using the geometric morphometric method, the estimation of ancestry in the South African Bantu and Caucasian American resulted in over 98% accuracy[6]. Geometric morphometrics itself is a technique that analyses form variations among the samples, which means that it can analyse both size and shape independently or dependently—therefore, it is able to visualise the object in a 3D form and overcome the limitations of both morphoscopic and morphometric methods[16,17,18,19,20], resulting in higher accuracy for both sex and ancestry estimations.

The present study aims to quantify the sexual dimorphism and population affinity of the adult mandible in Malaysian and Western Australian populations, as there is only a limited number of research in this particular field. However, it is not within the scope of this project to develop a population-specific standard for the estimation of sex and ancestry for either population. Though, the results of this study may be useful for the development of such standards in the future.

MATERIAL AND METHODS

Material

The present study examines 101 computed tomography (CT) scans of adult mandible from two different populations of known sex, age, and ancestry. The sample consists of 45 Malaysian (20 males and 25 females of Chinese and Malay/native Malaysian origins) and 56 (27 males and 29 females) Western Australian individuals acquired from the Centre for Forensic Anthropology collection. CT scans presenting abnormal morphology, including mandibular trauma, missing teeth, and/or extensive alveolar resorption were excluded since it would affect the ability to locate all required anatomical landmarks. The inclusion criteria were scans with a slice thickness of ≤1 mm and included the entire mandible and the external auditory meatus (for the purpose of anatomical positioning). Research ethics approval was granted by the Human Research Ethics Committee of the University of Western Australia (RA/4/1/4362).

Data Acquisition

A total of 32 bilateral and midline landmarks were obtained using OsiriX MD (version 11.0.2). While most of the landmarks are commonly used in traditional metrical and geometric morphometric systems[21], several new landmarks were included/adjusted to better characterise the shape of the mandible (Table 1 and Figure 1). Prior to landmark collection, the mandible was positioned in anatomical position (Frankfort plane), where the highest point on the upper margin of the external auditory meatus and the lowest point on the lower margin of the left orbit are on a horizontal plane[22].

Table 1. Landmark schema*

No		Landmark	Code	Operational Definition
Bilateral landmarks				
Right	Left			
01	19	Coronion	co	The most superior point on the coronoid process
02	20	Mandibular notch	mn	The most inferior point on the margin of the mandibular notch
03	21	Condylion mediale	cdm	The most medial point on the mandibular condyle
04	22	Condylion laterale	cdl	The most lateral point on the mandibular condyle
05	23	Posterior ramus**	pr	The middle point on the posterior border of the ramus (lateral view)
06	24	Gonion	go	The most lateral external point of the junction of the horizontal and ascending rami of the mandible
07	25	Base**	ba	The most inferior point on the lower border of the mandibular corpus perpendicular to the mental foramen
08	26	Mentale	ml	The most inferior point on the margin of the mandibular mental foramen
09	27	Lateral infradentale	lid	The midpoint of a line tangent to the outer margins of the cavities of the lateral incisor and canine teeth
10	28	Mental alveolar border**	mab	The most superior point on the lateral alveolar margin perpendicular to the mental foramen
11	29	Endocanine	enc	The most medio-posterior point on the alveolar margin of the canine
12	30	Ectomolare	ecm	The most lateral point on the alveolar margin on the buccal side, usually on the second molar
13	31	Endomolare	enm	The most lateral point of the lingual alveolar border in line (and/or) parallel to the plane between the ectomolares
14	32	Anterior ramus**	ar	The middle point on the anterior border of the ramus (lateral view)
Midline landmarks				
15		Gnathion	gn	The middle point on the lower border of the mandible in the midsagittal plane
16		Pogonion	pg	The most projecting point of the chin in the midsagittal plane
17		Mandibular symphisis	mns	The deepest point at the mandibular symphysis curvature (between infradentale and pogonion marks)
18		Infradentale	id	The midpoint of a line tangent to the outer margins of the cavities of the two mandibular central incisor teeth

**Landmarks were placed with the skull positioned in Frankfort plane*

***New/adjusted landmarks designed for the present study*

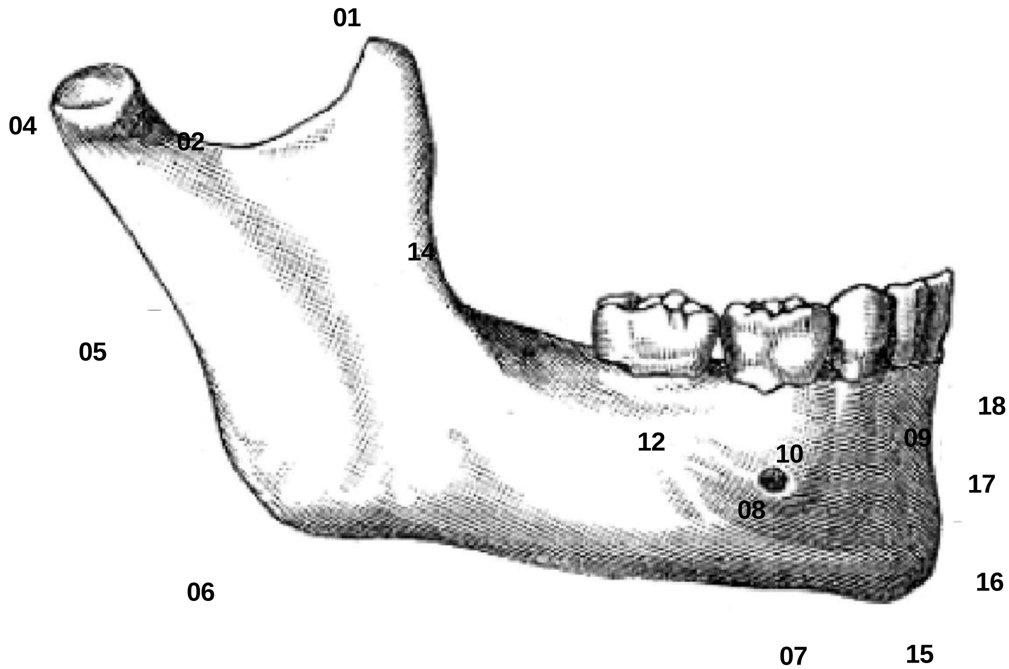

Figure 1. Lateral view of the mandible, showing several selected landmarks used in the present study

Geometric Morphometrics

Raw landmark coordinates were processed using MorphDB (version 2018) and exported to Morphologika (version 2.5) to analyse the three-dimensional coordinates of the landmarks. The raw landmarks from all samples were first processed using Generalised Procrustes Analysis (GPA), which involves translating, rescaling, and rotating the configurations to minimise a total sum of squares and acquire a unit centroid size. The next step was to explore the samples from different groups (male versus female and Malaysian versus Western Australian) using Principal Component Analyses (PCA). The results are presented in the Principal Component (PC) plots/graphs, wireframe, and rendered (polygon) models. The significant PCs were selected based on the cumulative value calculated by Morphologika, where the several first displayed PCs were taken until the cumulative value reached 80%.

Statistical analyses were performed for two types of assessment. For the centroid size, a one-way ANOVA was performed on the pooled sample to compare the means between the four groups (Malaysian female [F-MY], Western Australian female [F-WA], Malaysian male [M-MY], and Western Australian male [M-WA]). Independent t-test was performed to compare the means between two groups (see Table 2). Prior to the independent t-test, a test of equal variance between groups was conducted to see whether the variance between the groups tested was equal. For the PCA, one-way and two-way MANOVA were performed to compare multiple significant PCs in each group (Table 2). The results of MANOVA tests were presented in p-value, Wilk's lambda (Wilk's λ), and partial eta squared (partial η^2) to measure the significance, the between-group differences, and the effect size for group mean differences, respectively. The 3D-visualisations of the mandibles were then plotted on the two most significant PCs on the graph (selected based on the smallest p-value). A lower p-value (less than 5%) is mainly interpreted as a stronger correlation between two variables. However, statistical significance means that it is unlikely that the null hypothesis is true. The effect size is then calculated to understand the strength of the difference between two groups.

Finally, significant statistical results were followed by Discriminant Function Analysis (DFA) as a post-hoc analysis to predict the group membership of the samples. The statistical tests were performed using IBM® SPSS® version 21.0. An alpha level of 0.05 was used for all tests.

Table 2. Statistical analyses used in this study

Group	Comparison	Centroid Size	Principal Components
Pooled sample	F-MY, F-WA, M-MY M-WA	One-way ANOVA	One-way MANOVA
Pooled population	Female vs Male	Independent t-test	Two-way MANOVA
Pooled sex	Malaysian vs Western Australian	Independent t-test	Two-way MANOVA
Malaysian	F-MY vs M-MY	Independent t-test	One-way MANOVA
Western Australian	F-WA vs M-WA	Independent t-test	One-way MANOVA
Female	F-MY vs F-WA	Independent t-test	One-way MANOVA
Male	M-MY vs M-WA	Independent t-test	One-way MANOVA

Precision Study

Prior to data collection, a 4x4 precision study was conducted, in which four repetitions of placing the landmark sets were performed on four different samples, with a minimum of 24 hours between each repetition. The results were plotted on a PC graph to see if they cluster based on the individuals tested. The relative technical error of measurement (rTEM) and coefficient of reliability (R^2) were calculated for several specifically selected interlandmark distances (ILD) to assess the precision rate of landmark placing.

RESULTS

Precision Study

Landmarks collected from four different individuals (represented by different symbols) on four different occasions were plotted on a PC graph, which showed that the four repetitions clustered together (Figure 2), indicating acceptable precision of placing landmarks. Table 3 presents rTEM for 15 selected measurements, all of which were less than 5%. All measurements resulted in R^2 greater than 0.60, and 13 of them were greater than 0.80, indicating acceptable precision and low intra-observer error.

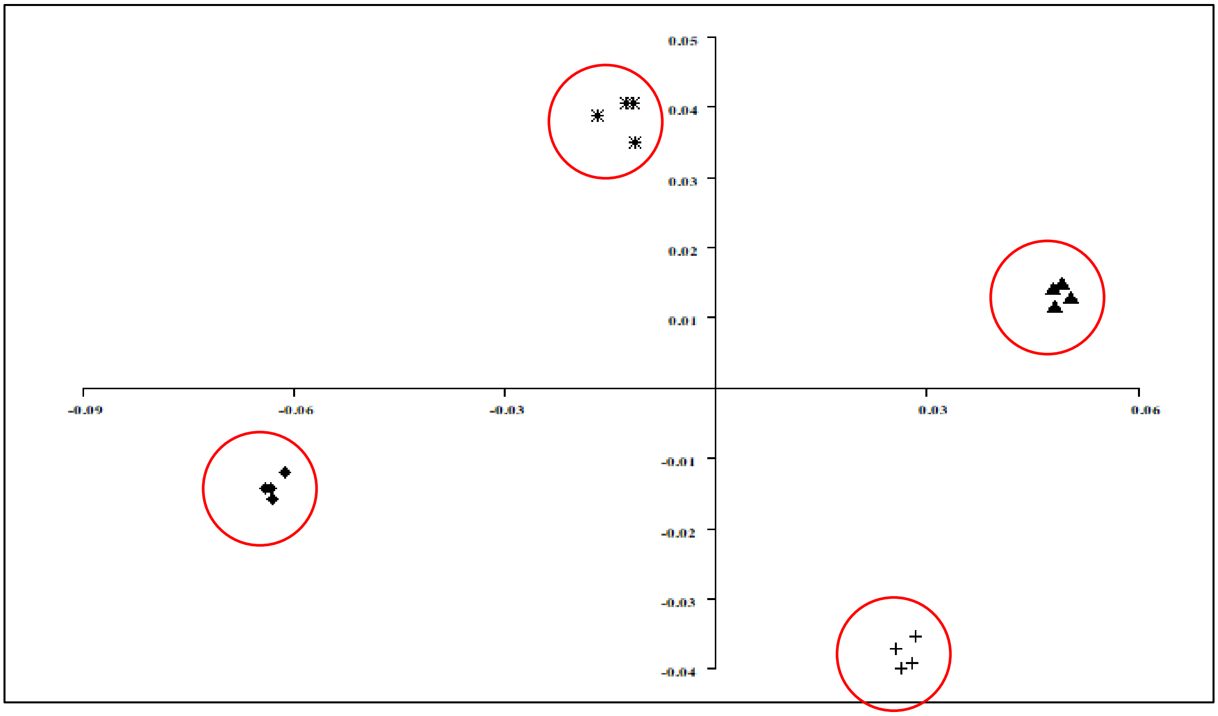

Figure 2. PC graph of the 4x4 precision study; four different individuals are represented by different symbols in red circles

Table 3. Descriptive statistics for the precision study

Measurement	Landmarks	R^2	rTEM (%)
Bicondylar breadth	cdl-cdl	0.997	0.3
Medial bicondylar breadth	cdm-cdm	0.997	0.3
Bigonial width	go-go	0.968	0.7
Alveolar breadth	ecm-ecm	0.972	0.6
Lingual breadth	enm-enm	0.741	2.9
Lingual intercanine distance	enc-enc	0.865	2.7
Chin height	id-gn	0.818	2.1
Mandibular height (R)	ba-mab (R)	0.854	2.1
Mandibular height (L)	ba-mab (L)	0.649	1.7
Ramus breadth (R)	ar-pr	0.973	0.8
Ramus breadth (L)	ar-pr (L)	0.969	1.0
Intercoronial distance*	co-co	0.929	1.2
Intermandibular notch distance*	mn-mn	0.996	0.3
Intermentale distance*	ml-ml	0.990	0.6

Bilateral infradentale breadth*	lid-lid	0.969	1.7

**specifically designed ILDs for the purpose of precision study*

Centroid Size

The means of the centroid size were statistically different between the four groups (F-MY, F-WA, M-MY, and M-WA), with a p-value < 0.001 ($F = 37.268$, $df = 3$). There were also significant differences between sex in the pooled population ($p < 0.001$, $df = 99$), between sex in the Malaysian population ($p < 0.001$, $df = 43$), and between sex in the Western Australian population ($p < 0.001$, $df = 54$).

Comparison between the populations in the pooled sex sample and between populations in female and male groups separately were not significant ($p = 0.723$, $p = 0.572$, and $p = 0.418$, respectively). The distributions of the samples and the descriptive statistics for each group are presented in Table 4.

Table 4. Sample distributions and descriptive statistics

Population	Sex	Number	Mean Centroid Size	Standard Deviation	Standard Error	95% CI	
						Lower Bound	Upper Bound
Malaysian	Female	25	5.648	0.032	0.006	5.635	5.661
Western Australian	Female	29	5.643	0.033	0.006	5.631	5.665
Malaysian	Male	20	5.708	0.036	0.007	5.694	5.722
Western Australian	Male	27	5.716	0.046	0.006	5.704	5.728
Total		**101**	**5.676**	**0.046**			

Principal Component Analysis

The results of the PCA are presented separately between the pooled sample, between-sex comparisons, and between-population comparisons. In all tests, the contribution/proportion of the most significant PC (PC1) ranges from 17.2 to 26.8%. The graphs were supposed to be plotted on the two most significant PCs with $p < 0.05$, but for results in which there was only one statistically significant PC with $p < 0.05$, the second most significant PC was selected to visualise the graph, even though the p-value was greater than 0.05. This applies to the sexual dimorphism in the Western Australian population and population variation in the male samples. The significant and selected PCs and their corresponding mandibular morphology are presented in Table 5.

a. <u>Pooled Sample</u>

There were 13 significant PCs found in the pooled sample. A one-way MANOVA was performed on these PCs to compare the four groups, resulting in a significant difference ($p < 0.001$, $F = 4.342$, Wilk's $\lambda = 0.219$, partial $\eta^2 = 0.397$). Figure 3 shows the mandibles when plotted on the PC graph on the two most significant PCs (PC1 and PC3, $p < 0.001$ for both), showing the most distinct shape variations from each group (red, yellow, green, and blue circles).

A two-way MANOVA was also performed to assess the interaction between the independent variables, with a result of p = 0.915, indicating there was no interaction between sex and population in the present study.

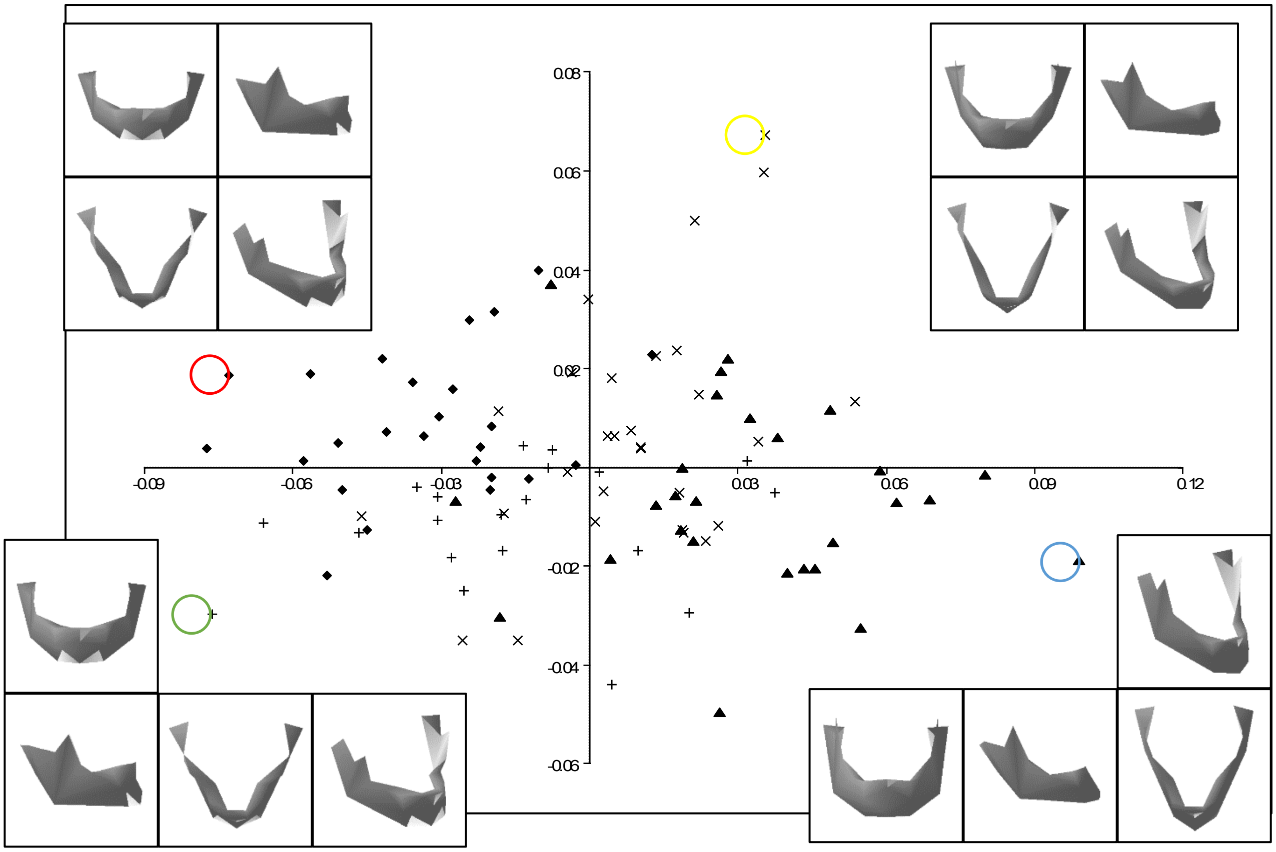

Figure 3. Pooled sample, PC1 v PC3 (♦: Malaysian female, x: Western Australian female, +: Malaysian male, ▲: Western Australian male); red, yellow, green, and blue circles represent individuals with the most distinct mandibular shapes in each group

b. Sexual Dimorphism

For the comparison between sexes in the pooled sample, there were 13 significant PCs. The two-way MANOVA resulted in p = 0.000, F = 4.605, Wilk's λ = 0.587, and partial η^2 = 0.413. The samples are plotted on PC1 and PC3 (both p < 0.001) (Figure 4).

In Malaysian and Western Australian populations, there were 10 and 11 significant PCs, respectively. The results were both significant for these analyses, with the one-way MANOVA resulting in p = 0.001, F = 4.216, Wilk's λ = 0.446, and partial η^2 = 0.554 for the Malaysian samples and p = 0.007, F = 2.854, Wilk's λ = 0.584, and partial η^2 = 0.416 for the Western Australian samples. Figure 5 shows the Malaysian mandibles plotted on PC1 (p = 0.019) and PC4 (p = 0.024), and Figure 6 shows the Western Australian mandibles plotted on PC1 (p = 0.001) and PC2 (p = 0.060).

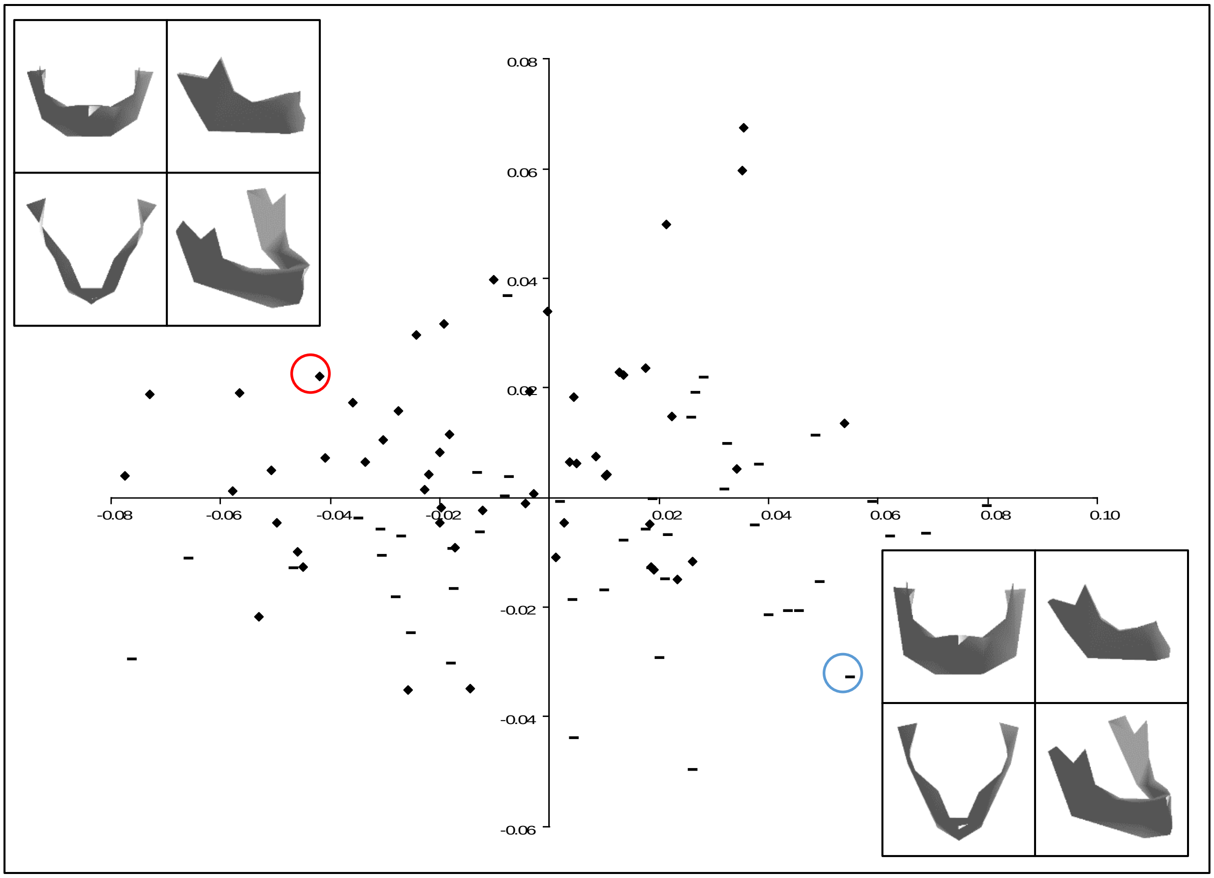

Figure 4. Sexual dimorphism in the pooled sample, PC1 v PC3 (♦: female, –: male); red and blue circles represent individuals with the most opposing mandibular shapes

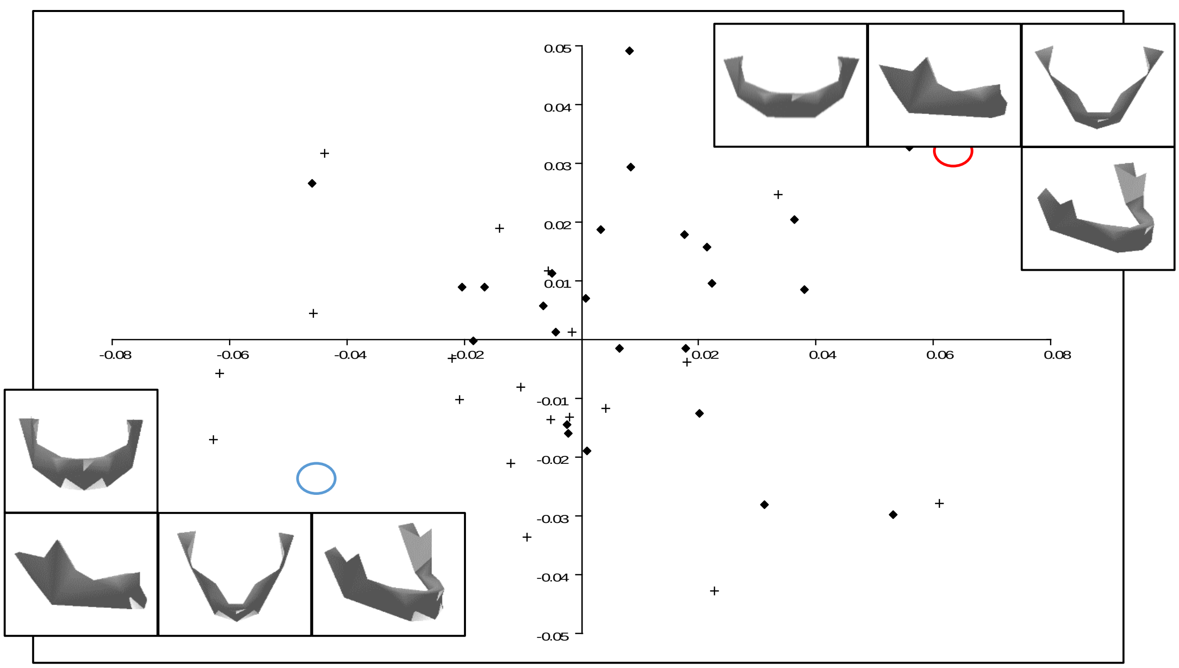

Figure 5. Sexual dimorphism in the Malaysian sample, PC1 v PC4 (♦: female. +: male); red and blue circles represent individuals with the most opposing mandibular shapes

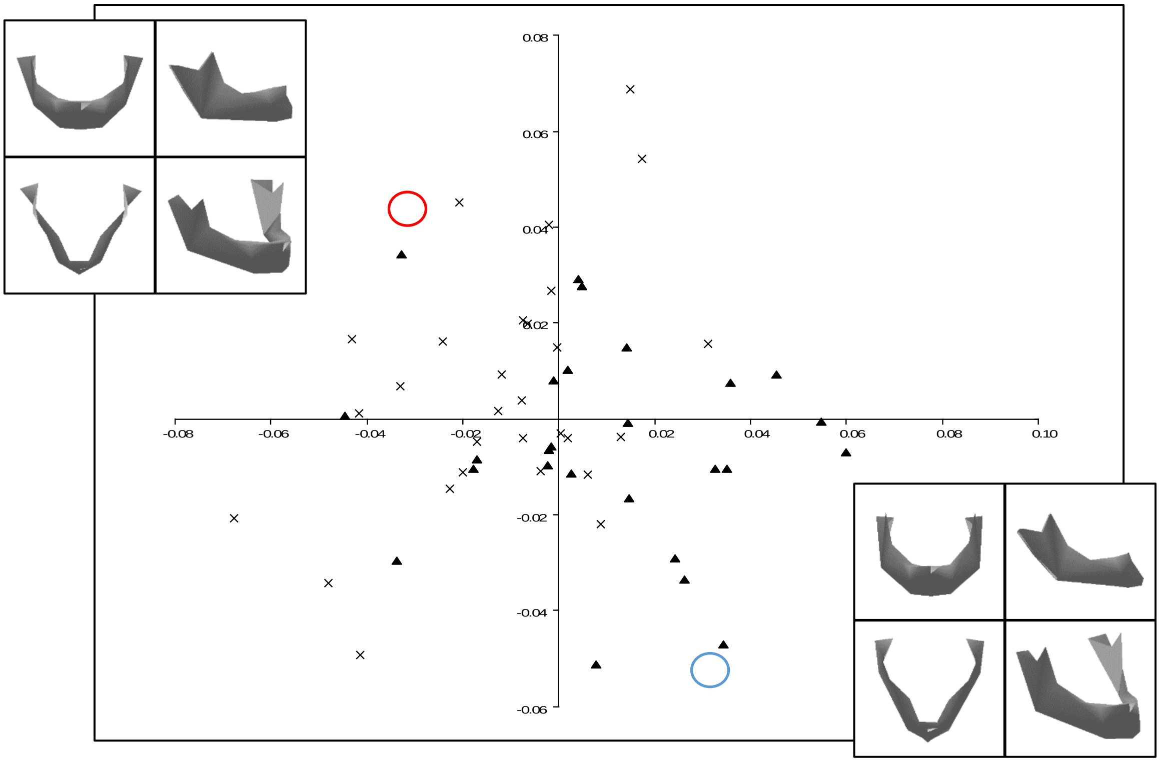

Figure 6. Sexual dimorphism in the Western Australian sample, PC1 v PC2 (x: female, ▲: male); red and blue circles represent individuals with the most opposing mandibular shapes

c. Population Variation

The comparison between both populations in the pooled sample resulted in 13 significant PCs, with a significant result on two-way MANOVA ($p < 0.001$, $F = 10.189$, Wilk's $\lambda = 0.391$, and partial $\eta^2 = 0.609$). The mandibles are plotted on PC1 and PC10 ($p < 0.001$ and $p = 0.018$, respectively) (Figure 7).

For the female samples, there were 12 significant PCs, with a significant difference between Malaysian and Western Australian populations ($p < 0.001$, $F = 9.214$, Wilk's $\lambda = 0.271$, and partial $\eta^2 = 0.729$). For the male samples, there were 9 significant PCs, and the difference between both populations was also significant with $p < 0.001$, $F = 5.440$, Wilk's $\lambda = 0.430$, and partial $\eta^2 = 0.570$. The female mandibles are plotted on PC1 and PC3 ($p < 0.001$ and $p = 0.022$, respectively) (Figure 8), while the male mandibles are plotted on PC1 and PC9 ($p < 0.001$ and $p = 0.127$, respectively) (Figure 9).

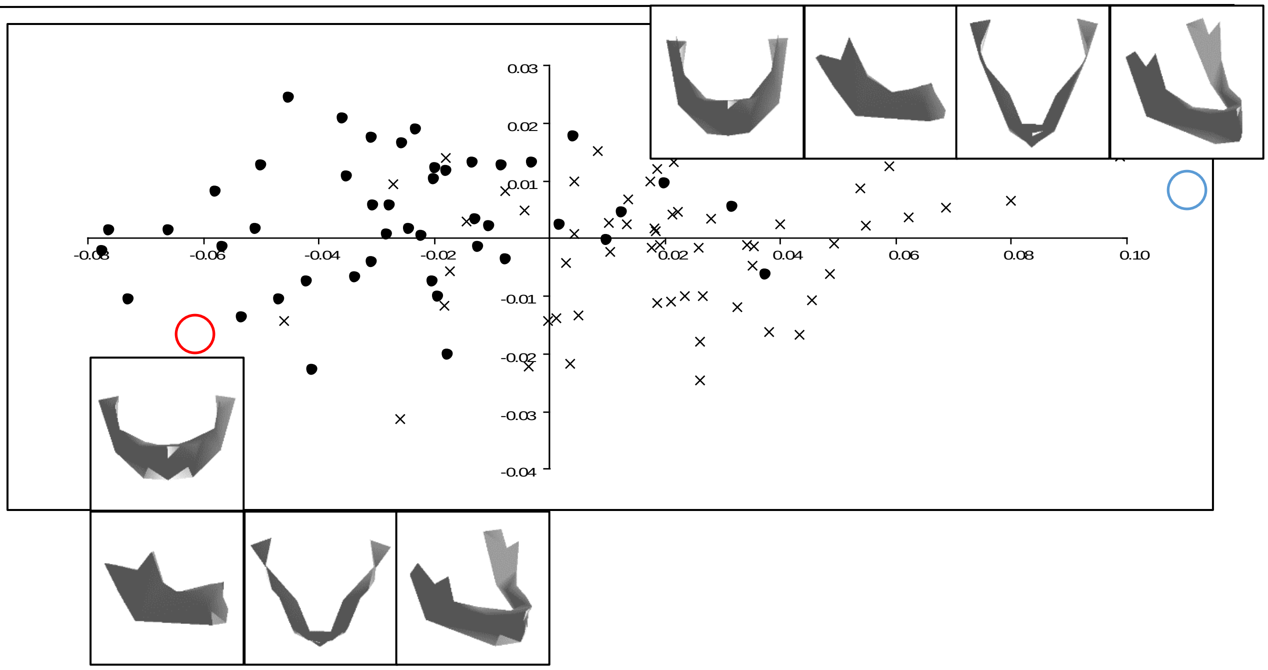

Figure 7. Population variation in the pooled sample, PC1 v PC10 (●: Malaysian, x: Western Australian); red and blue circles represent individuals with the most opposing mandibular shapes

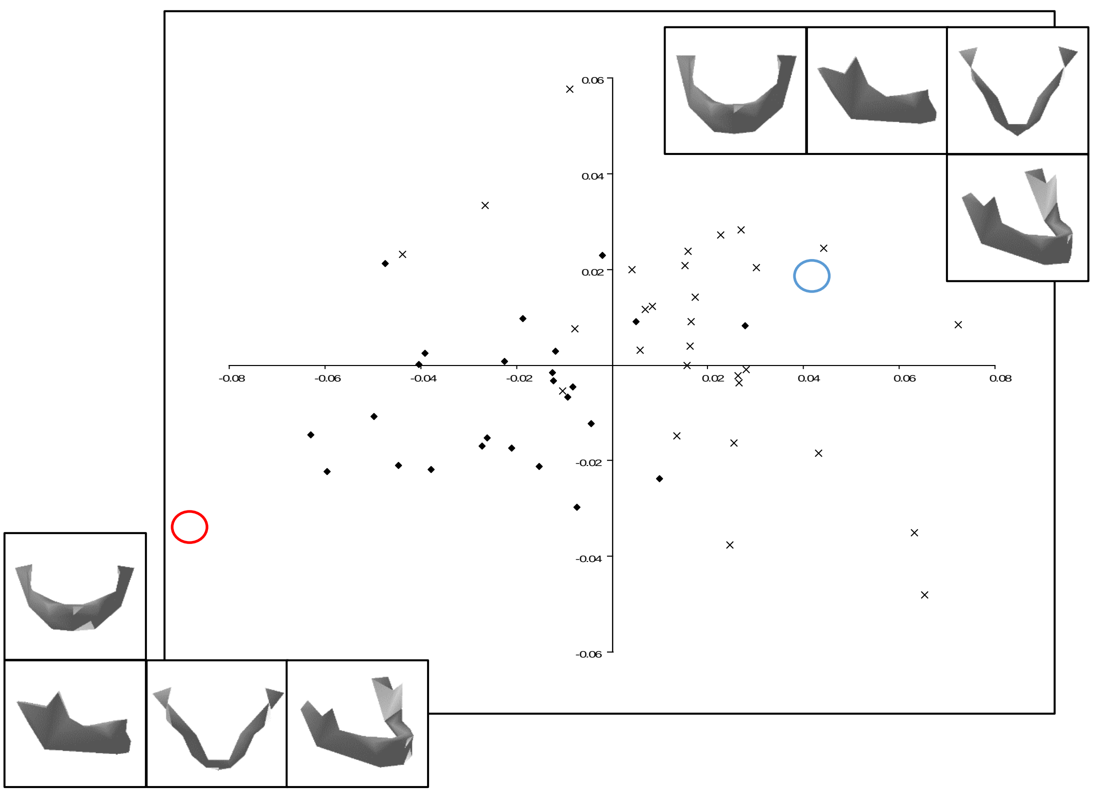

Figure 8. Population variation in female samples, PC1 v PC3 (♦: Malaysian, x: Western Australian); red and blue circles represent individuals with the most opposing mandibular shapes

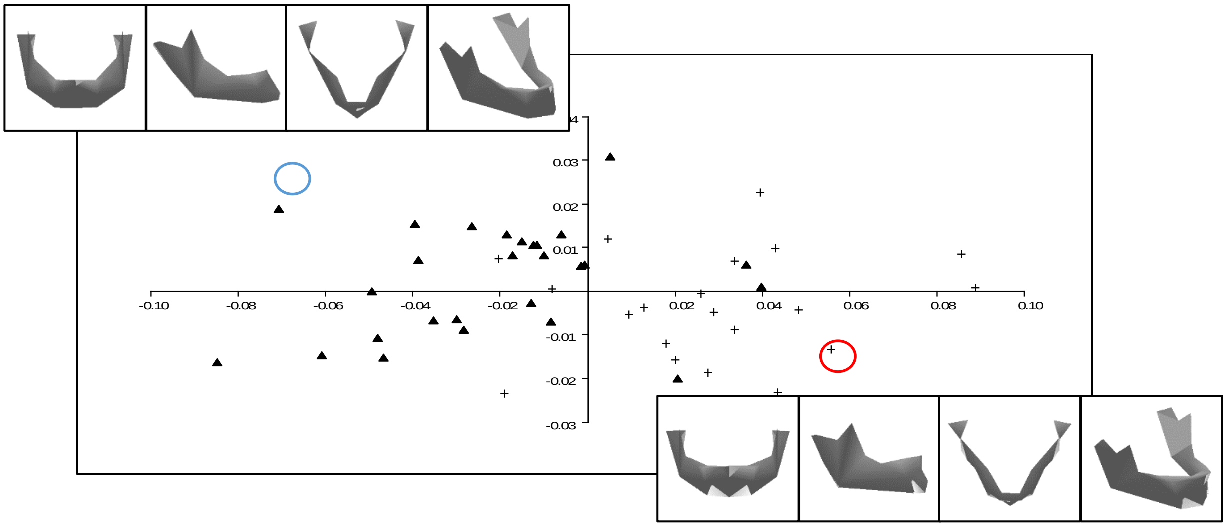

Figure 9. Population variation in male samples, PC1 v PC9 (+: Malaysian, ▲: Western Australian); red and blue circles represent individuals with the most opposing mandibular shapes

Table 5. Significant/Selected PCs and Corresponding Mandibular Morphology

Significant/ Selected PCs	Corresponding Morphological Features	
	Positive Correlation	**Negative Correlation**
Pooled Sample		
PC1 (p<0.001)	Ramus height, gonial angle, mandibular body length, pogonial projection (mental eminence), coronial height (compared to the condyles)	Intercondylar distance, bigonial width, infradentale projection
PC3 (p<0.001)	Coronial height, mandibular body length, infradentale projection	Ramus height, gonial angle, pogonial projection, bigonial width, mandibular body height
Sexual Dimorphism: Pooled Population		
PC1 (p<0.001)	Ramus height, gonial angle, mandibular body length, pogonial projection (mental eminence), coronial height (compared to the condyles)	Intercondylar distance, bigonial width, infradentale projection
PC3 (p<0.001)	Coronial height, mandibular body length, infradentale projection	Ramus height, gonial angle, pogonial projection, bigonial width, mandibular body height
Sexual Dimorphism: Malaysian		
PC1 (p=0.019)	Infradentale projection, intercondylar distance, bigonial width, and a V-shaped (rather than U-shaped) mandible on the superior view	Gonial angle, ramus height, and pogonial projection
PC4 (p=0.024)	-	Chin height and coronial height
PC6 (p=0.030)	Intercondylar distance	Ramus breadth, mandibular body height, and the level of pogonion (higher on the chin)
Sexual Dimorphism: Western Australian		
PC1 (p=0.001)	Coronial height, gonial angle, pogonial projection, and a more U-shaped mandible (superior view)	Intercondylar distance and ramus width
PC2 (p=0.060)	Coronial height	Ramus height, bigonial width, gonial angle, and pogonial projection

Population Affinity: Pooled Sex		
PC1 (p<0.001)	Ramus height, gonial angle, pogonial projection, coronial height, and a more V-shaped mandible (superior view)	Ramus breadth, interrcondylar distance, and bigonial width
PC10 (p=0.018)	Pogonial projection (mental eminence)	Gonial angle, mandibular body length, and condylar width
PC7 (p=0.049)	Pogonial projection and a more U-shaped mandible (superior view)	Intercondylar distance, ramus height, gonial angle, and coronial height
Population Affinity: Female		
PC1 (p<0.001)	Pogonial projection, coronial height, a more U-shaped mandible (superior view)	Ramus breadth
PC3 (p=0.022)	Ramus height, pogonial projection, and ramus breadth	Intercondylar distance
PC2 (p=0.044)	A more V-shaped mandible (superior view), pogonial projection, gonial angle, and coronial heigh	Infradentale projection and ramus breadth
Population Affinity: Male		
PC1 (p<0.001)	Ramus breadth, wider mandible, chin height, and a more V-shaped mandible (superior view)	Gonial angle, ramus height, and pogonial projection
PC9 (p=0.127)	Ramus height, thicker alveolar body, and a more posteriorly located medial condyles (compared to the lateral ones)	Mandibular body length and chin height

Discriminant Function Analysis

The results of the DFA are shown in Table 6. More detailed results can be seen in Appendix 1.

Table 6. Classification accuracy using discriminant function analysis (DFA)

Category	**Comparison**	**Percentage of correctly classified individuals based on Centroid Size (%)**	**Percentage of correctly classified individuals based on Principal Components (%)**
Pooled sample	F-MY, F-WA, M-MY M-WA	49.50	**71.30**
Pooled population	Female vs Male	**91.10**	76.20
Pooled sex	Malaysian vs Western Australian	52.50	**89.10**
Malaysian	F-MY vs M-MY	**93.30**	82.20
Western Australian	F-WA vs M-WA	**91.10**	78.60
Female	F-MY vs F-WA	55.60	**94.40**
Male	M-MY vs M-WA	48.90	**91.50**

DISCUSSION

Pooled Sample

Comparisons between the four groups in the pooled sample showed that both size and shape can be used to significantly distinguish each group. When plotted on a PC graph, individuals from each group tend to cluster together, with PC1 and PC3 being the most significant features. Malaysian mandibles were mainly clustered on the left quadrants (negative PC1 value) while Western Australians were on the right quadrants (positive PC1 value). Meanwhile, female samples tended to be on the upper quadrants (positive PC3 value) while male individuals were on the lower quadrants (negative PC3 value). This indicates that both sexual dimorphism and population variation contribute to mandibular shape variations, as demonstrated by Franklin et al.[6,21].

The 3D-visualisation showed that Malaysian female and male mandibles have broader/thicker mandibular ramus and body, and more divergent (V-shaped) on the superior view. On the other hand, while Western Australians have narrower ramus breadth and body height, appear to be more U-shaped on the superior view, and the coronion is located more superior (compared to the condyles). In terms of sexual dimorphism, male mandibles from both populations seemed to have a greater gonial angle and more prominent pogonial projection (mental eminence).

The classification accuracy for centroid size between the four groups resulted in 49.5% of correctly classified individuals, while for the PCA, the accuracy was 71.3%. A more detailed insight into the classification accuracy showed that for centroid size, misclassifications occurred more within the same sex rather than population, while for PCA, there were more misclassifications within the same population rather than sex (Appendix 1). This was also reflected in the findings that Malaysian and Western Australian females, as well as Malaysian and Western Australian males, had almost equal values of the centroid size, indicating that centroid size was only able to distinguish the sex without any predictive ability to distinguish the population of origin. The higher accuracy found for PCA result demonstrated that shape variation, rather than size, is a better indicator when it comes to distinguishing sex and ancestry of an individual in a mixed sample. Moreover, the results of the DFA also showed that the estimation of sex and ancestry should be performed individually or in a step-by-step approach rather than simultaneously, which is highlighted by the higher classification accuracy for the separate sex or ancestry estimations.

Sexual Dimorphism

Comparisons between sexes in the pooled population as well as in Malaysian and Western Australian populations separately resulted in significant differences for both centroid size and PCA, indicating that both size and shape variations were contributing to sex estimations of the populations in this study. However, classification accuracy rates for sexual dimorphism in all compared groups were higher for the centroid size (all greater than 90%) than PCA (76-82%). The sex biases for centroid size were 0.8%, 3.0%, and 2.9% for the pooled sample, Malaysian, and Western Australian populations, respectively, and for PCA were 0.7%, 14%, and -1.5% for the pooled sample, Malaysians, and Western Australians, respectively (Appendix 1). These findings indicated that for sex estimation, size variation was a more significant parameter than shape variation. Nonetheless, shape analysis may still provide accurate results, either as a complementary examination, or in conditions where the centroid size of the mandible cannot be calculated (e.g. incomplete remains).

In the Malaysian sample, the significant PCs were PC1, PC4, and PC6. Female samples are grouped on the upper-right area on the graph, while male samples tend to cluster on the lower-left region. In the Western Australian population, individuals are plotted on PC1 and PC2, where the females are clustered on the upper-left area while the males are on the lower-right region. Based on the results of the between-sex comparisons in both populations, it appears that in general, male mandibles are larger in size than female ones, as also shown by other similar studies on the mandible and crania[10,23]. On the superior view, female mandibles appeared to be more divergent (V-shaped) in all populations, showing the lateral condyles pointing more outward compared to the male samples. Female mandibles also had relatively shorter ramus height in proportion to the mandibular body length, while the ramus height in male mandibles is comparatively similar to the mandibular body length. Ramus size, mandibular body length, and bicondylar breadth also showed significant differences between males and females in the pooled sample. This is similar to other studies stating that the mandibular condyles[6,24], ramus[6,25], bigonial breadth[24,26,27,28], and mandibular length[27] are the most sexually dimorphic indicators to distinguish between male and female. A study from Fan et al[28]. even demonstrated that mandibles showed differences in size and shape since adolescence/juvenile age.

Meanwhile, the present study found that female mandibles had smaller gonial angle than males for both populations, similar with other findings[29],but opposing the general hypothesis stating that the gonial angle is greater in females than males[30,31]. This might be due to different sample origin (population) and generation (temporal variation) used in the studies or other biological factors such as the facial patterns[32].

Population Variation

For the pooled sex, the significant PCs were PC1, PC10, and PC7. On the plot of PC1 and PC10, Malaysian samples tended to be on the left side (negative PC1 value) while Western Australians were mostly on the right side (positive PC1 value). In the female samples, the significant PCs were PC1, PC3, and PC2. The plot of PC1 and PC3 showed the majority of Malaysian females in the left quadrants, while Western Australian females were mainly in the right quadrants. In the male samples, Malaysian males occupied the right area on the graph, while Western Australian males were mostly in the left quadrants. The 3D-visualisation showed that in all compared groups, Malaysian mandibles tended to have smaller gonial angle, more divergent (V-shaped) mandibles on the superior view, shorter ramus height (in proportion to the mandibular body length), and the lateral condyles were located more laterally (pointing outwards) compared to Western Australian samples.

Meanwhile, Western Australian mandibles had more protruded pogonion (mental eminence) in proportion to the infradentale compared to Malaysian individuals. It should be noted that in all between-populations comparisons, Malaysian mandibles displayed relatively more "feminine" morphology, while Western Australian mandibles tended to display more "masculine" features in all the between-sexes comparisons, which resembled the comparisons in the pooled sample, where Malaysian female and Western Australian male were the two most opposing individuals on the PC graph. This particular finding supports the argument that every population has different gradations of sexual dimorphism, highlighting the importance of population-specific standards[33,34]. Even though mandibular shape can be used to distinguish between population, anthropologists should also consider factors such as human evolution (biological relationship), as well as geographic pattern, temporal variation, and population admixture when estimating ancestry, as these would contribute to the clinal variations of craniofacial features, including in the mandible[34,35,36].

Comparisons between populations in all compared groups (pooled sex, female, and male samples) resulted in significant PCA but not centroid size, indicating that mandibular shape may be used to estimate ancestry, but not the size. This is supported by the DFA results, where the PCA classification accuracy rates for all between-population comparisons ranged from 89 to 94%, while for the centroid size, the accuracies were all less than 56%. It means that shape variations were good indicators for the estimation of the population of origin of human mandibles, while size was not a reliable predictor of ancestry. A more detailed result of the classification accuracy is presented in Appendix 1.

Study Limitations

The limitations of the present study include the relatively small sample size, with only 20-29 individuals in each group. While this number might be enough to provide initial observations on each sex and population group, it might be too few to represent the real populations. Moreover, while the Malaysian sample consists only of Chinese and Malay (native Malaysian), the Western Australian sample represents the geographical region but lacks a detailed breakdown into ancestral groups. Considering that Western Australian population is quite heterogeneous, this may have affected the results of this study. Due to these limitations, the study findings cannot be used to develop population-specific standards to estimate sex and ancestry from the mandible. However, they may serve as an insight for future studies to develop such standards.

CONCLUSION

In conclusion, it has been demonstrated that the adult mandible can be potentially used to estimate sex and ancestry of unknown remains using geometric morphometric methods. While mandibular size is more accurate at estimating sex, shape variations are better at distinguishing between populations. However, there is no feature that specifically points towards one particular population. The analysis of a larger sample with more detailed information on the ancestral structure of the Western Australian population may allow for creating population-specific standards for sex and ancestry estimations, particularly for Asia-Pacific populations in the future.

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APPENDICES

Appendix 1: DFA results for classification accuracy

a. Pooled Sample

Centroid Size

Group	Predicted Group Membership				Total
	F-MY	F-WA	M-MY	M-WA	
F-MY	10 (40.0%)	13 (52.0%)	1 (4.0%)	1 (4.0%)	**25 (100%)**
F-WA	8 (27.6%)	18 (62.1%)	2 (6.9%)	1 (3.4%)	**29 (100%)**
M-MY	0 (0%)	1 (5.0%)	9 (45.0%)	10 (50.0%)	**20 (100%)**
M-WA	1 (3.7%)	0 (0%)	13 (48.1%)	13 (48.1%)	**27 (100%)**

PCA

Group	Predicted Group Membership				Total
	F-MY	F-WA	M-MY	M-WA	
F-MY	18 (72.0%)	3 (12.0%)	4 (16.0%)	0 (0%)	**25 (100%)**
F-WA	2 (6.9%)	20 (69.0%)	1 (3.4%)	6 (20.7%)	**29 (100%)**
M-MY	4 (20.0%)	1 (5.0%)	14 (70.0%)	1 (5.0%)	**20 (100%)**
M-WA	1 (3.7%)	4 (14.8%)	2 (7.4%)	20 (74.1%)	**27 (100%)**

b. Sexual Dimorphism—Pooled Sample

Centroid Size

Sex	Predicted Group Membership		Total
	Female	Male	
Female	49 (90.7%)	5 (9.3%)	**54 (100%)**
Male	4 (8.5%)	43 (91.5%)	**47 (100%)**

PCA

Sex	Predicted Group Membership		Total
	Female	Male	
Female	41 (75.9%)	13 (24.1%)	**54 (100%)**
Male	11 (23.4%)	36 (76.6%)	**47 (100%)**

c. Sexual Dimorphism—Malaysian Sample

Centroid Size

Sex	Predicted Group Membership		Total
	Female	Male	
Female	23 (92.0%)	2 (8.0%)	**25 (100%)**
Male	1 (5.0%)	19 (95.0%)	**20 (100%)**

PCA

Sex	Predicted Group Membership		Total
	Female	Male	
Female	19 (76.0%)	6 (24.0%)	**25 (100%)**
Male	2 (10.0%)	18 (90.0%)	**20 (100%)**

d. Sexual Dimorphism—W

Centroid Size

Sex	Predicted Group Membership		Total
	Female	Male	
Female	26 (89.7%)	3 (10.3%)	**29 (100%)**
Male	2 (7.4%)	25 (92.6%)	**27 (100%)**

PCA

Sex	Predicted Group Membership		Total
	Female	Male	
Female	23 (79.3%)	6 (20.7%)	**29 (100%)**
Male	6 (22.2%)	21 (77.8%)	**27 (100%)**

estern Australian Sample

e. Population Affinity—Pooled Sample

Centroid Size

Population	Predicted Group Membership		Total
	Malaysian	Western Australian	
Malaysian	24 (53.3%)	21 (46.7%)	**45 (100%)**
Western Australian	27 (48.2%)	29 (51.8%)	**56 (100%)**

PCA

Population	Predicted Group Membership		Total
	Malaysian	Western Australian	
Malaysian	40 (88.9%)	5 (11.1%)	**45 (100%)**
Western Australian	6 (10.7%)	50 (89.3%)	**56 (100%)**

f. Population Affinity—Female Sample

Centroid Size

Population	Predicted Group Membership		Total
	Malaysian	Western Australian	
Malaysian	12 (48.0%)	13 (52.0%)	**25 (100%)**
Western Australian	11 (37.9%)	18 (62.1%)	**29 (100%)**

PCA

Population	Predicted Group Membership		Total
	Malaysian	Western Australian	
Malaysian	24 (96.0%)	1 (4.0%)	**25 (100%)**
Western Australian	2 (6.9%)	27 (93.1%)	**29 (100%)**

g. Population Affinity—Male Sample

Centroid Size

Population	Predicted Group Membership		Total
	Malaysian	Western Australian	
Malaysian	10 (50.0%)	10 (50.0%)	**20 (100%)**
Western Australian	14 (51.9%)	13 (48.1%)	**27 (100%)**

PCA

Population	Predicted Group Membership		Total
	Malaysian	Western Australian	
Malaysian	19 (95.0%)	1 (5.0%)	**20 (100%)**
Western Australian	3 (11.1%)	24 (88.9%)	**27 (100%)**