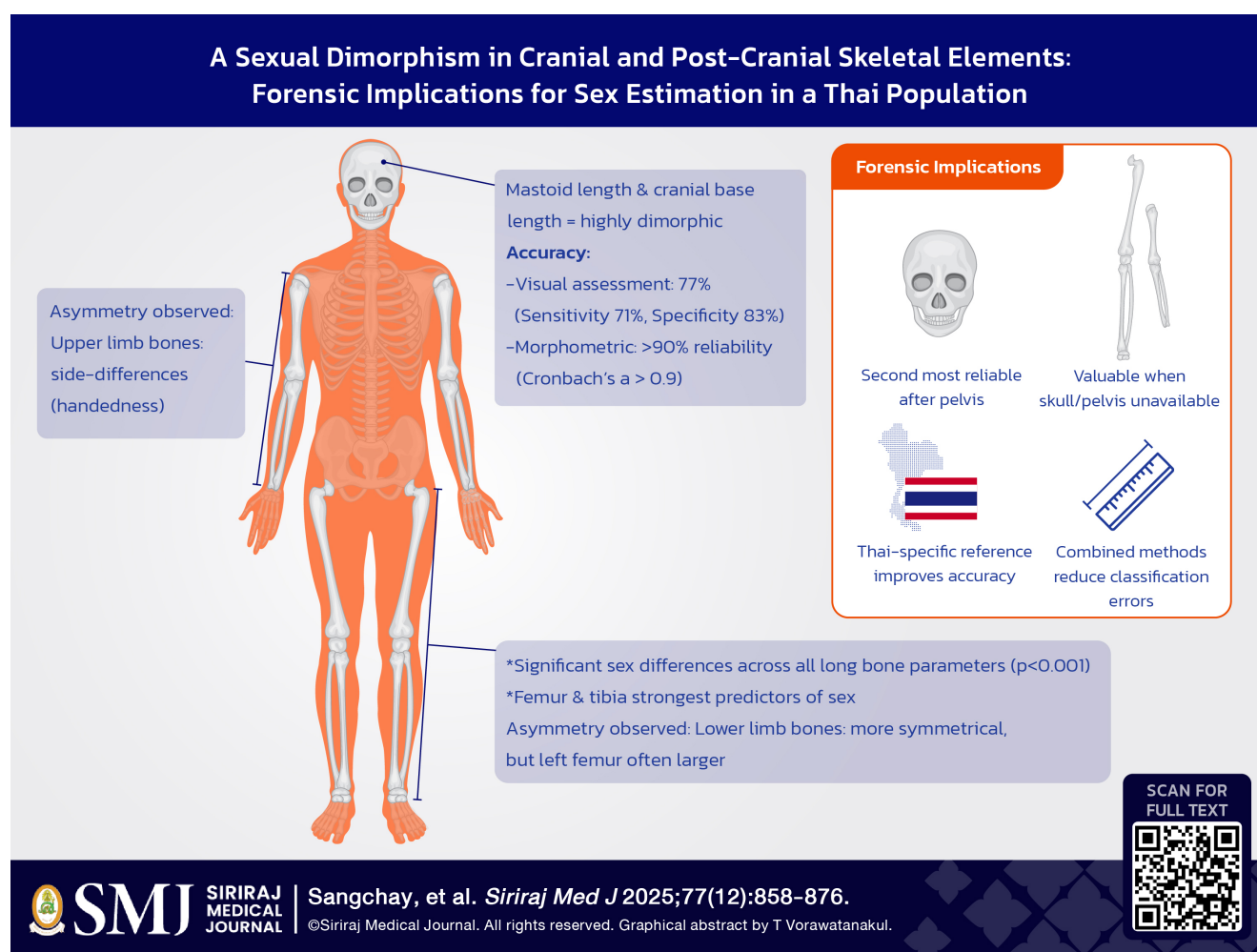


Sexual Dimorphism in Cranial and Post-Cranial Skeletal Elements: Forensic Implications for Sex Estimation in a Contemporary Thai Population

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ABSTRACT

Objective: The analysis of human skeletal remains is instrumental in forensic and anthropological contexts, particularly for establishing biological profiles of unidentified individuals. Determining sex via skeletal examination is a fundamental component of this process and traditionally involves morphological assessment and metric analysis of pelvic and cranial bones. Nevertheless, the precision and reliability of these methodologies—whether through morphological evaluation or morphometric analysis—remain subjects of ongoing debate and scrutiny. This research set forth to investigate the efficacy of morphological and morphometric analysis in sex estimation by focusing on cranial and post-cranial long bones within contemporary Thai population.

Materials and Methods: The study sample comprised 204 skulls (105 from males, and 99 from females) and 200 sets of long bones of upper (humerus, radius, and ulna) and lower extremities (femur and tibia). Multiple measurements were systematically obtained from various anatomical regions of each bone, and measurements of extremity long bones were compared between the left and right sides.

Results: The analysis revealed statistically significant differences in these metrics between sexes, indicating the potential utility of this approach for sex classification. However, despite achieving high levels of accuracy, the studied methodology yielded some classification errors, which suggests some potential limitations.

Conclusion: The findings suggest that both inherent skeletal variability in cranial and post-cranial morphology within contemporary Thai population and the specific analytical techniques employed can markedly influence the accuracy of sex determination. These factors harbor and confer important implications for forensic and anthropological applications.

Keywords: Sexual dimorphism; cranial and post-cranial skeletal elements; forensic implications for sex estimation; contemporary Thai population (Siriraj Med J 2025; 77: 858-876)

INTRODUCTION

In forensic anthropology, constructing a biological profile for unidentified human remains is crucial for identification, including sex, age, stature, and ancestry estimations. These parameters are essential for matching ante-mortem records and other data. Notably, a swift and organized skeletal analysis for sex determination is vital for refining subsequent biological profile estimations.¹

Skeletal sex estimation is a critical step that requires both expertise and precise decision-making due to its foundational role in the identification process. Sexual dimorphism in skeletal elements results from genetic and hormonal influences, which contribute to distinct traits differentiating male and female skeletons. To enhance accuracy and minimize subjectivity, sex assessments should be conducted and documented meticulously, thereby strengthening the identification process. The distinct skeletal traits influenced by genetic and hormonal factors differentiate males and females, underscoring the need for reliable and less subjective assessment techniques.²

In forensic investigations, accurate sex assessment from skeletal remains is essential for human identification. The pelvis and cranium are preferred for analysis due to their pronounced sexual dimorphism, enabling precise sex estimation. The os coxae is particularly favored for its marked dimorphism and high accuracy. However,

when these elements are absent or severely fragmented, alternative bones must be considered. Human long bones, or postcranial elements, serve as a secondary option due to their sexual dimorphism, though morphometric methods using long bones are limited, especially in databases focusing on Asian genetic and ethnic backgrounds.^{3,4}

The pelvis and skull are traditionally considered the most reliable skeletal components for sex determination due to well-defined dimorphism.⁵ Accurate assessment requires complete or intact structures, often compromised in forensic settings due to taphonomic degradation or recovery limitations. In such cases, long bones are valuable substitutes due to their sexually dimorphic features influenced by musculoskeletal development and hormonal regulation. However, they lack specific morphological traits indicative of sex, limiting their reliability for sex estimation.⁶

Numerous studies have examined the use of the human skull and long bones of the extremities for sex estimation. However, a critical limitation of many such investigations is the lack of intra- and inter-observer error assessments, which impedes a thorough evaluation of the reliability of the methodology being studied or the methodologies being compared.⁷ Cranial visual assessment of or for sexually dimorphic traits offers a rapid and practical method for sex estimation, but this

technique is highly subjective and less reproducible. This approach, particularly when evaluating the skull, involves analyzing varying morphological characteristics to distinguish between sexes, focusing on features such as the mastoid process, nuchal crest, and orbital traits.⁸

In addition to pelvic morphology, features in long bones, such as femoral head diameter, can indicate biological sex and are suitable for morphometric analysis. This method is effective with complete or nearly complete skeletal elements, but its reliability diminishes with fragmented remains due to taphonomic damage. Thus, exploring alternative methodologies, like digital imaging techniques to analyze long bone characteristics, becomes necessary.^{9,10}

While gross morphological analysis of the skull offers acceptable accuracy for sex estimation, it also has some associated limitations as follows. This technique is susceptible to intra- and inter-observer variability, leading to inconsistent assessments. The reliance on subjective judgment complicates its utility, particularly in skeletal specimens with ambiguous features. To enhance precision and reliability, direct pelvic morphometric measurements are proposed as a more refined and reliable approach.^{11,12}

Metric analysis of the skull provides greater precision in sex determination, often surpassing 90% accuracy, compared to visual morphological assessments. However, this method requires an intact or nearly complete skull to accurately assess landmarks. The mastoid process, in particular, demonstrates high reliability, with a sex determination success rate of 96.5%, supported by studies reporting approximately 90% accuracy from measurements in this region.¹³

Both non-metric and metric cranial analyses depend on identifiable skeletal features, which necessitate well-preserved bones. Current methodologies primarily derive from skeletal collections of specific populations, such as Black and White Americans, with limited data for Asian and other groups.¹⁴ Researchers underscore the critical need for population-specific databases to improve sex estimation accuracy in diverse populations.¹⁵

Asymmetry between the bones on the left and right sides is attributed to handedness. Approximately 90% of the population is right-handed. Handedness influences bone morphology, with the dominant limb generally exhibiting larger bones. Forensic studies also show that the right side, including hand bones, tends to be larger, particularly in right-handed individuals.¹⁶ Larger bones in the right hand are more common in right-handed individuals, with this difference being more pronounced in males. However, similar correlations are less consistent for left-handed individuals.¹⁷

This study evaluates sexual dimorphism in cranial and post-cranial elements for forensic sex estimation, assessing inter-observer reliability and error rates using gross morphological and metric approaches. Conducted in contemporary Thai population with dry skulls and extremity long bones, this study explores the method's applicability in forensic contexts. Additionally, morphometric analysis of the bilateral long bones examines the impact of asymmetry on sex estimation, considering the influence of sexual dimorphism.

MATERIALS AND METHODS

The study utilized human bone samples from the Siriraj Anatomical and Anthropological Bone Centre (Si-AABRC), of the Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The protocol for this study was exempted from Siriraj Institutional Review Board (SIRB) approval.

Cranial sexual dimorphism and morphometric measurements

Gross morphological assessment was conducted for each bone, focusing on traits such as the mastoid process, nuchal crest, and cranial contour, to estimate sex. Each cranium underwent detailed visual evaluation of specific dimensions and landmarks, including the supraorbital ridges, opisthocranium, and mastoid process. A sex classification was made if at least three traits indicated a particular sex. The supraorbital ridges were assessed for size and prominence, the opisthocranium for its most posterior point, and the mastoid process for size and robustness. All skulls were sequentially numbered and segregated to ensure unbiased subsequent craniometric analysis.

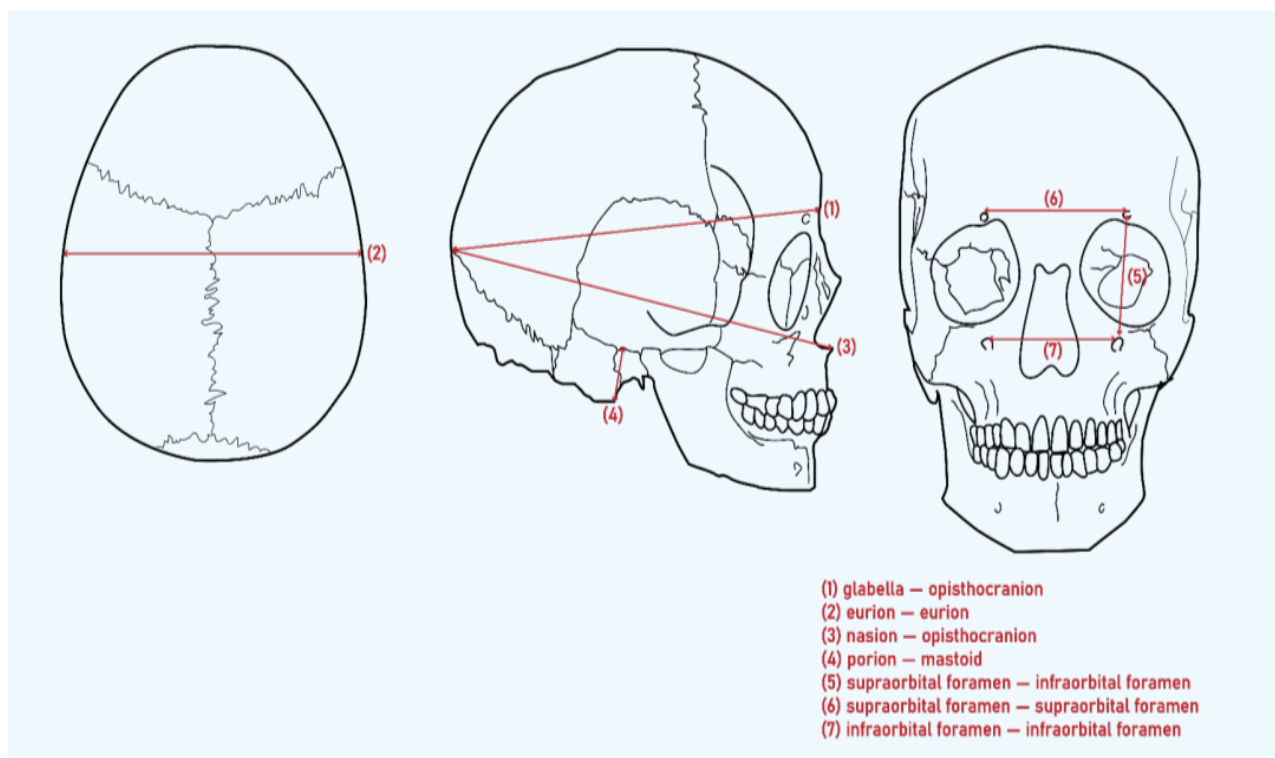
Following morphological assessment, cranial morphometric measurements were performed, as detailed in [Table 1](#). An independent re-evaluation by two blinded investigators was conducted to enhance accuracy and reduce inter-observer variability. The craniometric parameters assessed are illustrated in [Fig 1](#), with both observers unaware of prior measurements and the biological sex of each evaluated skull.

Post-cranial morphometric measurements

The upper extremity long bones included the humerus, radius, and ulna, on both sides, and the lower extremity bones included the femur and tibia, on both sides. Bones with deformities, fractures, osteophytes, or prosthetic modifications were excluded. Measurements were made using an osteometric board and digital vernier calliper. Post-cranial measurements are summarized in [Table 2](#).

TABLE 1. Description of cranial landmarks and morphometric measurements.

Measurement of Cranium	Description
Cranial Length (Front to Back)	Measurement of distance from glabella to opisthocranium
Cranial Width	Measurement of distance from left eurion to right eurion
Cranial Index	(Cranial Width/Cranial Length) x 100
Cranial Base Length	Measurement of distance from nasion to opisthocranium
Mastoid Height	Measurement of porion to end of mastoid process
Inter-orbital Foramen Length	Measurement of supraorbital foramen or notch to infraorbital foramen
Inter-supraorbital Foramen Distance	Measurement of left supraorbital foramen or notch to right
Inter-infraorbital Foramen Distance	Measurement of left infraorbital foramen to right

**Fig 1.** Cranial parameters used in cranial measurement method. All measurements were performed using the same digital vernier calliper and the same spreading calliper.

Statistical analyses

Cranial skeletal elements:

Prior to parameter measurements and analysis, two independent observers measured over 200 dry crania, yielding 204 viable samples. The study compared cranial measurements between sexes and evaluated classification accuracy via visual assessments measurement reliability. Statistical comparisons used either the independent t-test or Mann-Whitney U test, depending on data normality,

to identify significant sexual dimorphism. The primary goal was to identify cranial features with pronounced sexual dimorphism.

A contingency table assessed classification accuracy, comparing predicted sex with actual sex. Metrics such as sensitivity, specificity, precision, and overall accuracy evaluated the sex determination method. Measurement reliability was assessed with Cronbach's alpha, and intraclass correlation coefficients (ICC) were calculated for normally

TABLE 2. Description of post-cranial long bone landmarks and morphometric measurements.

Measurement of Humerus	Abbreviation	Description
Maximum Anatomical Length	H-MAL	Distance between the proximal end of humerus head to the distal end of trochlea
Humeral Head diameter	HHD	Distance between superior part of humerus head to the inferior humerus head
Anatomical Neck Diameter	AND	Maximum diameter of anatomical neck
Surgical Neck Diameter	SND	Maximum diameter of surgical neck
Humerus Midshaft Diameter	H-MD	Maximum diameter of midshaft
Epicondylar Breadth	EB	Distance between medial epicondyle to lateral epicondyle
Capitular Diameter	CD	Distance between superior to inferior of capitulum
Olecranon Fossa Diameter	OFD	Distance of the maximum diameter of olecranon fossa
Measurement of Radius	Abbreviation	Description
Maximum Anatomical Length	R-MAL	Distance between the proximal end of the head to the distal end of radius
Radial Head Diameter	RHD	Maximum diameter of head
Radial Neck Diameter	RND	Maximum diameter of surgical neck
Radial Midshaft Diameter	R-MD	Maximum diameter of midshaft
Distal Radial Breadth	DRB	Distance between styloid process to ulnar notch
Distal Radial Width	DRW	Distance between superior of distal breadth to inferior of distal breadth
Measurement of Ulna	Abbreviation	Description
Maximum Anatomical Length	U-MAL	Distance between the proximal end of olecranon process to distal end of styloid process
Ulna Midshaft Diameter	U-MD	Maximum diameter of midshaft
Maximum Olecranon Process Width	MOPW	Distance between maximum of olecranon process from medial to lateral side
Maximum Coronoid Process Width	MCPW	Distance between maximum of coronoid process from medial to lateral
Measurement of Femur	Abbreviation	Description
Maximum bone length	Fe-MBL	Maximum length of femur measured from the most superior part of the head of femur to the most inferior part of the furthest condyle
Maximum anatomical length	Fe-MAL	Maximum length of femur measured from the most superior part of the head of femur to the bicondylar contact point
Femoral midshaft diameter	Fe-MD	Maximum transverse diameter of femoral shaft measured at 50% length of Fe-MAL
Femoral head diameter	Fe-HD	Maximum diameter of the head of femur measured from the most superior to the most inferior points
Femoral neck diameter	Fe-ND	Maximum diameter of the neck of femur measured at the narrowest part from the most superior to the most inferior points
Bicondylar breadth	Fe-BB	Maximum length that crosses both femoral condyles, measured from the medialmost to the lateralmost points
Medial condylar width	Fe-MCW	Maximum width of medial condyle measured in vertical line from the most superior to the most inferior part of articular surface
Lateral condylar width	Fe-LCW	Maximum width of lateral condyle measured in vertical line from the most superior to the most inferior part of articular surface

TABLE 2. Description of post-cranial long bone landmarks and morphometric measurements. (Continue)

Measurement of Tibia	Abbreviation	Description
Maximum bone length	T-MBL	Maximum length of tibia measured from the most superior point of the furthest intercondylar tubercle on the tibial eminence to the most inferior point of the medial malleolus
Maximum anatomical length	T-MAL	Maximum length of tibia measured from the tibial bicondylar contact point to the most inferior point of the medial malleolus
Tibial midshaft diameter	T-MD	Maximum transverse diameter of tibial shaft measured at 50% length of T-MAL
Tibial plateau breadth	TPB	Maximum width of tibial plateau measured from the medialmost part of medial condyle to the lateralmost of lateral condyle
Tibial plateau medial width	TPMW	Maximum width of medial condyle measured from the most posterior to the most anterior part
Tibial plateau lateral width	TPLW	Maximum width of lateral condyle measured from the most posterior to the most anterior part
Distal tibial breadth	DTB	Maximum distance measured from the medialmost point of the medial malleolus to the medialmost point of the fibular notch
Distal tibial width	DTW	Maximum distance between the most anterior part to the most posterior part of inferior articular facet of distal tibia, measured at the middle of the inferior articular surface

distributed variables. ICC estimates the proportion of variance due to true differences, with reliability ranges, ensuring measurement consistency for valid comparisons.

Post-cranial skeletal elements:

The independent t-test compared two groups, with statistical significance set at $p < 0.001$ ($\alpha = 0.05$). Paired t-tests evaluated intra-individual differences between the left and right sides. Discriminant function analysis assessed sex prediction accuracy, selecting functions with the highest classification performance and significant variables.

Observation error and reliability analysis

Inter-observer measurement error was evaluated. Two observers independently measured the skeletal samples: the primary investigator and a faculty member of the Department of Anatomy with 10 years of experience. Each observer assessed all parameters blinded to the demographic data. The goal was to quantify measurement error and repeatability, as analyzed by SPSS Statistics software (SPSS, Inc., Chicago, IL, USA). Repeatability was evaluated using technical error of measurement (TEM), relative TEM (rTEM), and coefficient of reliability (R). An R-value approaching 1 indicates high agreement.¹⁸

RESULTS

Descriptive statistics for all measurements are summarized in **Tables 3-5**, including means and standard deviations. All measurements showed significant differences between males and females (all $p < 0.05$).

Cranial skeletal elements

Table 3 provides descriptive statistics for cranial variables by sex. The independent t-test revealed that males have significantly larger cranial length, width, cranial index (**Fig 2-A&B**), cranial base length (**Fig 2C**), mastoid length (**Fig 2D**), inter-orbital foramen distance (**Fig 2E**), and inter-infraorbital foramen distance (**Fig 2G**). The Mann-Whitney U test additionally showed that the inter-supraorbital foramen distance (**Fig 2F**) was significantly larger in males ($p = 0.005$), indicating strong sexual dimorphism suitable for sex estimation.

Diagnostic accuracy of visual assessment

Table 4 summarizes classification results: 75 male skulls correctly identified, 17 female skulls misclassified as male, 30 male skulls misclassified as female, and 82 female skulls correctly classified. The method's sensitivity was 71.43% (95% confidence interval [95% CI]: 61.79–79.82), and specificity was 82.83% (95% CI: 73.94–89.67), with a

TABLE 3. Descriptive statistics of cranial landmarks and morphometric variables (^a Independent *t*-test, ^b Mann-Whitney U test).

Descriptive statistics: Measurements of cranial landmarks					
Morphometric parameters	Male (n=105)		Female (n=99)		<i>p</i> -value
	Mean	Std Dev	Mean	Std Dev	
Cranial Length	17.15	0.73	16.24	0.61	< 0.001 ^a
Cranial Width	14.02	0.61	13.59	0.65	< 0.001 ^a
Cranial Index	81.83	4.34	83.74	4.36	< 0.01 ^a
Cranial Base Length	17.02	0.77	16.18	0.75	< 0.001 ^a
Mastoid Height	34.63	3.19	31.31	3.32	< 0.001 ^a
Inter-orbital Foramen Length	44.26	2.75	43.53	2.72	0.056 ^a
Inter-supraorbital Foramen Distance	113.8	4.56	90.52	5.12	0.005 ^b
Inter-infraorbital Foramen Distance	59.77	4.23	57.03	4.07	< 0.001 ^a

^a Independent *t*-test, ^b Mann-Whitney U test

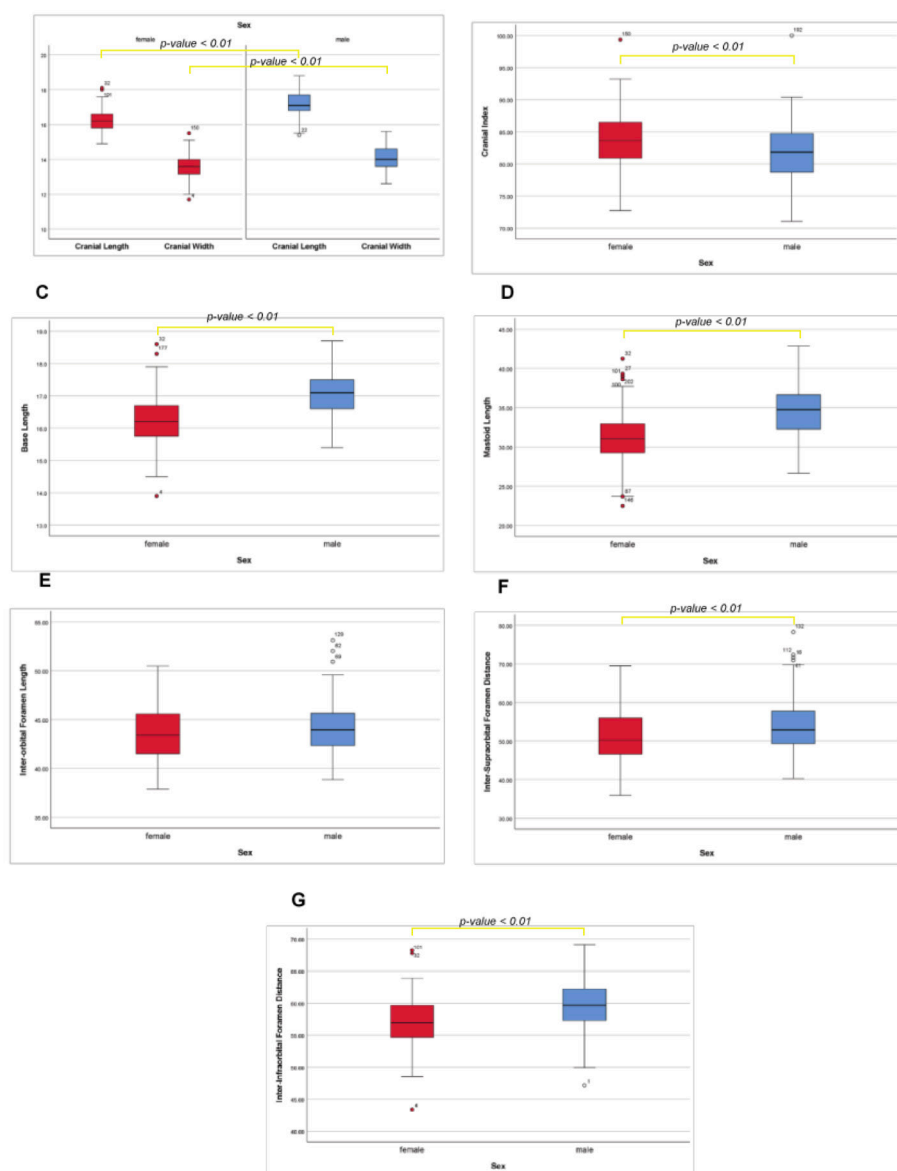


Fig 2. Comparative analysis of craniometric parameters. The figure displays a box and whisker plot comparing the means of craniometric parameters compared between male (n=105) and female (n=99) crania, namely: (A) Cranial length and width. (B) Cranial Index. (C) Cranial base length. (D) Mastoid length. (E) Inter-orbital foramen length. (F) Inter-supraorbital foramen distance. (G) Inter-infraorbital foramen distance. Significance levels ($p < 0.01$) are indicated for each parameter.

TABLE 4. Contingency table of sex classification and diagnostic accuracy by visual assessment and reliability of cranial morphometric assessment.

Contingency table of sex classification by visual assessment		
	Actual Male	Actual Female
<i>Predicted Male</i>	75	17
<i>Predicted Female</i>	30	82

Diagnostic accuracy test of sex classification by visual assessment		
Statistic	Value	95% C.I.
Sensitivity	71.43%	61.79%–79.82%
Specificity	82.83%	73.94%–89.67%
Positive Likelihood Ratio	4.16	2.65–6.52
Negative Likelihood Ratio	0.34	0.25–0.47
Positive Predictive Value	81.52%	44.39%–58.51%
Negative Predictive Value	73.21%	66.60%–78.93%
Accuracy	76.96%	70.57%–82.55%

Reliability of craniometric parameters with Cronbach's alpha		
Parameter	Cronbach's alpha	Interpretation
Cranial Length (cm)	0.97	Excellent
Cranial Width (cm)	0.87	Good
Cranial Index	0.86	Good
Cranial Base Length (cm)	0.89	Good
Mastoid Length (mm)	0.83	Good
Inter-orbital Foramen Distance (mm)	0.92	Excellent
Inter-supraorbital Foramen Distance (mm)	0.87	Good
Inter-infraorbital Foramen Distance (mm)	0.91	Excellent

TABLE 5. Descriptive statistics of post-cranial landmarks and morphometric variables.

Morphometric parameters	Humerus									
	Right		Female (n=94)		p-value	Left		Female (n=94)		p-value
	Male (n=106)		Mean	Std Dev		Male (n=106)		Mean	Std Dev	
	Mean	Std Dev	Mean	Std Dev	p-value	Mean	Std Dev	Mean	Std Dev	p-value
HMAL	30.66	1.38	28.52	1.91	< 0.001	30.66	1.38	28.52	1.91	< 0.001
HHM	45.08	3.22	40.16	2.37	< 0.001	44.79	3.31	39.59	4.11	< 0.001
AND	45.68	2.70	40.54	2.44	< 0.001	45.37	2.76	40.23	2.73	< 0.001
SND	33.73	2.36	29.96	2.37	< 0.001	32.74	2.45	29.22	2.49	< 0.001
HMD	21.79	2.03	19.35	1.97	< 0.001	21.51	2.04	18.84	1.83	< 0.001
EB	60.59	5.46	54.53	3.49	< 0.001	59.94	5.14	53.81	3.32	< 0.001
CD	21.15	4.29	18.41	1.24	< 0.001	20.69	1.54	18.43	1.41	< 0.001
OFD	21.71	2.36	19.94	2.03	< 0.001	22.15	2.04	20.56	1.72	< 0.001
Morphometric parameters	Radius									
	Right		Female (n=94)		p-value	Left		Female (n=94)		p-value
	Male (n=106)		Mean	Std Dev		Male (n=106)		Mean	Std Dev	
	Mean	Std Dev	Mean	Std Dev	p-value	Mean	Std Dev	Mean	Std Dev	p-value
RMAL	24.35	1.27	22.49	1.65	< 0.001	24.25	1.26	22.26	1.30	< 0.001
RHD	22.31	1.94	19.59	1.39	< 0.001	22.1	1.45	19.48	2.77	< 0.001
RND	15	1.47	13.13	2.47	< 0.001	15.42	2.11	13.32	1.59	< 0.001
RMD	15.02	1.38	13.83	2.39	< 0.001	14.5	1.29	13.23	1.69	< 0.001
DRB	27.3	2.14	24.82	2.68	< 0.001	26.81	2.17	24.18	1.63	< 0.001
DRW	20.19	1.97	17.41	1.55	< 0.001	18.87	2.08	16.69	1.71	< 0.001
Morphometric parameters	Ulna									
	Right		Female (n=94)		p-value	Left		Female (n=94)		p-value
	Male (n=106)		Mean	Std Dev		Male (n=106)		Mean	Std Dev	
	Mean	Std Dev	Mean	Std Dev	p-value	Mean	Std Dev	Mean	Std Dev	p-value
MOPW	24.68	2.40	20.86	1.79	< 0.001	24.25	2.19	20.55	1.87	< 0.001
MCPW	23.32	2.18	20.32	1.75	< 0.001	23.05	2.16	20.09	1.65	< 0.001
UMD	16.08	1.44	14.31	1.67	< 0.001	15.77	1.40	13.87	1.69	< 0.001
Morphometric parameters	Femur									
	Right		Female (n=94)		p-value	Left		Female (n=94)		p-value
	Male (n=106)		Mean	Std Dev		Male (n=106)		Mean	Std Dev	
	Mean	Std Dev	Mean	Std Dev	p-value	Mean	Std Dev	Mean	Std Dev	p-value
Fe-MAL (cm)	42.9	2	40.1	1.8	< 0.001	43.1	2.1	40.3	1.8	< 0.001
Fe-MD (mm)	26.91	2.08	24.62	1.85	< 0.001	26.94	2.15	24.6	1.84	< 0.001
Fe-HD (mm)	45.41	2.52	40.09	2.01	< 0.001	45.15	2.45	39.89	1.96	< 0.001
Fe-ND (mm)	31.25	2.22	27.09	2.04	< 0.001	30.74	2.43	26.79	2.09	< 0.001
Fe-BB (mm)	78.91	4.98	70.6	3.78	< 0.001	78.45	4.89	69.92	3.64	< 0.001
Fe-MCW (mm)	35.39	3.41	31.54	2.47	< 0.001	36.27	3.19	32.85	2.31	< 0.001
Fe-LCW (mm)	32.87	2.86	29.09	1.94	< 0.001	34.57	2.84	30.82	2.27	< 0.001
Morphometric parameters	Tibia									
	Right		Female (n=94)		p-value	Left		Female (n=94)		p-value
	Male (n=106)		Mean	Std Dev		Male (n=106)		Mean	Std Dev	
	Mean	Std Dev	Mean	Std Dev	p-value	Mean	Std Dev	Mean	Std Dev	p-value
T-MAL (cm)	35.8	1.9	33.6	1.7	< 0.001	36	1.9	33.6	1.7	< 0.001
T-MD (mm)	25.21	2.65	22.05	2.18	< 0.001	23.93	2.49	21.36	1.97	< 0.001
TPB (mm)	74.2	4.5	66.99	3.95	< 0.001	74.19	4.36	67.05	3.88	< 0.001
TPMW (mm)	48.9	3.49	44.15	2.72	< 0.001	48.35	3.37	43.68	2.95	< 0.001
TPLW (mm)	43.47	3.07	39.05	2.72	< 0.001	42.92	2.88	38.61	2.69	< 0.001
DTB (mm)	44.24	2.85	39.65	2.41	< 0.001	44.06	2.75	39.59	2.32	< 0.001
DTW (mm)	36.51	2.54	32.65	1.85	< 0.001	36.49	2.39	32.58	1.94	< 0.001

positive likelihood ratio of 4.16 and a negative likelihood ratio of 0.34. The positive predictive value (PPV) was 81.52%, negative predictive value (NPV) was 73.21%, and overall accuracy was 76.96%.

Extremity long bones

Descriptive statistics (Table 5) and boxplots (Fig 3) show significant sex differences ($p < 0.001$) across all humeral, radial, and ulnar parameters. Table 5 displays descriptive statistics for femoral and tibial measurements. Independent *t*-tests (Fig 5) confirmed significant sex differences for all parameters ($p < 0.001$).

Bilateral comparisons

Table 5 presents bilateral measurements. Paired *t*-tests (Fig 4) showed significant symmetry, except for HuSND, HuHMD, HuEB, HuOFD, RaDRW and UIMAL in females; and, HuHMAL, HuSND, RaRMD, RaDRB, RaDRW and UIUMAL in males.

Paired *t*-tests (Fig 6) showed significant asymmetry, except for Fe-MD ($p = 0.847$ in females, and $p = 0.817$ in males). In tibiae, males demonstrated greater variability, with females showing differences in T-MD ($p < 0.001$), T-PMW ($p = 0.008$), T-PLW ($p = 0.022$), while males differed significantly in five parameters, all $p < 0.01$, as illustrated in Fig 6.

Reliability analysis: Technical error of measurement

The coefficient of reliability (R) for both cranial and post-cranial measurements is shown in Tables 4 and 6, respectively.

Reliability of craniometric measurements

Table 4 reports inter-observer reliability. Cronbach's alpha indicated high internal consistency, with parameters such as cranial length, inter-orbital distance, and inter-infraorbital distances all exceeding 0.90. Other metrics, including cranial width, cranial index, cranial base length, mastoid length, and supraorbital distances, showed good reliability (α 0.75-0.90), confirming measurement consistency.

Reliability of osteometric measurements of the extremity long bones

Table 6 illustrates the R-values for this interobserver reliability analysis. Most upper and lower extremity long bone morphometrics show a reliability above the cut-off point, (high to very high). However, two tibial morphometrics exhibit a reliability below the cut-off point, including TPLW ($r = 0.64$) and DTW ($r = 0.64$).

DISCUSSION

Sex estimation from skeletal remains can be achieved

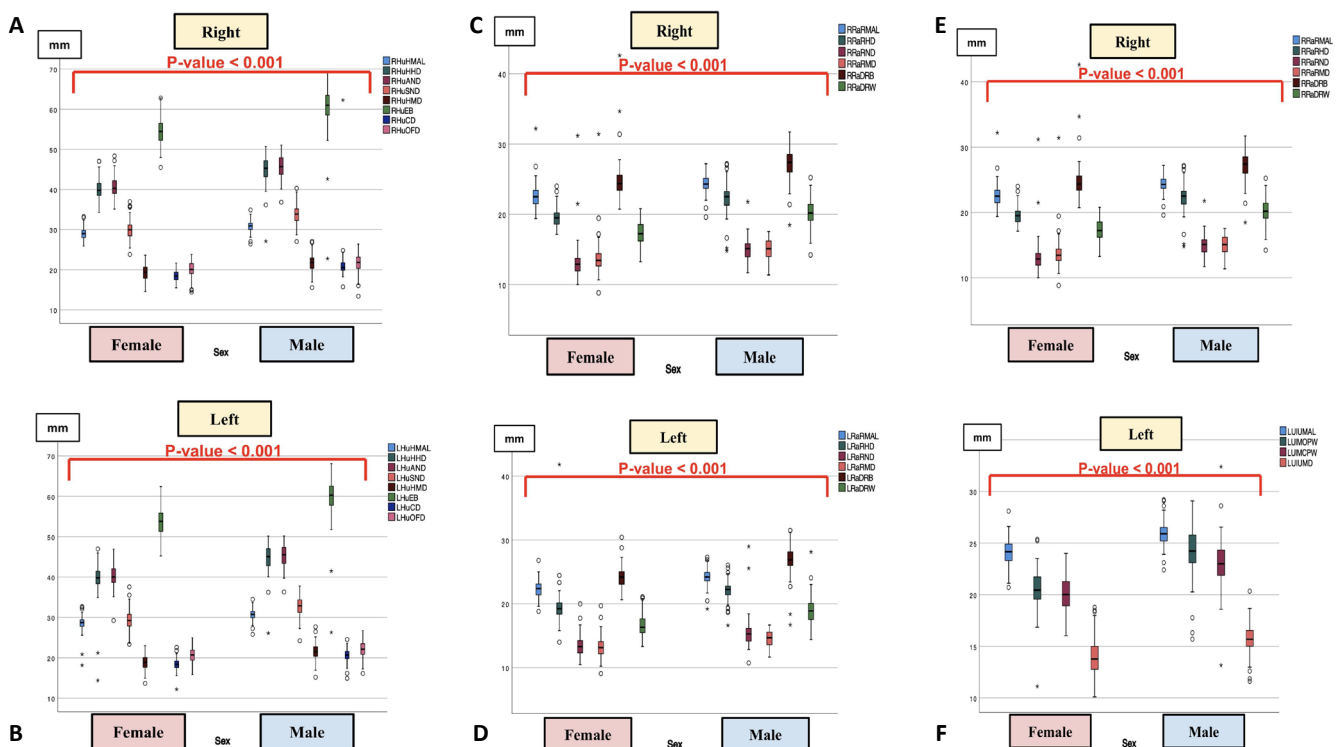
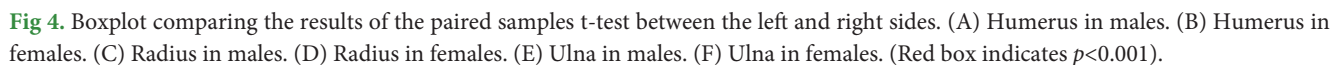


Fig 3. Boxplot comparing the results of the independent *t*-test between males and females. (A) Right side of humerus. (B) Left side of humerus. (C) Right side of radius. (D) Left side of radius. (E) Right side of ulna. (F) Left side of ulna.



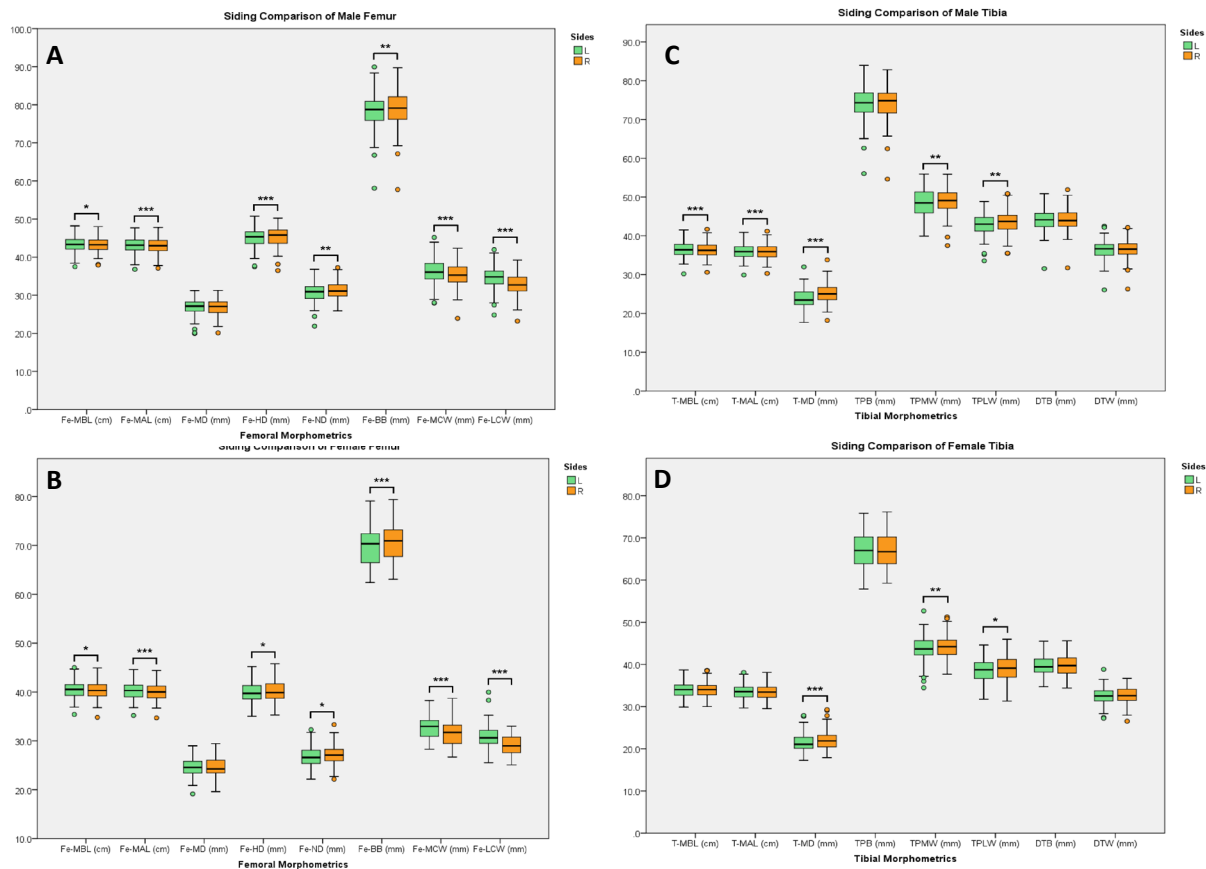


Fig 6. Boxplot comparing the results of the paired samples t-test between the left and right sides. (A) Femur in males. (B) Femur in females. (C) Tibia in males. (D) Tibia in females. (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

through the visual assessment of morphological variations stemming from sexual dimorphism. Key indicators include skeletal size and robustness, which are influenced by extrinsic factors such as biomechanical loads, as well as intrinsic factors like genetic makeup and hormonal influences. Both cranial and postcranial skeletal elements exhibit distinguishable sexual dimorphism in adult individuals. Among the available methodologies, combined non-metric and metric analysis of skull morphology is considered the second most reliable approach for sex determination in adult skeletal remains, following pelvic morphology assessment.¹⁹ Within the cranium, features of the mastoid region are regarded as particularly informative. Morphological characteristics of the nuchal crest and mental eminence form the basis of cranial morphological sex estimation techniques, which are recognized for their high accuracy. Subsequent validation studies have reinforced and refined the reliability of skull morphological traits, leading to enhanced predictive accuracy and decreased classification errors.^{20,21}

The findings of this study demonstrate that cranial measurements obtained by multiple observers, following anthropometric standards, exhibit high levels of reliability. Cronbach's alpha coefficients ranged from 0.832 to

0.971, indicating strong to excellent internal consistency between/among observers' measurements. These results are consistent with prior research, which recommended a reliability threshold of $R > 0.95$ to ensure measurement dependability and accuracy. Notably, cranial length, inter-orbital foramen distance, and inter-infraorbital foramen distance showed the highest reliability coefficients at 0.971, 0.920, and 0.908, respectively. The elevated reliability scores of these parameters suggest that they are among the most sexually dimorphic features in the cranium. Furthermore, these results support the premise that craniometric measurements, when conducted following standardized protocols and independent assessments, can be both consistent and reproducible across different observers, thereby serving as robust tools for forensic sex determination. Overall, the results endorse the reliability and reproducibility of craniometric methods in forensic contexts.

The statistical analyses, employing independent *t*-tests and Mann-Whitney U tests, revealed statistically significant differences in various cranial measurements between males and females. Consistent with existing literature on the Thai population, male crania demonstrated larger mean measurements than females.^{22,23} The high

TABLE 6. Assessment of the coefficients of reliability for post-cranial extremity long bones measurements.

Bone	Morphometric variables	Coefficient of reliability (r-value)	Interpretation
Humerus	HMAL	0.98	Very High
	HHD	0.96	Very High
	AND	0.94	Very High
	SND	0.85	High
	HMD	0.91	Very High
	EB	0.92	Very High
	CD	0.9	Very High
	OFD	0.85	High
Radius	RMAL	0.98	Very High
	RHD	0.91	Very High
	RND	0.82	High
	RMD	0.87	High
	DRB	0.86	High
	DRW	0.99	Very High
Ulna	UMAL	0.88	High
	MOPW	0.89	High
	MCPW	0.82	High
	UMD	0.88	High
Femur	Fe-MAL	0.91	Very High
	Fe-MD	0.92	Very High
	Fe-HD	0.96	Very High
	Fe-ND	0.94	Very High
	Fe-BB	0.94	Very High
	Fe-MCW	0.73	High
	Fe-LCW	0.79	High
Tibia	T-MBL	0.99	Very High
	T-MAL	0.92	Very High
	T-MD	0.68	Moderate
	TPB	0.84	High
	TPMW	0.78	High
	TPLW	0.64	Moderate
	DTB	0.92	Very High
	DTW	0.64	Moderate

reliability scores further reinforce the statistical validity of these findings. This concordance with prior studies underscores the efficacy of craniometric techniques in accurately sexing skulls and supports their application in forensic investigations. Although visual assessment yielded an accuracy of approximately 77%, with sensitivity and specificity of 71.43% and 82.83%, respectively, it remains inherently subjective and susceptible to inter-observer variability. This aligns with earlier research highlighting the potential for inaccuracies and examiner dependence associated with visual methods. In contrast, craniometric techniques offer objective, quantifiable data, reducing the likelihood of subjective bias. While direct comparison of the accuracy between these methods was limited by differing evaluation criteria, the superior reliability of craniometric measurement underscores its potential as an efficient and objective approach for forensic sex estimation.^{24,25}

The morphologies of the mastoid process, orbital region, and nuchal crest are key indicators of sexual dimorphism. Additionally, other cranial features have been identified as potential sex determinants. These anatomical areas are notably resilient, often withstanding destructive forces. Employing morphometric measurements combined with stepwise discriminant functions generally achieves more accurate sex classification than visual assessments alone. However, this study found that morphometric analysis can be less accurate in sex differentiation compared to direct observational methods.

Sexual dimorphism and morphometric analysis of extremity long bone

Sexual dimorphism in the femur and tibia is strongly influenced by both sex hormones and biomechanical factors, leading to generally larger skeletal dimensions in males compared to females. This study revealed statistically significant differences between sexes across all measured femoral and tibial parameters ($p < 0.001$), with males exhibiting greater mean values. The observed dimorphism is largely attributed to the influence of androgens and estrogens, particularly testosterone, which promote bone growth and increased bone mass in males, especially during puberty. This hormonal effect contributes to the development of more pronounced skeletal features, including key landmarks on the femur and tibia. Specifically, the femoral measurements in this study support findings from previous research in Thai population, showing similar values for midshaft diameter (Fe-MD), head diameter (Fe-HD), and neck diameter (Fe-ND).^{26,27} However, the current study found that the maximum bone length of the femur (Fe-MBL) is notably

greater, suggesting potential population-specific growth trends or methodological differences.

When comparing femoral bone lengths, including the maximum length (Fe-MBL) and anatomical length (Fe-MAL), the values observed in this study are smaller than those reported in Sri Lankan and Indian populations, yet larger than those found in Japanese population.²⁸ In comparison with an Ancient Anatolian population, the measurements for females are closely aligned, whereas male measurements are noticeably smaller. For midshaft diameter (Fe-MD), this study's results are slightly greater than those reported in Indian population and generally consistent with Sri Lankan data, although the Sri Lankan female values are slightly smaller.²⁹ Notably, in Sri Lankan population, male and female midshaft diameters are nearly identical, highlighting minimal dimorphism in that metric. Compared to ancient Anatolian population, midshaft diameters in this study are again closely aligned in females, but marginally smaller in males, suggesting possible population-specific variation or environmental influences on skeletal development.

On the distal femur (Fe-MCW and Fe-LCW), the measurements of this study were found to be slightly smaller in size compared to Korean population.^{30,31} Importantly, it must be noted there is no comparative analysis of Fe-BB due to a difference in measurement methods since other studies used epicondylar breadth – the maximum length between medialmost to lateral most points on the epicondyle of the femur – instead of the definition used in this study.

Similarly, the tibial measurements in this study demonstrate marked sexual dimorphism, aligning with findings from previous research. When compared to earlier data from Thai population, the maximum tibial length (T-MBL) and distal tibia breadth (DTB) in this study are slightly smaller, while the proximal tibial measurements—specifically the medial and lateral condylar widths (TPMW and TPLW)—are slightly larger. The tibial plateau breadth (TPB), however, remains comparable between studies. In comparison with Sri Lankan population, this study reports a notably smaller anatomical length (T-MAL), while TPB measurements are larger.³² Additionally, TPMW and TPLW values are slightly greater than those observed in Japanese and Brazilian populations, suggesting region-specific variation in proximal tibial morphology.³³ Compared to ancient Anatolian population, T-MBL values are marginally larger in females and slightly smaller in males, further highlighting population and sex-related differences in tibial dimensions.³⁴

The midshaft diameter (T-MD) of the tibia in the

present study was found to be notably larger than that reported in the Indian population. When compared with ancient Anatolian population, female measurements were distinctly larger, while overall values were generally comparable. It is important to acknowledge, however, that methodological variations exist across studies, particularly in the anatomical landmarks used for measurement—many researchers take the diameter at the level of the nutrient foramen, which may influence reported values. Regarding the distal tibia, the breadth of the inferior articular surface (TPB) closely aligns with data from Sri Lankan population but is significantly larger than values recorded in Japanese population.³⁵⁻³⁷ Similarly, the width of the inferior articular surface (DTW) in this study was found to be markedly greater than that of Kenyan population, highlighting inter-population variability in distal tibial morphology.³⁸

From all of the aforementioned points, the femur and tibia can both be regarded as effective and reliable indicators for sex determination, and the measurement methods used for both the femur and tibia can be used in the process of sex determination.

TEM and reliability

It is well-recognized that observer error arising from visual or metric variables can result in inconsistencies in evaluating sexual dimorphism and determining sex. Recent studies indicate that visual assessment is prone to substantial inter-observer error due to vague variable definitions, heavy reliance on the observer's prior experience, and the seriation process used to categorize individuals. This error analysis demonstrates that geometric morphometrics achieves high levels of intra- and inter-observer agreement.

The results of the technical error of measurement (TEM) analysis, which is used to assess inter-assessor reliability, indicate variable levels of agreement across different skeletal parameters. For the humerus, six out of eight parameters—namely HMAL, HHD, AND, HMD, EB, and CD—demonstrated very high reliability, whereas SND and OFD exhibited only high reliability scores. In the case of the radius, half of the parameters, including RMAL, RHD, and DRW, showed very high interpretative consistency, whereas RND, RMD, and DRB were characterized as having only high reliability. For the ulna, all parameters—UMAL, MOPW, MCPW, and UMD—exhibited high reliability. This variation can be attributed to differences in the anatomical aspects of each bone, such as the specific landmarks used for measurement, including SND and OFD in the humerus, RND, RMD, and RBD in the radius, and UMAL, MOPW, MCPW,

and UMD in the ulna. The instances where parameters yielded only high rather than very high reliability may stem from variability in landmark identification, often influenced by factors such as the absence of precise measurement landmarks—particularly in regions like the surgical neck of the humerus, where measurement relies heavily on the assessor's judgment.

Values reflect measurement consistency for both femoral and tibial parameters, except for the maximum bone length measurements of the femur (Fe-MBL) and tibia (T-MBL), due to the absence of comparable data. Using an R-value threshold of ≥ 0.90 , most femoral measurements exceeded this cutoff, indicating high interobserver reproducibility, except for Fe-MCW and Fe-LCW. In contrast, most tibial measurements did not reach this threshold with only two parameters—T-MAL and DTB—demonstrating high reliability. These results suggest that femoral morphometric measurements generally exhibit greater interobserver consistency, whereas tibial measurements show variable reliability, with only selected parameters achieving robust repeatability.

Diagrams were used to clarify the definitions of each parameter during measurements; however, despite standardization, measurement errors remained possible due to limitations in observer interpretation. A key source of error is the variation in observer experience. In this study, one observer had no prior experience in skeletal measurement, whereas the other was an expert in forensic anthropology, which likely contributed to discrepancies, particularly in the identification of anatomical landmarks. Variations in understanding and interpreting the diagrams may have further contributed to minor differences in landmark annotation. Additionally, the morphology of the landmarks themselves influences measurement reliability. Many tibial measurements, along with two femoral parameters (Fe-MCW and Fe-LCW), involve landmarks with curved or rounded contours, which can be ambiguous and challenging to delineate precisely, thereby reducing measurement accuracy.

Cranial morphologies can often be consistently distinguished even by observers without prior experience. However, morphologies that are not clearly defined present significant challenges for consistent determination. Our findings indicate that even experienced observers analyzing skulls for sexual dimorphism may inconsistently interpret coordinate landmarks for sex assessment. While the robustness of the mastoid process and the pronounced nuchal crest are strong indicators of male sex, morphological trait evaluation still leads to varying levels of prediction accuracy between/among observers. This suggests that anatomical landmarks on cranial

bones may be misinterpreted. The description of these landmarks varies across the literature. It is crucial to describe quantitative methodologies that utilize cranial features exhibiting sexual dimorphism since they are pertinent to forensic practitioners. Additionally, understanding the impact of cranial sexual dimorphism on classification accuracy in sex estimation is essential.³⁹⁻⁴¹

The results of the reliability analysis of craniometric measurements taken by two inter-observers (the intraclass correlation coefficient [ICC], specifically Cronbach's alpha), was used to assess the internal consistency and reliability of the measurements across various cranial parameters. That analysis revealed that the craniometric measurements exhibit high internal consistency, with all parameters showing good to excellent reliability. When interpreted according to the ranges established by Koo and Li, the Cronbach's alpha values for cranial length, inter-orbital foramen distance, and inter-infraorbital foramen distance indicate excellent reliability ($\alpha > 0.90$).⁴² Other parameters, including the cranial width, cranial index, cranial base length, mastoid length, and inter-supraorbital foramen distance showed good reliability ($0.75 \leq \alpha \leq 0.90$). These high reliability scores suggest that the measurements are consistent and repeatable across different observers.^{43,44}

Furthermore, the definition of specific landmarks measured on the cranium and long bones of the extremities, such as the tibia, presents a recurring challenge in certain regions. To ensure measurement accuracy, all assessments must be both reproducible and independent. Consequently, future research should include evaluations of interobserver error in these measurements to minimize the risk of sex misclassification.

Effects of skeletal asymmetry and siding comparison

The paired samples t-test results of the upper extremity long bones indicate that two of the eight parameters—HMAL and SND—exhibit statistically significant differences between the left and right sides in males, with p -values less than 0.001. Additionally, analysis of the radius bones revealed three parameters—RMD, DRB, and DRW—that demonstrate significant bilateral differences at p -values below 0.001. Conversely, examination of the ulna shows that only UMAL exhibits significant asymmetry with a p -value under 0.001. The variation in the degree of bilateral differences, ranging from high to very high significance, is likely influenced by factors, such as handedness, which predominantly affects bone size disparity between sides. Supporting this, Walters *et al.* (1998) reported that right-handed individuals tend to have larger bones in their dominant

hand, highlighting the functional role of dominance in skeletal asymmetry.⁴⁵⁻⁴⁷

Humans, as bipeds, typically distribute body weight evenly across the lower limbs during upright stance. Minimal bilateral differences in femoral and tibial morphometrics are, therefore, generally expected, so many previous studies have therefore analyzed only one limb for sex estimation. However, our findings revealed significant asymmetry between sides for both femoral and tibial measurements, which can be attributed to factors such as differential biomechanical loading, environmental influences, habitual limb preferences, and localized mechanical stress - all of which can induce side-specific bone remodeling. Degenerative joint conditions, notably osteoarthritis affecting the hip and knee, may further contribute to asymmetries, with parameters, such as femoral head diameter, bicondylar breadth, and proximal tibial measurements, showing consistent side differences. In the femur, almost all parameters demonstrated significant asymmetry with p -values below 0.05, which is consistent with prior studies that reported greater measurements on the left side, particularly in the lower limbs.^{48,49}

Similarly, most femoral measurements in our study were larger on the left side in both sexes. Carvallo and Retamal (2020) studied a Chilean population and found bilateral symmetry predominantly in proximal femoral parameters, but they did not explore the underlying causes.⁵⁰ In the tibia, asymmetry was also present, but less pronounced, with males showing greater asymmetry than females. These findings align with van der Gaast, *et al.* (2022), who identified asymmetry in the tibial plateau potentially related to previous injuries, while the distal tibia exhibited high symmetry, consistent with the findings of Verbakel, *et al.* (2024). Nonetheless, caution in interpretation is warranted due to the relatively limited sample size.^{51,52}

Given the observed inherent asymmetry, measurements should be taken bilaterally to ensure accuracy - especially for the femur. Despite the tibia generally demonstrating greater symmetry than the femur, bilateral assessment of the tibia remains advisable. Due to the possibility of unpredictable laterality in fragmentary remains at scene investigations, it is prudent to prioritize measurements of parameters known for bilateral symmetry when only partial femoral or tibial fragments are available.

Although most adult skeletons display characteristic sexual dimorphism, the accuracy of sex estimation is influenced by several factors, including population variability, age, and pathological or taphonomic changes. The degree and expression of sexual dimorphism can vary significantly both within and across different populations. Therefore,

it is crucial to employ population-specific data when applying these techniques in forensic contexts. The extent of sexual dimorphism and the differences between sexes differ across populations, and both morphological and metric methods are used for sex estimation. Morphological attributes—such as shape, specific traits, and relative size differences—are key indicators. Techniques focusing on pelvic shape, measurements, and the presence or absence of distinctive pelvic features are generally preferred since these tend to demonstrate greater sexual dimorphism and higher accuracy. Other morphological traits may also suggest sex, but they are often less reliable than those with pronounced dimorphic features. Employing appropriate instrumentation, standardized protocols, advanced analytical software, and integrating multiple measurements through multivariate approaches can improve the reliability of sex assessments, although individual measurements can still yield reasonably accurate results.

Sample size is a crucial consideration since the validation of classification functions necessitates cross-population studies to assess their robustness and generalizability. The use of approximately 400 individuals in this study may affect prediction accuracy and elevate the risk of technical errors. Expanding the sample size could mitigate these issues and enhance the overall effectiveness of the method.

Lastly, understanding the factors that influence prediction accuracy and classification errors—particularly in cranial and post-cranial sex estimation—is vital, given that the cranium is primarily governed by hormonal rather than mechanical factors - unlike the extremity long bones. This makes it especially useful in cases where the population of origin from which an unidentified skeleton originates from is unknown. Future research should focus on collecting and analyzing cooperative datasets from diverse populations, both for pelvic parameters and other skeletal features that display sexual dimorphism, to refine and validate forensic sex estimation methods across different population groups.

Limitations and suggestions

Future research should include measurements of additional osteometric landmarks, such as cranial landmark distances of cranium and fibular morphometrics of post-cranial bones and assess both intra- and interobserver reliability across all measurement methods.

Larger regionally diverse, and ethnically varied samples are recommended to evaluate the reliability, repeatability, and accuracy of sex estimation methods. The resulting data may facilitate the development of

discriminant functions and help to estimate other biological profiles, such as stature.

The fact that our sample size of 204 crania is slightly lower than Yamane's suggested 214 likely exerted minimal impact on the results of our study. However, variation in observer expertise significantly influenced measurement accuracy in our study. The author's limited experience may have affected the accuracy of visual assessment, and despite efforts to reduce variability, differences in examiner skill and physical and mental condition contribute to inter-observer variability. Standardized training and calibration are, therefore, necessary to enhance consistency.

Outliers in descriptive statistics, particularly for the inter-supraorbital foramen distance ($p=0.056$), can be attributed to population-specific variations, such as the presence of multiple foramina or notches. These variations may have affected measurement accuracy, highlighting the need for further research to account for such differences in diverse populations.

CONCLUSION

Determining sex from skeletal remains is a fundamental component of human identification in forensic investigations. Although the skull is regarded as the second most preferable skeletal element for sex estimation, it is essential to account for measurement errors, which can impact the accuracy of sex determination. The adoption of alternative morphometric approaches, such as direct measurement of long bones of the extremities, may offer a viable alternative with superior accuracy—particularly when the pelvis or skull are not available. It is crucial to identify and understand the instrumental and methodological factors that influence accurate sex prediction. The selection of an appropriate analytical approach is paramount since it significantly influences the overall process of forensic human identification and helps reduce the likelihood of misclassification.

Data Availability Statement

The data used in this study was obtained from the Siriraj Anatomical and Anthropological Bone Research Centre (Si-AABRC) database, Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol university, Bangkok, Thailand. Access to this data was approved through Si-AABRC data access procedures. The data is not publicly available but can be requested from the Si-AABRC with appropriate permissions.

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Conflicts of Interest

None.

Registration Number of Clinical Trial

Not Applicable.

Author Contributions

Conceptualization and methodology, N.S. and J.C.; Investigation, K.T., S.B., S.W., N.C. and J.C.; Formal analysis, K.T., S.B., S.W., N.C. and P.R.; Visualization and writing – original draft, K.T., S.B., S.W., N.C. and P.R.; Writing – review and editing, N.S.; Supervision, N.S. All authors have read and agreed to the final version of the manuscript.

Use of Artificial Intelligence

No artificial intelligence was used in this study.

Ethics/IRB Approval

SIRB Protocol No. 195/2564 (Date of Proof: 22 March 2021) and 680/2564 (Date of Proof: 25 August 2021).

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