



**UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA**

MARCOS PAULO SALLES MACHADO

**APLICAÇÃO E VALIDAÇÃO DA FERRAMENTA DIAGNÓSTICO
PROBABILÍSTICO DO SEXO (DSP V2) EM UMA POPULAÇÃO DO
SUDESTE BRASILEIRO**

*APPLICATION AND VALIDATION OF DIAGNOSE SEXUELLE
PROBABILISTE V2 TOOL IN A SOUTHEASTERN BRAZILIAN POPULATION*

PIRACICABA

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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Biologia Buco-dental, na Área de Anatomia.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Buco-dental Biology, in Anatomy Area.

Orientador: Profa. Dra. ANA CLÁUDIA ROSSI

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A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

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“O objetivo da vida não é ser feliz. É ser útil, honrado, compassivo, fazendo com que nossa vida, bem vivida, faça alguma diferença.”

(Ralph Waldo Emerson)

“Pois o Deus todo-poderoso, por ser soberanamente bom, nunca deixaria qualquer mal existir nas suas obras se não fosse bastante poderoso e bom para fazer resultar o bem do próprio mal.”

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RESUMO

O osso do quadril é a parte do esqueleto que apresenta maior dimorfismo sexual. Os métodos baseados neste segmento oferecem os resultados mais precisos, sendo o DSP2 uma das ferramentas mais utilizadas nessa análise. O objetivo deste estudo foi validar a aplicação do DSP2 na obtenção do sexo, a partir de uma coleção de osteológica brasileira. Uma amostra de 103 ossos do quadril (53 masculino e 50 feminino) pertencente à coleção osteológica da área de Odontologia Legal da Faculdade de Odontologia de Piracicaba –UNICAMP foi mensurada de acordo com as instruções da ferramenta DSP2. A coleção consiste em recentes e modernos ossos catalogados, compilados de indivíduos falecidos entre 2008 e 2010 de uma população do sudeste brasileiro. A média de idade dos indivíduos foi de 55 anos. As medidas foram realizadas com um paquímetro digital 150-mm (Starrett, Athol, Massachussetts, EUA) e com um paquímetro de 600-mm para regiões onde o paquímetro digital não permitiu alcance. As calibrações foram realizadas por dois experts forenses, com duplo cegamento. Apenas o osso do quadril esquerdo foi avaliado. A aquisição dos dados ocorreu em dois passos: a) análise métrica pelo método do DSP2; b) comparação entre a estimativa do sexo pelo DSP2 e a informação presente na coleção osteológica catalogada. A análise dos erros de mensuração foi feita por meio de três indicadores frequentemente utilizados em estudos osteométricos e antropométricos. O erro técnico de medição (TEM), o erro técnico relativo (% TEM) e o coeficiente de confiabilidade (R) foram calculados para avaliar a precisão e a confiabilidade das medições. Os resultados foram expressos como média e desvio padrão. As análises estatísticas foram realizadas utilizando o Bioestat® (Instituto Mamirauá - Conservação na Amazônia, Brasil). Para cada amostra, a suposição de normalidade univariada foi verificada pelo teste de Kolmogorov-Smirnov. As diferenças de significância estatística entre homens e mulheres para cada medição foram avaliadas usando o teste t de amostras não pareadas. O nível de significância foi estabelecido em $p < 0,05$. Os resultados revelaram que as diferenças entre os sexos foram estatisticamente significantes para todas as medidas, com exceção dos comprimentos acetábulo-sinfise e espino-auriculares. Dos 103 ossos do quadril analisados, houve erro em 9,43% dos indivíduos do sexo masculino e em 14% dos indivíduos do sexo feminino. Foi demonstrado que o DSP2 pode ser aplicado à população brasileira, extremamente miscigenada, com um bom grau de precisão, apesar desta se mostrar menor do que quando o estudo foi aplicado em outras amostras populacionais. Este estudo, além de demonstrar claramente que a variação métrica dos ossos do quadril é extremamente útil na estimativa do sexo, reforça o entendimento de que o dimorfismo sexual pélvico não é específico pra determinadas populações.

Palavras-chaves: Dados de População Antropológica Forense; Diagnóstico Sexual Probabilístico; Ossos do quadril; Osteometria; Estimativa Sexual; Bioarqueologia; Morfologia Óssea.

ABSTRACT

The hip bone (*os coxae*) is the skeletal element that presents the greatest level of sexual dimorphism. Therefore, methods involving the analysis of the *os coxae* provide the most accurate sex estimation, and DSP2 (Diagnose Sexuelle Probabiliste v.2) is one of the most accurate tools used in this identification. The aim of this study was to apply and validate DSP2 in the identification of 103 *os coxae* (53 male and 50 female) belonging to a Brazilian-identified skeletal collection. A sample of 103 hip bones (53 male and 50 female) belonging to the osteological collection of the Forensic Dentistry area of the School of Dentistry of Piracicaba - UNICAMP was measured according to the instructions of the DSP2 tool. The collection consists of recent and modern cataloged bones, compiled from individuals who died between 2008 and 2010 of a population of southeastern Brazil. The mean age of the subjects was 55 years. Measurements were performed with a 150-mm digital caliper (Starrett, Athol, Massachusetts, USA) and with a 600-mm caliper for regions where the digital caliper did not allow reaching. Calibrations were performed by two forensic experts, with double blindness. Only the left hip bones were evaluated. The data acquisition was obtained in two steps: a) metric analysis by the DSP2 method; b) comparison between the estimation of sex by DSP2 and the information present in the cataloged osteological collection. The analysis of the measurement errors was made through three indicators frequently used in osteometric and anthropometric studies. The technical error of measurement (TEM), the relative technical error (% TEM) and the reliability coefficient (R) were calculated to evaluate the accuracy and reliability of the measurements. The results were expressed as mean and standard deviation. Statistical analyzes were performed using the Bioestat ® (Mamirauá Institute - Conservation in the Amazon, Brazil). For each sample, the assumption of univariate normality was verified by the Kolmogorov-Smirnov test. The differences in statistical significance between men and women for each measurement were evaluated using the t-test of unpaired samples. The level of significance was set at $p < 0.05$. Differences between sexes were statistically significant for all measurements, except for the acetabulo-symphyseal and spino-auricular lengths. From the 103 *os coxae* analyzed, there was a 9.43% error in male individuals and a 14% error in females. The results revealed that DSP2 can be applied to Brazilian-mixed populations with a good index of accuracy, although at a lower accuracy than other population samples. This study also clearly demonstrates that metric variation of the *os coxae* is extremely useful in sex estimation and reinforces the notion that pelvic sexual dimorphism is not population-specific.

Keywords: Forensic Anthropology Population Data; Probabilistic Sex Diagnosis; *Os Coxae*; Osteometrics; Sex Estimation; Bioarcheology; Bone Morphology.

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1 INTRODUÇÃO

A estimativa do sexo é um dos pilares do perfil biológico (Steyn & Iscan, 2008; Gómez-Valdés et al., 2012; Small et al., 2012; Albanese, 2013). A avaliação do sexo de um indivíduo é essencial quando se busca a identificação de restos humanos (Gómez-Valdés et al., 2012). Os diferentes autores pesquisados concordam que os ossos pélvicos oferecem alta precisão e os melhores resultados para a estimativa do sexo, representando os mais dimórficos dentre os ossos do corpo humano (Gómez-Valdés et al., 2012; Small et al., 2012; Albanese, 2013). Muitas vezes, os métodos métricos são usados na estimativa do sexo devido à sua assertividade (Chapman, 2014). No entanto, uma crítica significativa ao emprego de métodos quantitativos na estimativa do sexo é que esses métodos foram previamente demonstrados como específicos para a população da qual a amostra se originou (Chapman, 2014). Estudos anteriores também sustentam que as análises métricas possuem certo grau de subjetividade devido à dificuldade de localizar os pontos anatômicos de mensuração. Além disso, vários estudos defenderam a ideia de que os métodos devem ser validados antes de serem aplicados em cada grupo geográfico (Fortes de Oliveira et al., 2012). Alguns autores afirmam que padrões específicos otimizam a precisão da estimativa de sexo, porque as características do esqueleto variam entre cada população devido à variabilidade regional (Steyn & Iscan, 2008).

Na prática, a maioria das análises em laboratórios utiliza uma combinação dos métodos visual (também conhecido como morfoscópico) e métrico. É importante notar que há críticos e defensores dos dois métodos. Ambos foram anteriormente criticados por apresentarem altos erros interobservadores. As deficiências creditadas ao método visual são que o método é subjetivo e a consistência pode depender da experiência do observador, o que pode criar altos erros interobservadores (Murail et al., 2005). No entanto, esses métodos são citados como sendo fáceis de usar e não exigem equipamentos especializados ou conhecimento de estatística (Walker, 2005). Os métodos métricos geralmente são considerados de maior precisão, embora tenha sido observado que eles consomem mais tempo (MacLaughlin & Bruce, 1990).

Diagnóstico Sexual Probabilístico (DSP) é uma ferramenta para estimar o sexo com base na análise antropométrica dos ossos pélvicos. A técnica foi desenvolvida em 2005 e testada nos países da Europa, África, América do Norte e Ásia, nomeadamente, França, Portugal, Lituânia, EUA e Tailândia. A técnica é apresentada como simples, prática e com alto índice de assertividade nas diferentes populações onde foi testada (Murail et al., 2005).

Na Bélgica, o método comum de sexagem visual em departamentos antropológicos consiste no exame visual de oito variáveis pélvicas (Maat et al., 1999). Uma vantagem do DSP consiste em ser frequentemente utilizado e já ter demonstrado alta precisão (Nielsen, 2011; Bruzek & Veleminsky, 2006; Baillif-Ducros et al., 2012) ou seja, é citado como um método de escolha em vários manuais (Beauthier, 2011; Grant & Fibiger, 2011).

A técnica é relativamente fácil de aprender e aplicar, de modo que se torna adequada para observadores inexperientes (Bruzek, 2002). O objetivo do método é fornecer determinação sexual para qualquer humano anatomicamente moderno, independentemente da população a que pertence o espécime e isso foi adequadamente demonstrado. O diagnóstico sexual pode ser realizado em ossos do quadril bem preservados ou danificados (Murail et al., 2005).

Recentemente, o DSP2, Diagnóstico Sexual Probabilístico v.2, (Brůžek et al., 2017) foi desenvolvido e está disponível gratuitamente para diferentes softwares. Os dados podem ser inseridos facilmente, manualmente ou transferidos de outra planilha. A nova versão acrescenta avisos de cores para indicar quando os dados estão fora do intervalo, são fáceis de operar, são robustos e menos sensíveis ao viés. Embora os testes tenham sido realizados em grupos europeus, asiáticos, norte-americanos e africanos, não houve experimento em nenhum grupo populacional sul-americano. A população brasileira é caracterizada pela miscigenação, sendo importante a aplicação do método DSP2 neste grupo populacional para fornecer validação geográfica.

Diante do exposto, e a necessidade de aplicação do DSP2 em diferentes populações, o presente estudo teve como objetivo aplicar e validar o DSP2 em uma população miscigenada, utilizando uma coleção osteológica pertencente a uma população contemporânea representativa do sudeste brasileiro.

2 ARTIGO: Application and validation of Diagnose Sexuelle Probabiliste V2 tool in a miscegenated population

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ABSTRACT

The hip bone (*os coxae*) is the skeletal element that presents the greatest level of sexual dimorphism. Therefore, methods involving the analysis of the *os coxae* provide the most accurate sex estimation, and DSP2 (Diagnose Sexuelle Probabiliste v.2) is one of the most accurate tools used in this identification. The goal of this study is to apply and validate DSP2 in the identification of 103 *os coxae* (53 male and 50 female) belonging to a Brazilian-identified skeletal collection. Differences between sexes were statistically significant for all measurements, except for the acetabulo-symphyseal and spino-auricular lengths. From the 103 *os coxae* analyzed, there was a 9.43% error in male individuals and a 14% error in females. The results revealed that DSP2 can be applied to Brazilian-mixed populations with a good index of accuracy, although at a lower accuracy than other population samples. This study also clearly demonstrates that metric variation of the *os coxae* is extremely useful in sex estimation and reinforces the notion that pelvic sexual dimorphism is not population-specific.

1. INTRODUCTION

Sex estimation is one of the pillars of the biological profile [1], [2], [3], [4]. The assessment of an individual's sex is essential when first identifying human remains [2]. The literature agrees that os coxae provide a high accuracy and the best results for sex estimation, as it has been accepted that the os coxae is the most dimorphic bone in the body [2], [3], [4]. Often, metric methods are used in sex estimation due to their assertiveness [5]. However, a significant criticism of employing quantitative methods in sex estimation is that these methods have been previously demonstrated to be specific to the population from which the sample originated [5]. These previous studies also support that metric analyses possess a degree of subjectivity due to the difficulty of locating points of measurement. Additionally, several studies have defended the notion that methods must be validated before they can be used in each geographical group [6]. Specific standards optimize the accuracy of sex estimation because skeletal characteristics vary among each population due to regional variability [1].

Diagnose Sexuelle Probabiliste (DSP) is a tool to estimate sex based on the anthropometric analysis of the os coxae. The technique was developed in 2005 and tested in the countries of Europe, Africa, North America and Asia, namely, France, Portugal, Lithuania, USA and Thailand. The technique is presented as simple, practical and possessing a very high assertiveness index in the different populations where it was tested [7]. Recently, DSP2, Diagnose Sexuelle Probabiliste v.2, [8] was developed and is freely available for different software. Data can be easily entered, either manually or by pasting from another spreadsheet. The new version adds color warnings to indicate when data are out of range, is easy to operate, is robust and is less sensitive to bias. Although the tests were performed in European, Asian, North American and African groups, there was no experiment in any South American population group. The Brazilian population is characterized by miscegenation, making it important to apply the DSP2 method in this population group in order to provide geographical validation.

The present study aimed to apply and validate DSP2 in a mixed population using the osteological collection belongs to a contemporary Southeast Brazilian population.

2 MATERIALS AND METHODS

2.1 Study Sample

A hundred three os coxae (53 male and 50 female) belonging to the Bone Collection housed at Piracicaba Dental School from the University of Campinas (UNICAMP) were measured according to DSP2 instructions. This study was approved by Committee for Ethics of Research of the School of Dentistry of Piracicaba (protocol number: 100/2015) (ANEXO 2).

The collection consists of recent and modern cataloged bones, compiled from 250 skeletons from individuals who died between 2008 and 2010, that were exhumed between 2013 and 2014. The skeletons belong to a contemporary Southeast Brazilian population, are of the actual population and surroundings, are in a good state of preservation, and were identified by death certificates. The Southeast Brazilian population is one of the most miscegenated populations, as it contains a mixture of European, African, Asiatic and the indigenous population [6]. This population is unlike that of many other countries, as Brazilian miscegenation has occurred over centuries, which transforms the scenario in a peculiar case study.

The mean age-at-death of the individuals used in this study was 55 years. The female mean was 63 years ($\pm 20,43$), and the male mean was 48 years ($\pm 15,24$) (Table 1).

Table 1. Age distribution according to males and females.

Sex	Age Range and Percentage							
	17-30	31-40	41-50	51-60	61-70	71-80	81-100	Unknown
Male	6 (5.82%)	6 (5.82%)	13 (12.62%)	12 (11.65%)	7 (6.79%)	3 (2.91%)	-	-
Female	4 (3.88%)	3 (2.91%)	8 (7.76%)	6 (5.82%)	4 (3.88%)	15 (14.56%)	7 (6.79%)	9 (8.73%)

Os coxae with macroscopic anomalies, such as trauma, ankylosis, gross asymmetry, malformations or pathologies, were excluded from this study. Only adult individuals were included.

Minimal information was taken from skeletons to ensure the anonymity of the sample. Information was accessed only after the measurements had been taken and restricted to sex and age. Essentially, the study was performed as a blind test regarding the identification of data.

2.1 Protocol Measurements According to DSP

Measurements were taken with a 150-mm digital caliper (Starrett, Athol, Massachusetts, EUA) and with a 600-mm outside caliper for regions where the digital caliper could not be used.

Assessments and measurement calibrations were made by two forensic experts, one with 5 years' experience and the other 15 years', both of whom were double blinded. Only the left side of the coxae was analyzed. To ascertain intra-observer and inter-observer reliability and reproducibility, twenty os coxae were randomly chosen and re-measured by both researchers after an interval of two months.

Data acquisition occurred in two steps: (a) the metric analysis via the DSP2 method and (b) the comparison between the DSP2 sex estimation result and information from the Bone Collection. All measurements were made according to the DSP2 detailed description of all 10 variables, as defined in Table 2 [7], [8].

Table 2. Definition of the variables used in the study.

Variables	Definition
PUM (Brauer 1988)	Acetabulo-symphyseal pubic length: minimum distance from the superior and medial point of the pubic symphysis to the nearest point on the acetabular rim at the level of the lunate surface
SPU (Gaillard 1960)	Cotylo-pubic breadth: pubic breadth between the most lateral acetabular point and the medial aspect of the pubis.
DCOX (Brauer 1988)	Maximum pelvic height: measured from the inferior border of the os coxae to the most superior portion of the iliac crest.
IIMT (Brauer 1988)	Depth of the great sciatic notch: distance from the posterior-inferior iliac spine to the anterior border of the great sciatic notch.
ISMM (Schulter-Ellis et al 1983)	Post acetabular ischium length: distance from the most anterior and inferior point of the ischial tuberosity to the furthest point on the acetabular border.
SCOX (Brauer 1988)	Iliac breadth: distance between the antero-superior iliac spine and the postero superior iliac spine.
SS (Gaillard 1960)	Spino-sciatic length: minimum distance between the anterior inferior iliac spine and the deepest point in the greater sciatic notch.
SA (Gaillard 1960)	Spino auricular length: distance between the anterior inferior iliac spine

	and the auricular point.
SIS (Brauer 1988)	Cotylo-sciatic breadth: distance between the lateral border of the acetabulum and the midpoint of the anterior portion of the great sciatic notch.
VEAC (Brauer 1988)	Vertical acetabular diameter: maximum vertical diameter of the acetabulum, measured on the acetabular rim, as a prolongation of the longitudinal axis of the ischium.

Data were included in the DSP2 simple spreadsheet program to automatically calculate the probability of each specimen's being female or male. Comparisons between estimated and known sex were made afterward.

2.2 Statistical Analysis

There are no guidelines regarding the sample size necessary for a validation study of a sex estimation tool. However, the sample size was calculated by considering the following parameters: 5% standard error, 80% power, and a confidence level of 95%. Taking these parameters into consideration, as well as a possible loss due to exclusion criteria, the minimum sample size that would be necessary to satisfy these requirements was estimated to be 100 os coxae.

The measurement error analysis was made using three indicators frequently used in osteometric and anthropometric studies, which were proposed by Ulijaszek and Kerr [9]. The technical error of measurement (TEM), the relative technical error (% TEM) and the reliability coefficient (R) were calculated in order to evaluate the accuracy and reliability of measurements [10].

$$TEM = \frac{\sqrt{\sum_{i=1}^n d_i^2}}{2n} \quad (1)$$

$$\%TEM = \frac{TEM}{\bar{x}} \quad (2)$$

$$R = 1 - \frac{TEM^2}{\sigma^2} \quad (3)$$

Descriptive analyses were obtained for all measurements. The results were expressed as the mean and standard deviation. Statistical analyses were performed using Bioestat® (Mamirauá Institute - Conservação na Amazônia, Brazil). For each sample,

assumption of univariate normality was verified by the Kolmogorov-Smirnov test. Statistically significance differences between males and females for each measurement were evaluated by using the unpaired samples t- test. The significance level was set at $p < 0.05$.

3 RESULTS

Absolute TEM is interpreted as the typical magnitude of error associated with a measurement and was used to estimate intra-observer precision. The absolute TEM for intra-observer measurements ranged between 0.83 and 2.69 and for inter-observer measurements between 1.02 and 2.68. Relative TEM represents an estimate of error magnitude as a percentage of object size. The intra-observer relative TEM had values among 0.90 and 3.86, while inter-observer relative TEM values ranged from 1.38 to 3.86 (Table 3). The coefficient of reliability R represents the proportion of between-subject variance, free from measurement error. R ranges from 0 (not reliable) to 1 (complete reliability). The minimal value was 0.68, and the maximum value was 0.98.

Table 3. Intra and inter observer variation results for the calculation of the technical error of measurement (TEM).

	TEM intra (mm)	TEM inter (mm)	%TEM intra	%TEM inter	R intra	R inter
PUM	2.69	2.44	3.86	3.51	0.68	0.70
SPU	1.64	1.13	6.02	4.22	0.77	0.80
DCOX	2.59	2.19	1.23	1.06	0.97	0.98
IIMT	2.60	2.97	6.26	7.09	0.73	0.69
ISMM	2.14	2.06	1.94	1.86	0.92	0.93
SCOX	1.40	2.68	0.90	1.75	0.97	0.89
SS	0.83	1.01	1.14	1.38	0.98	0.97
SA	0.93	1.80	1.23	2.45	0.96	0.77
SIS	1.26	1.02	3.35	2.74	0.93	0.92
VEAC	1.92	1.17	3.18	1.99	0.84	0.89

Descriptive analysis for female and male os coxae, as well as Student's t-test results, are described in table 4. Means between sexes are different, except for PUM and SA measurements, which are less than 2 mm distinct.

Table 4. Descriptive statistics (by sex) for the os coxae variables in the Piracicaba sample.

Variables	n (F)	Mean (F)	SD (F)	Min (F)	Max (F)	n (M)	Mean (M)	SD (M)	Min (M)	Max (M)	P - value	T - value
PUM	50	70.14	5.46	55.00	90.70	52	69.09	5.05	60.00	79.40	0.1575	1.0099
SPU	50	24.62	3.49	18.55	39.10	53	28.62	3.29	21.40	38.30	<0.0001	-5.9873
DOCX	50	194.88	13.08	166.40	228.20	52	210.01	12.64	174.90	231.70	<0.0001	-5.9416
IIMT	49	47.94	7.11	31.40	72.50	52	42.47	6.25	29.80	61.70	<0.0001	4.1151
ISMM	50	101.93	7.37	88.50	120.70	52	112.22	6.59	92.40	126.00	<0.0001	-7.4389
SCOX	48	150.70	9.03	127.80	170.80	52	156.30	8.62	136.40	180.10	0.0010	-3.1736
SS	50	68.66	5.20	59.30	81.80	53	74.59	5.54	62.80	87.70	<0.0001	-5.5963
SA	50	74.62	5.12	65.00	88.80	53	76.06	6.34	63.20	90.10	0.1035	-1.2699
SIS	50	36.00	3.21	28.00	43.40	53	38.33	4.15	25.70	50.00	0.0010	-3.1852
VEAC	50	55.15	4.35	45.50	65.50	52	61.06	3.59	52.40	67.70	<0.0001	-7.4927

All p-values were considered statistically significant, demonstrating differences between females and males, except for the measurements PUM and SA.

The knowledge that PUM and SA measurements were not statistically significant led the present authors to excluded them in attempt to verify a different variable combination for our population. Besides, since individual's height was not take into account, we also tried to verify the results without DCOX measurements (Table 5). The DCOX variable is influenced by height, since is a sagittal measurement. The total classification sexing rates decreased, and the accuracy increased, but it was not enough to be considered significant. Since there was no succeed, the values discussed are considering all the 10 variables utilized in DSP2.

Table 5. Results with various combinations of variables

	% sexing total	% accuracy total	% sexing (F only)	% accuracy (F only)	% sexing (M only)	% accuracy (M only)
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10 variables	85.43%	88.34%	98.05%	86.00%	89.32%	90.57%
8 variables (without PUM and SA)	76.69%	92.23%	84.00%	90.00%	73.58%	92.45%
9 variables (without DCOX)	85.43%	89.32%	92.00%	86.00%	79.24%	92.45%
Best 4 vars (DCOX, PUM, SPU, IIMT)	82.52%	90.29%	92.00%	88.00%	73.58%	73.58%
Worst 4 vars (SIS, VEAC, SA, SS)	60.19%	93.20%	38.00%	82.00%	79.24%	100%

In comparing the results provided by the DSP2 and the real individual sex that the os coxae belonged, 5 (9.43%) errors were observed among males and 7 (14%) among females.

4 DISCUSSION

In forensic anthropology, there are two possible study outcomes: (a) enrichment of already accepted methods or (b) development of new ones [1], [10]. We believe that our study improved the utilization of DSP2. Originally, this method was designed to be non-population-specific [7] but did not take into account the possibility of sex estimation in miscegenated populations. DSP2 refers to a reference sample composed of a multiethnic population [8]. Nevertheless, none of the populations on their own display a miscegenation similar to that seen in Brazil, which occurred over centuries. Currently, mixed populations are particularly relevant due to globalization [5], and skeletons may be discovered in a specific country but belong to another population group area [5].

First, DSP2 provided excellent reproducibility with a 95% threshold [7], [8], demonstrating its usefulness in excluding any previous or prejudged attribution to a particular population. The authors of this study achieved their goal to validate the hypothesis that all of the different populations of modern humans, from Africa, Asia, North America and distinct parts of Europe, share a common pattern of sexual dimorphism in os coxae. In the present study, the DSP2 tool was also shown to be highly efficient and practical, as the assertiveness index was 90.57% for males and 86% for females. Accuracy and sexing classification was lower in the Bone Collection used, possibly because variables can behave distinctly when applied in different populations. Some other authors, such as Mestekova et al. [11] and Chapman et al. [5] that used the DSP on their study, also achieved distinct values. Mestekova et al. [11] have not found misclassifications as us and Bruzek et al. [8]. On the other hand, Chapman et al. [5] only found one specimen that could not had sex determined by DSP.

Comparatively, this is a much higher value than Bruzek et al. [8], Mestekova et al. [11] and our study. Besides, Mestekova et al. [11] found results even lower than ours for measurements that represent bone fragments simulations.

DSP2 was developed using different degree of admixture, as well as the Maxwell collection. The European genetic contribution to populations of African descent in the United States using nine autosomal DNA markers show that European genetic ancestry ranged from 6.8% to 22.5% (Parra et al. 1998). It is necessary to take into account the miscegenation of the Southeast Brazilian population, as there was mixing among European, African and Asiatic immigrants and the indigenous population already living in the country [6]. Reduction in homogeneity might be due to miscegenation among populations, leading to a decrease or variation of the peculiarities typical for a given population [3]. Albanese [4] states that race must be estimated before sex estimation, although recognizing that sex estimation methods that take into account race are limited and problematic. Small et al. [3], testing subpubic angle in South African populations, observed differences between same sex individuals belonging to different population groups in addition to the typical differences between males and females. Karakas et al. [10] agree that sexual dimorphism can be affected by a population's differences. Vacca and Di Vella [12] defend using a calibration method when determining the sex of a skeleton according to the variability of the populations to which it belongs.

It is also important to emphasize that the stature of the individuals was not used as a parameter and could lead to a confounding factor, as men tend to be larger than women and, consequently, to exhibit proportionally larger bone size. In fact, for the individuals for whom there was disagreement between the DSP and the real results, the DCOX measurement, defined as the maximum height measured from the inferior border of the os coxae to the most superior point of the iliac crest [7], had values that were discrepant of the mean presented by both men and women. In most cases, where a female was incorrectly classified as male, the DCOX values ranged from between 200.20 mm to 228.20 mm, which represents values almost 17.09% above the female mean for a DCOX measurement. In the male group, the DCOX values in specimens incorrectly assigned as females ranged between 174.90 mm to 211.45 mm, representing values 20.07% below average. Biwasaka et al. [13] states that differences are more pronounced in sagittal elements, such as the DCOX measurement. Additionally, Albanese [4], when trying to estimate the sex through distinct bones of the pelvis, observed that the males consistently classified as females were among the smallest males in every dimension.

Locating the reference regions for the measurements is notably simple. The difficulty is to reproduce an exact anatomical landmark for taking measurements, generating an inter and intra-observer error of several millimeters. However, this observed difference does not change the final results of the analysis.

This study clearly demonstrates that metric variation of the human pelvis is highly useful in sex estimation and reinforces the notion that pelvic sexual dimorphism is not population specific, with a broadly similar pattern of sexual dimorphism being observed across different populations, including those that are highly miscegenated.

5 CONCLUSION

The results revealed that DSP2 can be applied to a miscegenated population, as seen in the Brazilian population, which has a good index of assertiveness, although the assertiveness is lower than that of other bone collections already tested by DSP and DSP2 tools. The measurements PUM and SA did not project demonstrable differences between females and males that were statistically significant. The major divergent values were in the DCOX measurement, probably due to the influence of stature.

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3 CONCLUSÃO

Os resultados revelaram que o DSP2 pode ser aplicado a uma população miscigenada, como visto na população do sudeste brasileiro estudada, e que possui um bom índice de assertividade, embora a assertividade seja menor que a de outras coleções ósseas já testadas pelas ferramentas DSP e DSP2.

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Ref.: Ms. No. FSI-D-18-00032R3

Application and validation of Diagnose Sexuelle Probabiliste V2 tool in a
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Dear Professor Rossi,

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Thank you for submitting your work to this journal.

With kind regards

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27/11/2017

Comitê de Ética em Pesquisa - Certificado



COMITÊ DE ÉTICA EM PESQUISA
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CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Validação da ferramenta diagnóstica para determinação do sexo - DSP, no Brasil"**, protocolo nº 100/2015, dos pesquisadores Marcos Paulo Salles Machado, Ana Cláudia Rossi e Sarah Teixeira Costa, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 19/10/2015.

The Ethics Committee in Research of the Piracicaba Dental School - University of Campinas, certify that the project **"Validation of diagnostic tool for sex determining - DSP in Brazil"**, register number 100/2015, of Marcos Paulo Salles Machado, Ana Cláudia Rossi and Sarah Teixeira Costa, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee on Oct 19, 2015.

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Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
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