ANTHROPOLOGIE • XLIII/1 • pp. 77–85 • 2005

PETRA URBANOVÁ, VLADIMÍR NOVOTNÝ

DISTINGUISHING BETWEEN HUMAN AND NON-HUMAN BONES: HISTOMETRIC METHOD FOR FORENSIC ANTHROPOLOGY

ABSTRACT: In order to develop an identification key for distinguishing between human and non-human osteological samples, bone structure of several animal taxa was studied using quantitative microscopy. Both domestic and wild species were included in the sample, analysed at both micrometric (13 variable parameters) and macrometric (3 variable parameters) scales. The observed data was first used to evaluate inter- and intra-species diversity. The least determinative parameters were then eliminated via stepwise discriminant function analysis. The most discriminating micrometric properties of compact bone tissue appeared to be: number of osteons in 1 mm^2, maximum osteon diameter, maximum diameter and area of Haversian canal, and mid-shaft femoral cortical thickness.
Ultimately, two different equations for discriminating between human and non-human bone are formulated. The first type uses only histometric properties of bone structure. The second type of equations combines histometric measurement and grossest morphometric parameter – cortical thickness. The latter equations correctly predict taxonomic classification in 100% of cases.

KEY WORDS: Bone tissue – Histometry – Human vs. non-human origin – Image analysis – Discriminant function analysis

INTRODUCTION

Since Carl Linné first formulated his systematic classificatory nomenclature, *Homo sapiens*, the object anthropology as a science focuses on, has been established as an integral part of the animal kingdom sharing similar biological affinities with more or less related species. Yet, in practice and theory, various scientific disciplines regularly deal with problems of precise, valid and mainly reliable determination of biological samples. Likewise, distinguishing between human samples and samples of any other origin comes as a primary precondition for any anthropological expertise. Forensic anthropological investigations especially bear on this issue. Being of service to the legal system and criminalistics, this discipline unconditionally requires knowledge of the exact origin of recovered osteological remains.

Comparative samples of osteological materials are currently the forensic anthropologist's simplest and most reliable method for taxonomic determination. Main anatomical characteristics such as bone size and shape are considered principal attributes in evaluation. Nonetheless, many taxa share similar morphological structures and, together with the frequently fragmented condition of many bone samples, the discriminative capability of such characteristics may be compromised. Recently, the application of immunochemical or DNA analytical techniques have blossomed as a means of addressing these methodological problems, but they are becoming increasingly and commonly known to be over-sensitive to the biological integrity of the osteological materials studied (Bartlett, Davidson 1992, Cattaneo *et al*. 1992, Evison *et al*. 1997, Lee, Pagliaro 2000, Poetsch *et al*. 2001). What method, then, can be recommended to the analyst dealing

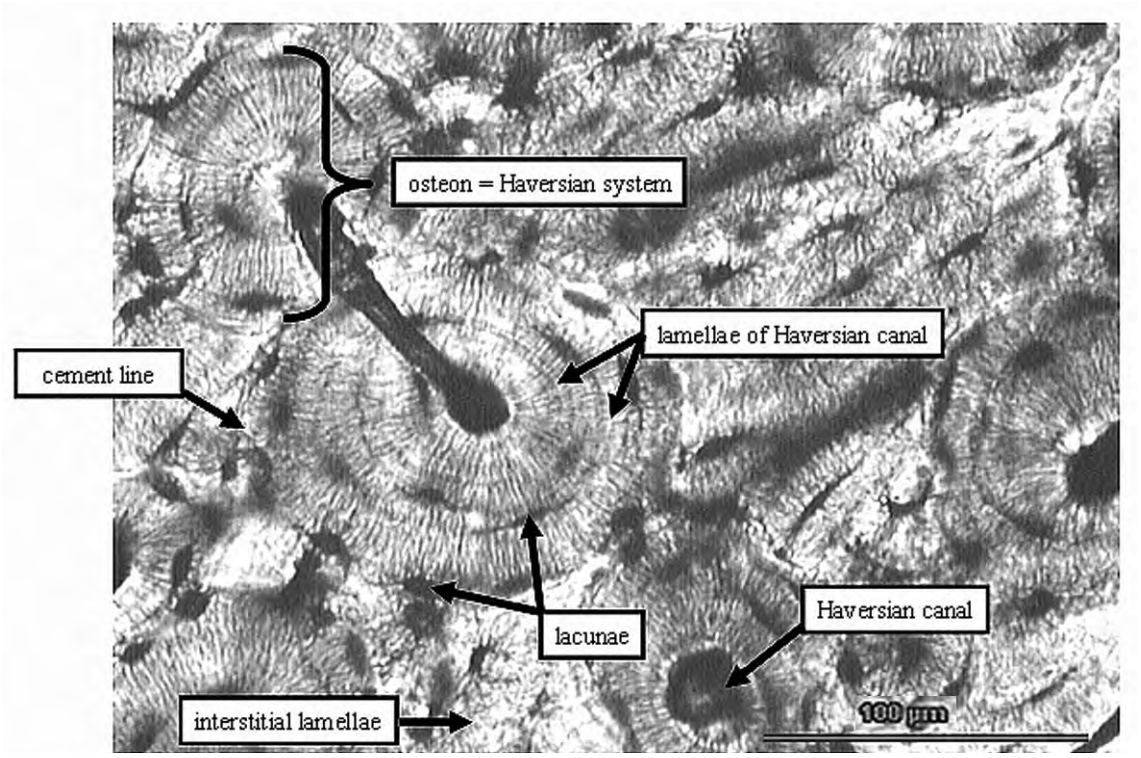


FIGURE 1. Cross section of tubular bone as appeared in a transmit light microscope.

with heavily degraded or cremated remains, when all previously mentioned techniques have been exhausted with little, if any, analytical utility? Can the question of taxonomic origin be resolved with the application of any other approach? Histological examination may provide an appropriate solution.

It could be said that with an increase in analytical resolution, there is a concomitant increase in the material, technical and financial requirements of analysis. Concerning this issue, however, histological methods may represent a "golden mean". Despite being falsely disregarded on supposed technical and material grounds, histology is an extremely useful resource in standard anthropological examination.

Our research had been initiated by contemporary needs of the Institute of Criminalistics in Prague, Czech Republic that had been left without an adequate identification key or suitable tables for distinguishing between human and non-human bone samples by using histological approaches. We had been asked to fill up the methodological vacuum.

Together with tooth enamel, bone is classified as the hardest tissue of the mammalian body. The bone tissue of an adult human male, as well as of many other mammals, generally occurs in two extreme forms: compact (cortical) bone tissue (*substantia compacta*) and cancellous (trabecular) bone tissue (*substantia spongiosa*). Compact bone forms mainly the shafts of long (tubular) bones, the surfaces of their extremities, short bones, and the outer and inner layer (*lamina externa et interna*) of the skull vault. Cancellous bone constitutes the internal parts of the long bone extremities (epiphyses) and the middle layer (diploë) of the skull vault. These structures, together with the organization of other, functionally dependent osseous components, differentiate each type of bone. Cancellous bone consists of a system of trabeculae and griddles arranged in the direction of biomechanical stress. Conversely, the functional and structural unit of compact bone is a secondary osteon or Haversian system. An osteon consists of concentrically arranged, nested lamellae, paralleling the longitudinal axis of a bone and running in long, drawn-out spirals or bending into curves. The resistance of bone to stress is determined by the orientation of osteons as well as on biomechanical properties of the bone itself. In the central portion of each osteon, concentric lamellae enclose a so-called Haversian canal (*canalis centralis*). The canal is composed of a vascular and lymphatic bundle running simultaneously with nerve fibers. Bone cells (osteocytes, osteoblasts) in lacunae occupy the spaces or gaps between lamellae. Cement line, ring of highly mineralized amorphous substances, separates the Haversian system from its surroundings. These surroundings consist of interstitial lamellae representing relicts of older lamellae previously incorporated into one of the regular osteons or primary bone (non-Haversian bone occurring in the development). Both components contribute to the formation of an integrated structure which is delimited in the inner (endostal) and outer (periostal) surfaces of the bone by a third system of lamellae, called inner (outer) circumferential lamellar systems.

In forensic anthropology, and anthropology in general, histological methods have been most useful as a means of estimating age at death (Bouvier, Ubelaker 1977, Ericksen

TABLE 1. List of micrometric parameters included in the study.

Parameter	Acronym
Number of secondary osteons[1] in 1mm^2	NO
Minimum osteon diameter	DO_{min}
Maximum osteon diameter	DO_{max}
Osteon area	AO
Osteon perimeter	PO
Minimum Haversian canal diameter	DHC_{min}
Maximum Haversian canal diameter	DHC_{max}
Haversian canal area	AHC
Haversian canal perimeter	PHC
Minimum osteon and Haversian canal diameter ratio	Rt_{min}
Maximum osteon and Haversian canal diameter ratio	Rt_{max}
Feret diameter[2] of osteon	FO
Feret diameter of Haversian canal	FHC

[1] Secondary osteons are here defined as objects roughly circular in shape, which do not show any signs of resorption and which are presented as complete in the visual field.

[2] Feret diameter can be specified as the theoretical diameter of the object as if it were circular in shape.

1991, Kerley 1965, Singh, Gunberg 1970), detecting pathological conditions as expressed in bone tissue (Schultz 2001, Stout, Gehlert 1979), and most recently in determining the degree of bone preservation as an indicator estimating the amplificability of DNA from osteological materials (Cipollaro *et al.* 1998, Guarino *et al.* 2000, Kolman, Tuross 2000).

Nevertheless, the application of histological methods based exclusively on metric characteristics of compact bone tissue for distinguishing between human and non-human origin of samples is quite a controversial topic. Ever since the first publication of papers dealing with histological observations of inter-species differences (Keneyres, Hegyi 1903 cited by Hunger, Leopold 1978) the scientific community has been divided into several branches. These represent both a principled rejection (Kernbach 1925) as well as an acceptation of the morphological and metric parameters of the method (Goldbach, Hinüber 1955, Rämsch, Zerndt 1963). More recently, plexiform bone has been considered to be a general determinant of non-human bone tissue (Owsley *et al.* 1985). Similarly, Haversian canals with diameters less than 50 μm refer to non-human origin (Sauer, Lackey 2000).

MATERIAL

The analysed sample consisted of 53 human bones (45 femora and 8 tibiae) and bones from 10 animal taxa. Osteological material from both wild and domestic living species of animals were included in the sample: ox (*Bos taurus*), horse (*Equus caballus*), dog (*Canis familiaris*), sheep (*Ovis aries*), pig (*Sus scrofa domestica*), Euroasian wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), European roe deer (*Capreolus capreolus*) and two species of birds for comparison with mammals, domestic turkey (*Meleagris gallopavo*) and domestic fowl (*Gallus gallus*). These taxa were chosen in order to cover the range of animals for which the analyst might encounter taxonomic ambiguity when compared to human samples (i.e., of various age categories, such as mature, sub-adult/juvenile or foetal) and in accordance with the criterion of the Institute of Criminalistics in Prague (e.g., cases of suspected poaching). The osteological material sampled was obtained from anatomical autopsies and then macerated (i.e., manually cleaned of adhering soft tissue, degreased in acetone, and bleached in a 10% solution of hydrogen peroxide). Archaeological bones were also included in the sample.

METHODS

With a metallurgical saw, cross-section wafers of approximately 0.5 cm were removed from anterior, posterior respectively, mid-shaft portions of the femora and tibiae. Neither determination nor emphasis was given to the anatomical side form, which a bone was derived. The wafers were dehydrated in 70%, 90% and 98% ethanol solution and then in acetone, for 8 hours each. Undecalcified thin-sections were prepared manually by grinding (carbide sandpaper with 220, 600 and 2000 grains) and polishing to a final thickness of approximately 80 μm. The thin-sections were then analysed in a standard transmit light microscope. The microscopic views were digitised using a JVC TK-C1381 black and white camera and measurements were accomplished in PC. Aware of possible structural variation from the endosteal to the periosteal border, the entire surface of each cross-section was considered in analysis. Sigma Scan Pro Version 5.0 image analysis software was used for automatic measurement parameters of 3,297 objects (1,381 secondary osteons and 1,916 Haversian canals). The parameters included in measurement and evaluation are shown in *Table 1.*

Apart from the previously mentioned micrometric measurements, bone mid-shafts were also evaluated macrometrically. Anterior-posterior (AP) diameter, mediolateral (ML) diameter, and cortical thickness (CT) were determined for each.

The acquired data was first analysed to demarcate inter- and intra-species variability within the sample. Next, stepwise discriminant function analysis, a method of multidimensional statistics, was used to identify the most discriminating histometric variables of cortical bone, eliminate predictor variables that poorly characterize differences among taxa, and ultimately to develop an identification key for distinguishing between human and non-human osteological samples. Wilks' *Lambda* (Wilks' λ) was applied to point out to the significance of each variable in predicting the origin of the sample. The significance of all statistics was evaluated at the $p \leq 0.05$ or 5% level.

TABLE 2. Summary statistics for parameters of Haversian canals.

Taxon	Maximum diameter			Minimum diameter			Perimeter			Area			Feret diameter		
	Mean	S.E.	Median	Mean	S.E.	Median	Mean	S.E.	Median	Mean	S.E.	Median	Mean	S.E.	Median
Homo sapiens sapiens	68.73	1.10	62.55	47.15	0.77	42.72	196.73	3.01	178.16	2877.37	90.68	2089.84	56.89	0.85	51.58
Bos taurus	42.56	1.38	42.03	30.99	1.16	28.82	123.41	4.01	119.58	1176.37	79.35	957.03	36.58	1.18	34.91
Equus caballus	45.12	1.17	41.69	29.37	0.76	26.88	128.93	3.10	119.95	1213.83	69.53	934.04	37.03	0.85	34.49
Sus scrofa domestica	36.18	1.37	35.15	26.23	1.39	24.50	106.03	4.28	101.58	826.45	66.88	717.77	31.40	1.24	30.23
Sus scrofa	32.36	1.24	31.67	23.36	0.92	22.44	95.02	3.35	93.09	672.01	47.99	568.17	27.81	0.97	26.90
Capreolus capreolus	23.72	0.92	21.36	15.13	0.63	14.67	69.34	2.38	61.82	327.58	23.08	253.96	19.65	0.64	17.98
Cervus elaphus	24.97	0.74	25.07	17.80	0.58	16.73	73.75	2.08	73.77	409.35	22.26	383.09	22.01	0.59	22.09
Canis familiaris	34.42	0.68	32.17	21.11	0.40	19.57	98.23	1.79	92.59	694.37	26.03	537.11	27.81	0.47	26.15
Ovis aries	31.76	1.14	27.95	18.36	0.60	16.83	88.55	2.97	79.43	574.13	38.44	408.91	25.21	0.76	22.82

TABLE 3. Summary statistics for parameters of osteons.

Taxon	Maximum diameter			Minimum diameter			Perimeter			Area			Feret diameter		
	Mean	S.E.	Median	Mean	S.E.	Median	Mean	S.E.	Median	Mean	S.E.	Median	Mean	S.E.	Median
Homo sapiens sapiens	263.91	3.89	254.58	206.44	3.04	197.80	837.59	13.92	783.01	44119.88	1301.64	37709.96	227.25	3.24	219.12
Bos taurus	238.46	8.07	225.40	181.49	5.65	175.28	698.71	22.86	673.22	36067.23	2951.58	28647.53	202.79	6.30	190.98
Equus caballus	238.50	5.17	224.69	183.74	4.16	174.32	700.80	15.02	677.12	35506.87	1602.37	30287.48	204.65	4.39	196.37
Sus scrofa domestica	232.26	11.95	234.24	180.72	9.88	183.81	681.48	34.49	700.44	33118.87	3239.81	32616.13	200.50	10.18	203.77
Sus scrofa	207.78	8.23	208.11	162.56	5.92	157.41	610.24	22.76	614.69	27168.05	1907.80	24022.51	179.05	6.55	174.81
Cervus elaphus	110.11	2.75	104.68	85.01	2.10	84.54	321.99	7.72	310.25	7410.84	357.90	6695.40	95.25	2.26	92.33
Capreolus capreolus	127.72	3.37	126.40	100.96	2.51	101.74	514.30	13.09	507.36	9900.04	455.86	9577.15	109.76	2.68	110.43
Canis familiaris	151.59	2.35	146.48	117.15	1.74	113.24	444.74	6.48	431.59	14034.94	405.39	12607.42	130.39	1.81	126.70
Ovis aries	169.66	3.50	166.09	123.79	2.42	120.06	486.09	9.28	474.59	16457.60	599.95	14867.19	141.11	2.60	137.58

RESULTS

Individual, intra- and inter-species variability

The variable parameters of 1,381 secondary osteons and 1,916 Haversian canals were measured. As expected, the results show great variability in analysed characteristics for each taxon. Individual variability of the measured parameters can be considered relatively uniform. With few exceptions, no statistically significant differences were recognized at the level of a single bone specimen, neither among sectors of a thin-section nor between anterior and posterior portions of mid-shafts. Only a single horse bone (specimen number 38) and a bone from ox (specimen number 20) revealed significant differences for both secondary osteons and Haversian canals at anterior and posterior surfaces of the femur. Furthermore, except for an ox, a comparison between an individual's femur and tibia did not expose significant differences.

Although no significant differences attached to sex were detected, insufficient documentation of age, sex and clinical history unfortunately prohibited satisfactory evaluation of intra-species variability. Regardless, analysis did reveal significant differences between individuals with mature secondary osteon tissue and those with osteon tissue only, randomly distributed in preserved primary bone. Inter-species analysis confirmed the suspected existence of similarities among some of the taxa analysed for all measured parameters. Consequently, following these results, studied taxa were initially partitioned into 5 groups. The first group consists only of human samples, while the others are comprised of all other animal taxa, arranged as follows:

Group 1: *Homo sapiens*
Group 2: *Bos taurus, Equus caballus*
Group 3: *Sus scrofa domestica, Sus scrofa*
Group 4: *Ovis aries, Canis familiaris*
Group 5: *Capreolus capreolus, Cervus elaphus*

Subsequent analysis, however, obliged the fusion of groups 4 and 5, so that both roe deer and red deer occur in the same group as sheep and dog.

Summary statistics for each taxon are showed in *Tables 2, 3,* and *4*. Human samples show the highest value for both Haversian canals and osteons and the lowest value for number of secondary osteons in 1 mm^2. Conversely, the parameters for red deer show an inverse relationship to human samples.

Macrometric evaluation of femoral mid-shafts revealed the mean values of AP diameter and ML diameter for human adult individuals to be 28.60±0.89 mm and 27.58±0.89 mm, respectively. Our collection of animal bone exposed comparable measurements in two taxa – red deer (AP: 33.21 mm; ML: 28.08 mm) and domestic pig (AP: 28.03±0.30 mm; ML: 25.59±0.34 mm). All other taxa clustered into a group with markedly lower values (sheep, dog, roe deer, and pig/boar), or attained values almost twice as large as those for human femora (ox, horse). The third parameter observed, CT, revealed common femoral characteristics among humans (5.55±0.34 mm) and pigs (5.08±0.18 mm) only. As expected, the bones of juvenile and neonate individuals resembled those of birds (domestic turkey and domestic fowl) in all three macrometric parameters.

TABLE 4. Summary statistics for number of osteons in 1 mm^2.

Taxon	Mean	S.E.	Median
Homo sapiens sapiens	4.00	0.18	4.00
Bos taurus	9.61	0.63	9.00
Equus caballus	14.06	5.46	9.00
Sus scrofa domestica	7.00	0.58	7.00
Sus scrofa	8.17	0.95	8.00
Canis familiaris	25.58	7.02	20.00
Ovis aries	31.17	14.39	18.00
Cervus elaphus	26.00	0.58	26.00
Capreolus capreolus	18.67	1.67	17.00

Discriminant function analysis

In performing the stepwise discriminant analysis for the elimination of least discriminative parameters and the development of an identification key for determining the origin of the sample, all previously acquired data had to be abandoned. Instead, the median values measured for each thin-section were subjected to discriminant function analysis.

In the first step of the stepwise analysis, only micrometric variables were included. The greatest predictive histometric properties appeared to be: number of osteons in 1 mm^2 (NO), maximum diameter of Haversian canal (DHC_{max}) and area of Haversian canal (AHC) (*Table 5*). In conclusion, four classification equations were formulated. The greatest classification score calculated determines the origin of the sample. The equations predicted correct classification in 94% of cases.

$$S_1 = -107.178 + 1.931 \times NO + 6.014 \times DHC_{max} - 0.078 \times AHC$$

$$S_2 = -91.442 + 2.498 \times NO + 5.450 \times DHC_{max} - 0.072 \times AHC$$

$$S_3 = -63.284 + 2.098 \times NO + 4.523 \times DHC_{max} - 0.060 \times AHC$$

$$S_4 = -91.645 + 4.001 \times NO + 4.674 \times DHC_{max} - 0.061 \times AHC$$

In addition to micrometric parameters, the second step incorporated the cortical thickness (CT) of the mid-shaft of femur. In this step, number of osteons in 1 mm^2 (NO), maximum diameter of Haversian canal (DHC_{max}), area of Haversian canal (AHC), maximum osteon diameter (DO_{max}) and cortical thickness (CT) appeared to be the most discriminative variable parameters (*Table 6*). As in the previous case, four classification equations with the selected parameters were formulated. These equations predicted correct classification in 100% of cases.

TABLE 5. Summary statistics of discriminant function analysis for the parameters included in the model.

Parameter	Wilks' λ	F-remove	p-level
Haversian canal area	0.10762	6.26425	0.00181
Maximum Haversian canal diameter	0.12105	8.37802	0.00030
Number of osteons in 1mm^2	0.19967	20.74597	0.00000

TABLE 6. Summary statistics of discriminant function analysis for the parameters included in the model.

Parameter	Wilks' λ	F-remove	p-level
Maximum osteon diameter	0.009223	4.08482	0.015178
Haversian canal area	0.009907	5.12955	0.005537
Maximum Haversian canal diameter	0.011283	7.23147	0.000862
Number of secondary osteons in 1mm^2	0.013398	10.46146	0.000071
Cortical thickness	0.061466	83.86802	0.000000

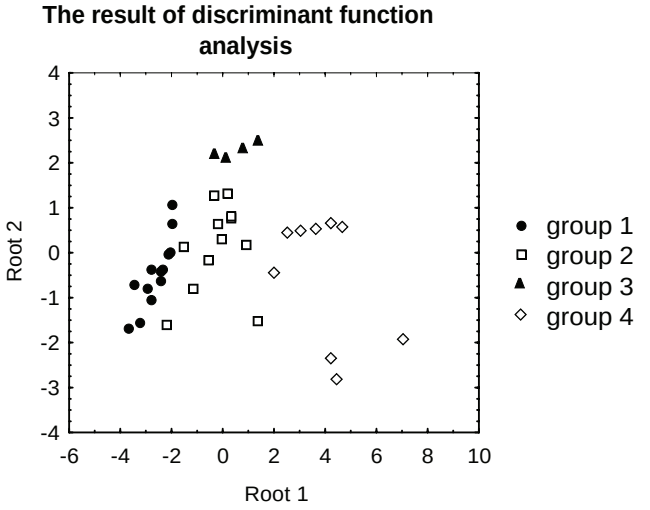


FIGURE 2. Scatterplot of canonical scores for the first type of discriminant function.

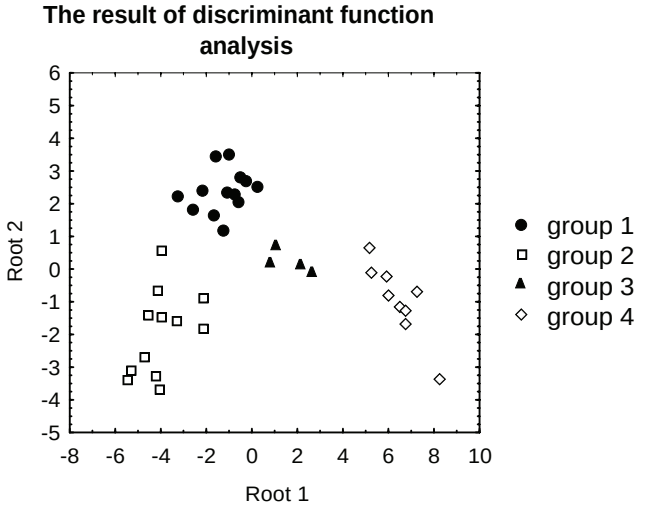


FIGURE 3. Scatterplot of canonical scores for the second type of discriminant function.

S_1=–216.780+0.638×DO_{max}+4.150×NO+6.453×DHC_{max}–
–0.091×AHC+0.009×CT

S_2=–264.731+0.760×DO_{max}+4.753×NO+6.011×DHC_{max}–
–0.087×AHC+0.013×CT

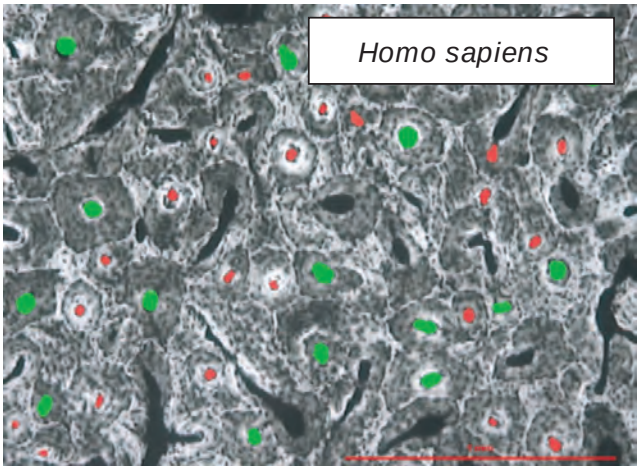
S_3=–156.635+0.602×DO_{max}+4.376×NO+4.919×DHC_{max}–
–0.072×AHC+0.007×CT

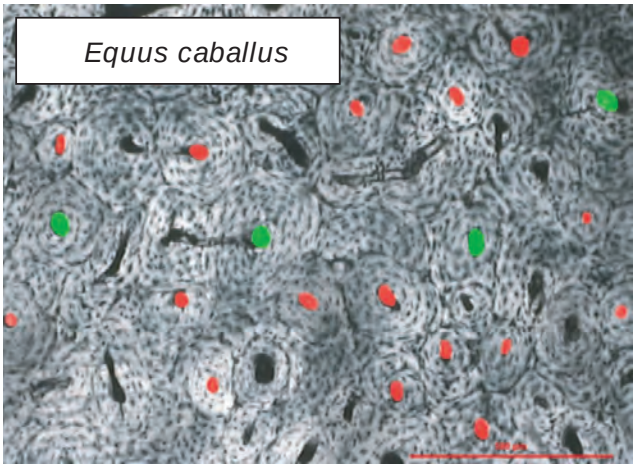
S_4=–160.953+0.530×DO_{max}+6.382×NO+4.984×DHC_{max}–
–0.071×AHC+0.005×CT

DISCUSSION

Histomorphometrics has had a long tradition in anthropology. Yet, the perceived utility of this method for distinguishing between human and non-human bones has been impeded by various prevailing prejudices and intellectual biases which still exist today, as well as methodological misunderstandings and shortcomings that inhibit its application in practice. One of the major "fossilized" errors in practical application has been the established assumption of considering a Haversian canal diameter value of 50 μm as a borderline criterion for distinguishing between humans and other animals. *Figure 4* illustrates the apparent fact that using this single determinative variable alone is rather misleading. Although commonly considered methodologically acceptable or tolerable, especially in legal investigations, the presumed Haversian canal diameter borderline is clearly shown here to be exceeded by human bones.

Nonetheless, the authors do agree with previous researchers that Haversian canal dimensions do, in fact, reflect the taxonomic origin of bone. Additionally, however, Haversian canal area also appears to have similar potential





category	N	mean
< 50 µm	23	36.24
> 50 µm	17	64.78

category	N	mean
< 50 µm	19	38.33
> 50 µm	4	50.36

NO	5
DHC_{max}	64.3477
AHC	2311.43

S_1	109.1725
S_2	105.9820
S_3	99.7843
S_4	87.8863

NO	14
DHC_{max}	44.7432
AHC	1166.992

S_1	87.9162
S_2	103.6863
S_3	98.5536
S_4	102.1901

FIGURE 4. Scheme of evaluation of human and non-human sample by using 50 µm of the Haversian canal diameter for a borderline between animals and humans (upper line) and by classification equations (lower line).

as a discriminative variable. In accord with Rämsch, Zerndt (1963), we assert that the number of secondary osteons in a visual field (in our study in $1mm^2$) represents a strong discriminative parameter.

Recently, one can detect a new trend to combine micrometric and morphometric approaches in an attempt to increase the accuracy of conventional methods (e.g., Thomas *et al.* 2000). In this vein, a cortical thickness parameter was included in this analysis. CT measurements provide applicability of fragmentary osteological material, so common in forensic and archaeological contexts. CT appeared to be a very strong predictive discriminating variable. Yet, when applied to fragmented bone it could potentially be affected by numerous taphonomic factors as well as dependent upon many circumferences, such as changes caused by the biological background of the individual (Bertelsen *et al.* 1995, Ruff 1984).

Together with CT, maximum osteon diameter also appears to be relatively important for distinguishing between the taxonomic groups constructed in this analysis.

Distinguishing between human and animal bone has always been the primary goal of histomorphometric investigations similar to that performed here. With the current increasing demands in forensic science for greater analytical reliability and resolution, it has become even more so important to thoroughly specify the taxonomic classification of recovered remains. Following the results of the inter-species evaluation performed in this analysis, individual taxa were studied as representatives of four major groups. However, it cannot at this time be concluded as to whether these similarities definitively correspond to taxonomic affinities or to similar physical proportions (weight bearing stress, locomotor patterns, bone robusticity, etc.). Consequently, group 4 consists of both domestic and wild living taxa of various taxonomic sub-groups (e.g., carnivores and even-toed ungulates). Yet, the wild living species of this group, red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), can be easily distinguished from group 2 which is composed of domestic animals.

Macrometric evaluation of the bones studied revealed a metric similarity between the bones of an adult human and those of pig. This finding should not come as a surprise as it is not uncommonly known even outside scientific circles. Even Pardoner, a character from one of Chaucer's Canterbury Tales, mistook pig bones as being the remains of saints.

Biostatistical methods addressing problems of quantitative evaluation of bone structure have not been a common part of histological examinations dealing with the taxonomic origin of osteological samples. Only a single

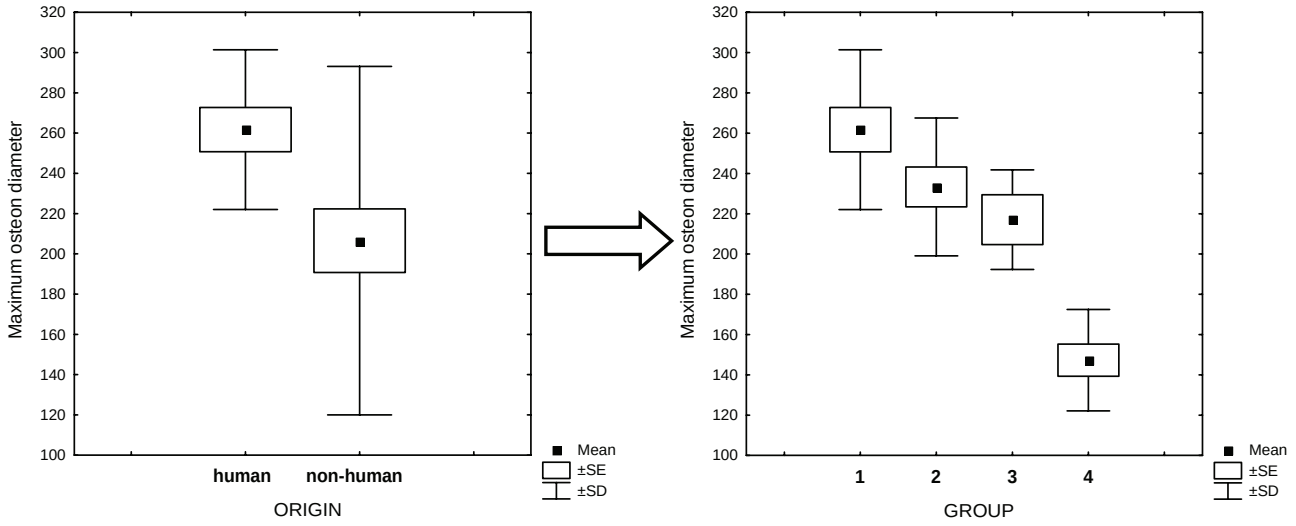


FIGURE 5. Box-whiskers of micrometric parameter according to origin of specimens (left) and according to the formed groups of taxons (right).

paper recently published by Cattaneo *et al*. (1999) formulated a canonical equation for the separation of humans and other non-human animals. But it should not be considered a practical guide for evaluating the taxonomic classification of osteological remains because the authors do not provide specific guidelines for the measurement of bone microstructure (e.g., how many objects should be measured or what kind of values should be put into the equation). In contrast, our paper is strictly orientated to the practical side of the problem. We suggest a "recipe" that could be followed by the investigator in the laboratory, and which has been shown to work with considerable accuracy. In order to eliminate mistakes associated with automatic measurement to the highest possible degree, mean statistics were abandoned in favour of the statistical median. This decision allowed for the elimination of exceptions in bone structure and/or mistakes in measurement and computer analysis that may occur in practice. In the equations formed, then, the investigator does not count with mean values but with median values.

Cattaneo *et al*. (1999) constructed one discriminant canonical equation for the separation of human samples from samples of any other origin. We implemented and suggest an alternative method. *Figure 5* shows the mean and variation around the mean of the measured parameter (DO_{max}) of human origin and those of all animal taxa in the study. When comparing the box plot on the left with that on he right, it is clear that the substantial discrepancy in degree of variability between human and non-human animals is greatly diminished with the sub-division of all non-human taxa into groups 2, 3, and 4. Furthermore, this decision opens up the possibility for new, previously mentioned methodological applications in the forensic sciences.

CONCLUSION

In this analysis, a practical identification key for distinguishing between human and non-human bone remains was formulated. The methods outlined may be applied to various disciplines of scientific inquiry, such as forensic anthropology, zooarchaeology, human paleontology (or paleoanthropology), and zoology. Application of this identification key requires the execution of seven compulsory stages of analysis:

1. Measurement of cortical thickness (CT), when degree of sample fragmentation permits.
2. Preparation of undecalcified thin-section.
3. Digitisation of microscopic view (at least 3 images recommended, in order to include the entire surface of a cross-section, from the endosteal to the periosteal border) and labelling of images with calibrated scale.
4. Establishment of required variable parameters (number of secondary osteons in 1 mm^2, maximum osteon diameter, maximum diameter and area of Haversian canal).
5. Calculation of each parameter median.
6. Calculation of score for each classification equation.
7. Comparison of the acquired data:
   a) if $S_1 > S_2$, S_3 and S_4 then the bone or the fragment belongs to an individual of **human** origin
   b) if $S_1 < S_2$ or S_3 or S_4 then the bone or the fragment belongs to an individual of **non-human** origin
   c) the index of the highest classification score reflects taxonomic group of individual.

ACKNOWLEDGEMENTS

We would like to thank the head and employees of the Institute of Anatomy, Faculty of Medicine, Masaryk University in Brno and the Department of Pathological Morphology, Veterinary and Pharmaceutical University in Brno, for the generous accessibility of the osteological material studied.

REFERENCES

BARTLETT S. E., DAVIDSON W. S., 1992: FINS (Forensically Informative Nucleotide Sequencing): A Procedure for identifying the animal origin of biological specimens. *Biotechniques* 12: 408–411.

BERTELSEN P. K., CLEMENT J. G., THOMAS C. D. L., 1995: A morphological study of the cortex of the human femur from early childhood to advanced old age. *Forensic Science International* 74: 63–77.

BOUVIER M., UBELAKER D., 1977: A comparison of two methods for the microscopic determination of age at death. *Amer. J. of Phys. Anthrop.* 46: 391–394.

CATTANEO C., DIMARTINO S., SCALI S., CRAIG O. E., GRANDI M., SOKOL R. J., 1999: Determining the human origin of fragments of burnt bone: A comparative study of histological, immunological, and DNA techniques. *Forensic Science International* 102: 181–191.

CATTANEO C., GELSTHORPE K., SOKOL R. J., 1992: Reliable identification of human albumin in ancient bone using ELISA and monoclonal antibodies. *Amer. J. of Phys. Anthrop.* 87: 366–372.

CIPOLLARO M., DI BERNARDO G., GALANO G., GALDERISI U., GUARINO F., ANGELINI F., CASCINO A., 1998: Ancient DNA in human bone remains from Pompeii archaeological site. *Biochemical and Biophysical Research Communications* 247: 901–904.

ERIKSEN M. F., 1991: Histological estimation of age at death using the anterior cortex of femur. *Amer. J. of Phys. Anthrop.* 84: 171–179.

EVISON M. P., SMILLIE D. M., CHAMBERLAIN A. T., 1997: Extraction of single-copy nuclear DNA from forensic specimens with variety of postmortem histories. *J. of Forensic Sciences* 42: 1032–1038.

GOLDBACH H. J., HINÜBER H., 1955: Versuch einer Systematik der Formelemente des Säugetierknochens. *Deutsche Zeitschrift für die Gesamte Gerichtliche Medizin* 44: 578–588.

GUARINO F. M., ANGELINI F., ODIERNA G., BIANCO M. R., DI BERNARDO G., FORTE A., CASCINO A., CIPOLLARO M., 2000: Detection of DNA in ancient bones using histochemical methods. *Biotechnic and Histochemistry* 75: 110–117.

HUNGER H., LEOPOLD D., 1978: *Identifikation*. Johann Ambrosius Barth, Leipzig. 520 pp.

KENYERES B., HEGYI M., 1903: Unterscheidung des menschlichen u. tierischen Knochengewebes. *Vjschr. gerichtl. Med*. 25: 225–232.

KERLEY E. R., 1965: The microscopic determination of age in human bone. *Amer. J. of Phys. Anthrop.* 23: 149–164.

KERNBACH M., 1925: *Étude sur l'identification des os*. Cartea Românească, Bucuresti. 135 pp.

KOLMAN C. J., TUROSS N., 2000: Ancient DNA analysis of human populations. *Amer. J. of Phys. Anthrop.* 111: 5–23.

LEE H. C., PAGLIARO E. M., 2000: Serology: Blood identification. In: J. A. Siegel, P. J. Saukko, G. C. Knupfer (Eds.): *Encyclopaedia of Forensic Sciences*. Pp. 1331–1338. Academic Press. A Hartcourt Science and Technology Company, online version, www.academicpress.com/forensic/forline.htm, 7. 3. 2002.

OWSLEY D. W., MIRES A. M., KEITH M. S., 1985: Case involving differentiation of deer and human bone fragments. *J. of Forensic Sciences* 28: 572–578.

POETSCH M., SEEFELDT S., MASCHKE M., LIGNITZ E., 2001: Analysis of microsatellite polymorphism in red deer, roe deer, and fallow deer – possible employment in forensic applications. *Forensic Science International* 116: 1–8.

SCHULTZ M., 2001: Paleohistopathology of bone: A new approach to the study of ancient diseases. *Yearbook of Phys. Anthrop.* 44: 106–147.

SINGH I. J., GUNBERG D. L., 1970: Estimation of age at death in human males from quantitative histology of bone fragments. *Amer. J. of Phys. Anthrop.* 33: 373–382.

STOUT D. S., GEHLERT S. J., 1979: Histomorphological identification of individuals among mixed skeletons. *Curr. Anthrop.* 20: 803–805.

RÄMSCH R., ZERNDT B., 1963: Vergleichende Untersuchungen der Havers'schen Kanäle zwischen Menschen und Haustieren. *Archiv für Kriminologie* 131: 74–82.

RUFF C. B., 1984: Allometry between length and cross-sectional dimensions of the femur and tibia in Homo sapiens sapiens. *Amer. J. of Phys. Anthrop.* 65: 347–358.

SAUER N. J., LACKEY M. W. L., 2000: Anthropology: skeletal analysis. In: J. A. Siegel, P. J. Saukko, G. C. Knupfer (Eds.): *Encyclopaedia of Forensic Sciences*. Pp. 261–270. Academic Press. A Hartcourt Science and Technology Company, online version, www.academicpress.com/forensic/forline.htm, 7. 3. 2002.

THOMAS C. D. L., STEIN M. S., FEIK S. A., WARK J. D., CLEMENT J. G., 2000: Determination of age at death using combined morphology and histology of the femur. *J. of Anatomy* 196: 463–471.

Petra Urbanová
Vladimír Novotný
Department of Anthropology
Faculty of Science
Masaryk University in Brno
Vinařská 5
603 00 Brno, Czech Republic
E-mail: urbanova@sci.muni.cz
E-mail: vnovotny@sci.muni.cz