VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 72, No. 3, pp. 239–248
DOI: 10.5603/FM.2013.0039

ISSN 0015–5659
www.fm.viamedica.pl

Histomorphometric, fractal and lacunarity comparative analysis of sheep (*Ovis aries*), goat (*Capra hircus*) and roe deer (*Capreolus capreolus*) compact bone samples

A.I. Gudea[1], A.C. Ștefan[2]

[1]*Department of Comparative Anatomy, Faculty of Veterinary Medicine, Cluj Napoca, Romania*
[2]*Department of Pathophysiology, Faculty of Veterinary Medicine, Cluj Napoca, Romania*

[Received 15 January 2013, Accepted 25 February 2013]

*Quantitative and qualitative studies dealing with histomorphometry of the bone tissue play a new role in modern legal medicine/forensic medicine and archaeozoology nowadays. This study deals with the differences found in case of humerus and metapodial bones of recent sheep (*Ovis aries*), goat (*Capra hircus*) and roe deer (*Capreolus capreolus*) specimens, both from a qualitative point of view, but mainly from a quantitative perspective. A novel perspective given by the fractal analysis performed on the digital histological images is approached. This study shows that the qualitative assessment may not be a reliable one due to the close resemblance of the structures. From the quantitative perspective (several measurements performed on osteonal units and statistical processing of data), some of the elements measured show significant differences among 3 species (the primary osteonal diameter, etc.). The fractal analysis and the lacunarity of the images show a great deal of potential, proving that this type of analysis can be of great help in the separation of the material from this perspective.* (Folia Morphol 2013; 72, 3: 239–248)

Key words: microscopic structure of bone, lacunarity, fractals, legal medicine, archaeozoology

INTRODUCTION

The difference at the level of the microstructure of the bone is a subject that has been approached quite a long time ago. There have been studies that deal with the differences at this level starting from the late 1950s [11–13, 15, 17] and continue in the last period with more detailed studies concerning microstructural differences among humans and animals or among different species [4–8, 20–24, 28].

This type of approach is a consequence of the new perspectives opened by the forensic and legal medicine investigations or the archaeozoological demands of the bone studies. Most of these studies focused on the distinction between human and nonhuman bony material (even trying to provide mathematical formulae for the distinction of fragments of bone of human and nonhuman origin material [2] or onto the distinction among some groups of species). As far as we know, there are few attempts in differentiating related species on the basis of the microhistomorphometric data [9, 10, 19]. A more recent work tries to gather several data in order to give a better view over the histomorphometric available data, being in fact a collection of the scientific histological methods

Address for correspondence: A.I. Gudea, Department of Comparative Anatomy, Faculty of Veterinary Medicine, Cluj Napoca, 3-5 Manașstur str. 400372, Romania, tel: 040 0264 596 384, fax: 040 0264 593792, e-mail: alexandru.gudea@usamvcluj.ro, stefan.andrei.vet@gmail.com

used in conjunction with bones, mainly from the anthropological perspective [3].

Most of the previously-mentioned studies focus on 2 distinctive aspects of the bone — the qualitative aspects (characterisation of the bone tissues, the frequencies of the Haversian systems, etc.) or on the quantitative aspects (primary and secondary osteonal features). We also tried to see to what extent the fractal analysis (a method for quantifying the degree of complexity) and lacunarity (a method for quantifying the emptiness) can be used in conjunction with the other methods in order to distinguish pattern characteristics between 3 types of bone samples.

Fractal analysis and lacunarity represent powerful tools for describing quantitatively types of biological systems [16, 27, 29]. The fractal dimension gives a number for how a structure fills up the space, being an indicator of the boundary irregularity and roughness [30]. The lacunarity values indicate the degree of gap distribution over a certain surface [25, 31].

To the best of our knowledge, the differential aspects regarding fractal analysis and lacunarity and their potential for the dissociation of the species have not yet been investigated.

Therefore, in the present study we have addressed the potential of the previously-mentioned methods in the respect of species differentiation.

MATERIALS AND METHODS

Preparation of the materials

The studied material consisted of the bone fragments originating from recent sheep, goat and roe deer bone specimens. The most of the sheep (*Ovis aries*) specimens used in our study originate from the collection of the Comparative Anatomy Department of the Faculty of Veterinary Medicine of Cluj Napoca, Romania, from individuals with known age and gender. The individuals originate mostly from Zackel-type local breeds. The goat (*Capra hircus*) bone specimens were collected throughout a period of 1 year from some local owners that slaughtered mature individuals of common unimproved breed for their own personal consumption. The roe deer (*Capreolus capreolus*) specimens used in this study originate from regional Hunter's Association members that were kind to provide the material itself and important information regarding gender, age and health status of the yielding harvested through annual population control measures from the nearby hunting areas.

In order to achieve the comparable results, the study limited the choice of materials only to humerus and metapodials of the previously-mentioned species. In total, 9 humeri and 7 metapodials were used in this study.

The bones were defleshed and mechanically cleaned. The preferred method for obtaining clean bones was maceration, a longer process that provides nice defatted pieces, ready to be used in such a study. Another method for the preparation of bones was the use of dermestid beetles, process that provided us with the most of our complete study. We used these methods as a consequence of the goals of our project.

The bones were cut transversely with a fine handsaw at the mid-diaphyseal part in order to obtain 4–10 mm thick bone rings or half rings. The pieces were ground with a circular grinding machine in order to reduce their thickness up to a few millimetres. The pieces were identified and marked by storing them in small histological cassettes. Each of the specimens was manually ground [26] using a slightly modified method suggested by Maat et al. [18]. We preferred this simple method due to the fact that it requires very simple instruments and proved to be a fast and reliable method for our needs. Basically, the procedure consists of the repeated series of manual grinding of the bone pieces by using a large glass stab that serves as a basis on which a sheet of waterproof abrasive paper is glued with Vaseline or any other glycerin-based hand cream (at least 2 grits — 100/350 up to 1000/1500), while the bone piece is kept in contact with the grinding surface by means of "Frost's gripping device" (consisting of a slip of abrasive paper placed transversely across the central part of a glass microscope slide). By grinding the specimen up to the moment that the piece becomes almost transparent, we obtained good quality specimens that were further on cleaned in alcohol (20–60 min) and then glued onto the normal microscopic slides with regular mounting medium and glass cover slips.

Imaging

The obtained specimens were examined in normal light microscope, starting with a 5 × magnification for the general evaluation of the specimen and the qualitative assessment of the sample, then with 10 × and 20 × for the measurements (quantitative assessment). The bone sections were photographed using an Olympus BX14 (Olympus America Inc., USA) microscope with an Olympus UC30 digital camera attached (Olympus America Inc., USA) and the

Table 1. Classification system of the bone structure types (medium-sized mammals) (taken from [5])

Primary (periosteal) bone type
1.a. lamellar non vascular
1.b. lamellar simple (primary) vascular canals
1.b.1 longitudinal
1.b.2 circular
1.b.3 reticular
1.b.4 radial
1.c lamellar with primary osteons
1.c.1 longitudinal primary osteons
1.c.2 longitudinal primary osteons with radial canals
1.c.3 longitudinal primary osteons with reticular canals
1.c.4 longitudinal primary osteons and radial simple vascular canals
1.c.5 longitudinal primary osteons in circular rows
1.d fibrous non vascular bone
1.e fibrous bone with simple (primary) vascular canals
1.e.1 longitudinal
1.e.2 circular
1.e.3 reticular
1.e.4 radial
1.f fibrous bone with primary osteons (fibrolamellar complex)
1.f.1 laminal
1.f.2 plexiform
1.f.3 reticular
1.f.4 radial
1.f.5 laminar/plexiform with longitudinal primary osteons
1.f.5.a in circular rows
1.f.5.b in a band
1.f.6 radial with primary osteons in radial rows
1.f.7 longitudinal primary osteons
1.f.8 longitudinal primary osteons in circular rows
1f/1a-c pseudo fibrolamellar complex
Secondary periostal bone types
2.a.1 scattered osteons
2.a.1.a scattered osteons with no organisation
2.a.1.b circular rows of scattered osteons
2.a.2 dense osteons
2.a.2.a dense osteons with no organisation
2.a.2.b circular rows of dense osteons

OLYMPUS Stream Basic™ software (Olympus America Inc., USA). The magnification used for photo acquisition was 10 × and the image resolution was 2080 px/ /1544 px. The representative images were saved with the calibration scale.

Most of the histological images were assessed qualitatively according to the classification system of bone structure types (adapted from Riqueles' system) regularly used by modern studies, especially those of Cuijpers [4–6] in a short descriptive form or similar to the one used by Morris [24]. A special note on Cuijpers's paper [5] has to be made, as it provides a specific human-sheep/goat and pig description of the bone structure in the perspective of interspecific differentiation (Table 1).

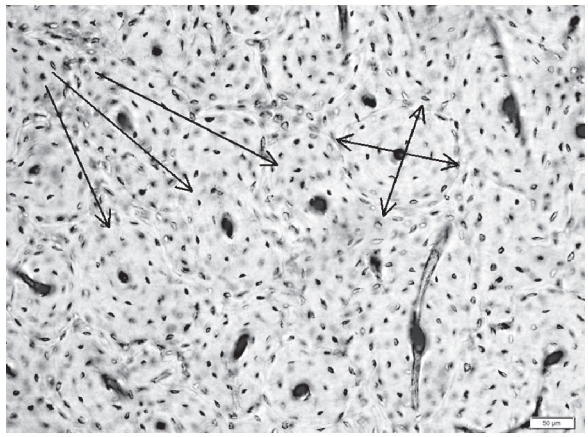

Figure 1. Obtained images from the CC03 mclp sample (*Capreolus capreolus metacarpal*). Haverisan systems and osteon banding (20 ×).

The following measurements were taken by means of the Image J computer software at the level of primary and secondary osteonal units:

— maximum and minimum primary osteonal diameter;
— primary osteonal circumference (perimeter);
— secondary osteonal area and perimeter;
— maximum and minimum haversian canal diameter (Fig. 1);
— haversian canal circumference;
— haversian canal area.

All measurements ($n \geq 100$) for each of the variables were expressed in μm or μm^2 according to the type of measurement.

For the fractal analysis and lacunarity, the samples were analysed under the light microscope and digital images of the bone structures were made, using the same digital setting as described previously. From each digital image, 500 px/500 px ROIs (regions of interest) were cropped from the middle of the bone sample using PhotoScape v3.6.2 software. The cropped ROI's were then opened with Image J 1.46q software and using the FracLac 2.5 plug-in the fractal dimension and lacunarity were calculated. The cropped ROIs and the binarisation output are shown below (Figs. 2, 3).

Statistical analysis

The data obtained from the sample measurements, fractal analysis and lacunarity were statistically analysed using the M.S. Excel (Microsoft™, USA) with statistiXL add-on (www.statistixl.com) and GraphPad InStat™v3.05 (GraphPad™, USA) software. As data passed the normal distribution test, a one-way ANOVA test was performed, followed by Tukey-Kramer or Scheffe's multiple comparisons test, if the difference

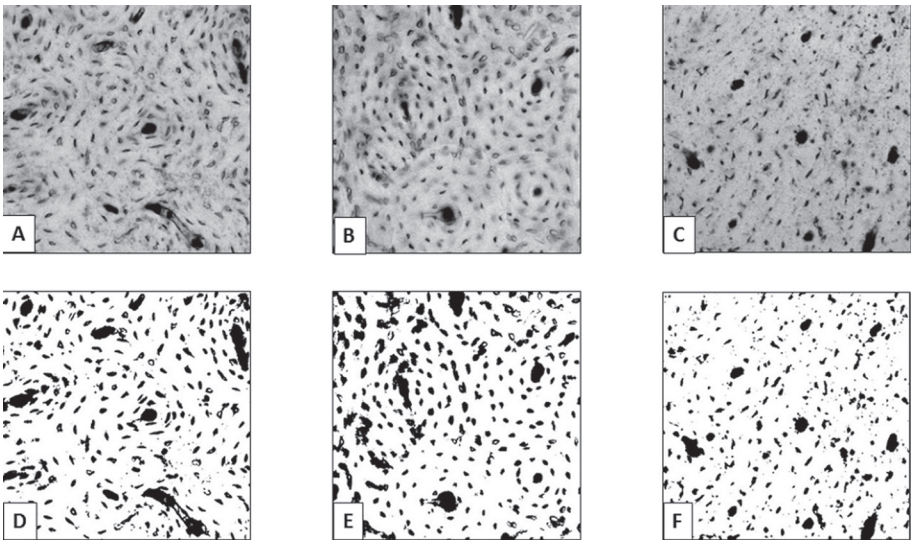

Figure 2. Humerus, sample ROIs and binarization; **A, D.** *Capreolus capreolus*; **B, E.** *Capra hircus*; **C, F.** *Ovis aries*.

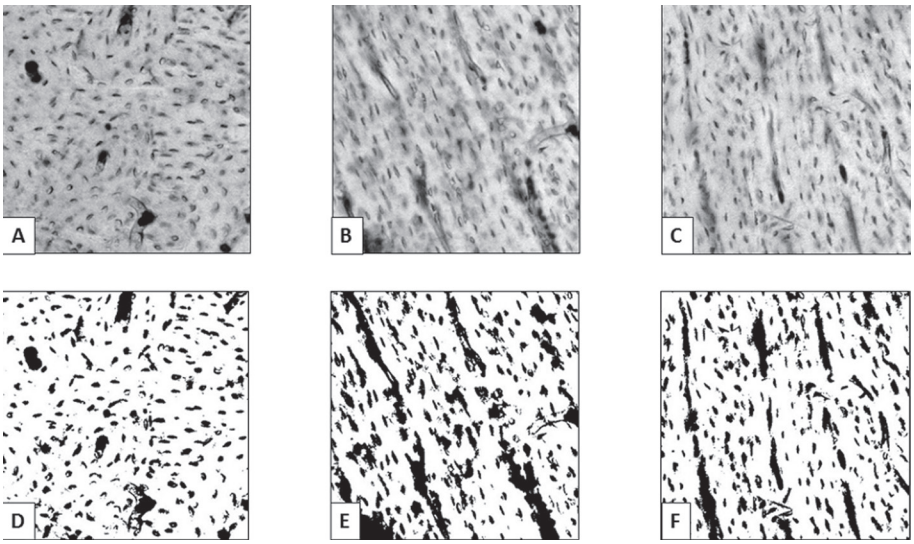

Figure 3. Metacarpus, sample ROI's and binarization; **A, D.** *Capreolus capreolus*; **B, E.** *Capra hircus*; **C, F.** *Ovis aries*.

was statistically significant. When 2 rows of data needed to be compared, unpaired t test with Welch correction was done. The significance level was set for $p < 0.05$.

RESULTS

Qualitative assessment

For the qualitative assessment of the studied pieces, we tried to limit our study to some histological specimens of the same type. This is the reason that led us to the restriction of the number of the studied specimens to those of the midshaft humerus. A number of specimens were examined and the characteristics observed were recorded as in Table 2.

Quantitative assessment

The measurements taken are summarised into Table 3. The median values are used alongside the standard deviation values.

According to the goal of this paper, the metrical data within these 3 species are compared to see whether these species can be differentiated on the basis of osteonal data. Several other statistical tests were applied in order to check the validity and variance of the series (ANOVA, POST-HOC/Tukey and Scheffe tests).

For the secondary osteon's area, there are no major differences when the mean values are compared for sheep and goat, while in roe deer these values

Table 2. Qualitative assessment of the studied specimens

Species, age, sex, slide no	Bone (segments of the mid-diaphyseal ring)	Lamellar bone types	Fibrous bone types	Composition	Scattered osteons	Dense osteons	Haversian canals
Ovis, mature, gender unknown** Oa2001 HLA	Humerus, anterior half	1a, 1c1, 1c3	1f1, 1f2, 1f3	F	2a1b, 2a1a	–	HC1, HC3
Ovis, mature, male, Oa, HLP	Humerus, posterior half	1a, 1c1	1f1, 1f2	F	2a1b, 2a1a	2a1a	HC1, HC3
Capra, mature, CH02 HLA	Humerus, posterior half	1a, 1c3, 1c1	1f3, 1f5b (2697)*	F	2a1b, 2a1a	2a1a	HC1, HC3
Capra, mature, CH02 HLA	Humerus, posterior half	1a, 1c1	1f1, 1f2, 1f3 (3114, 3115)*	F	2a1a	–	HC1, HC3
Capreolus, mature, CC03 HLA	Humerus, posterior half	1c1	1f3,1f2, 1f4 (3245)*	F/L?	2a1a	–	HC1
Capreolus, mature, CC03 HLA	Humerus, posterior half	1c3	1f2, 1f3, 1f4	F	2a1b (3080, 3092)*	–	HC1

*Designated number of the digital image in our collection; **Comparative collection specimen, Faculty of Veterinary Medicine Cluj Napoca (FMV) ossuary

Table 3. Statistical assessment of the measurements performed on different osteonal structures

Measured structures	Species	Area	Perimeter	Max. diameter	Min. diameter	Circularity
Haversian (vascular) canals	Ovis aries	363.93 ± 135.6	70.2 ± 16.8	25.41 ± 6.71	19.03 ± 3.9	0.968 ± 0.02
	Capra hircus	326.35 ± 134.2	64.9 ± 14.27	22.70 ± 5.17	18.48 ± 4.2	0.977 ± 0.02
	Capreolus capreolus	305.12 ± 122.6	64.3 ± 16.5	23.14 ± 6.46	17.48 ± 4.6	0.962 ± 0.03
	ANOVA	H1	H0	H1	H0	H1
	POST-HOC	ovis vs. Capreolus	–	ovis vs. capra	–	ovis vs. capra; capra vs. Capreolus
Haversian systems (Secondary osteons)	Ovis aries	16513.75 ± 6542	464.05 ± 76.5	164.23 ± 25.8	127.29 ± 22.2	0.968 ± 0.0283
	Capra hircus	16354.28 ± 4562	457.92 ± 64.5	168.58 ± 37.4	124.87 ± 18.4	0.962 ± 0.0342
	Capreolus capreolus	18325.47 ± 7621	476.32 ± 99.8	166.23 ± 28.2	132.96 ± 26.5	0.974 ± 0.0188
	ANOVA	H0	H0	H1	H0	H1
	POST-HOC	–	–	Ovis:capra	–	Non parametric values for ovis, capra
Primary osteons	Ovis aries	275.79 ± 115.3	59.11 ± 11.8	21.5 ± 4.9	15.8 ± 3.1	0.95 ± 0.04
	Capra hircus	255.28 ± 136.3	55.8 ± 14.6	19.9 ± 5.6	15.3 ± 3.9	0.96 ± 0.03
	Capreolus capreolus	281.45 ± 117.6	59.1 ± 12.4	21.0 ± 4.7	16.3 ± 3.4	0.97 ± 0.02
	ANOVA	H0	H1	H0	H1	H1
	POST-HOC	–	capra:Capreolus	–	capra:Capreolus	cvis:Capreolus

are less than 10% higher, but without reaching the statistical significance.

The perimeter of the same structures (secondary osteons) shows similar values for all species (less than 10% differences among species). The ANOVA test confirms the null hypothesis (no significant differences in our sample).

The diameter for the secondary osteon shows for the maximal osteonal diameters a statistically significant difference in case of *ovis* vs. *capra pair*. The average values are different, the ANOVA test rejecting the null hypothesis and the Tukey and Scheffe test points to this pair as being the one with statistically different values. The minimal osteonal diameter shows no significant differences among the studied series of data.

The circularity (a more complex indicator, describing the roundness of the structure, automatically computed when values are collected) shows differences among series (ANOVA), but the data analysis shows non-parametric values, making the POST-HOC test useless.

Table 4. Humerus and metacarpus — fractal dimensions and lacunarity, descriptive statistics

	No. of ROIs measured	Mean	Standard deviation	Standard error of mean
Humerus — Fractal dimensions, descriptive statistics				
cc04hlp	84	1.687	0.02314	0.002524
ch02hla	108	1.697	0.02293	0.002207
oa2011hlp	166	1.673	0.03176	0.002465
Humerus — Lacunarity, descriptive statistics				
cc04hlp	84	0.4390	0.05566	0.006073
ch02hla	108	0.4889	0.10110	0.009726
oa2012hlp	166	0.4917	0.08368	0.006495
Metacarpus — Fractal dimension, descriptive statistics				
cc02mcla	35	1.635	0.02371	0.004007
ch01mcl	86	1.679	0.02070	0.002232
oa2012mclp	81	1.664	0.03889	0.004321
Metacarpus — Lacunarity, descriptive statistics				
cc02mcla	35	0.4443	0.03953	0.006682
ch01mcl	86	0.4446	0.04857	0.005237
oa2012mclp	81	0.5878	0.12030	0.01337

The area of the vascular canal of the secondary osteons shows differences among all 3 species. The highest average value is seen in sheep (*Ovis aries*) (363.9 μ^2), followed by goat (*Capra hircus*) (326.3 μ^2) and then by roe deer (*Capreolus capreolus*) (305.1 μ^2). Statistically, the series were computed initially with ANOVA and then with Tukey/Scheffe tests which showed that the highest difference is found between sheep (*Ovis aries*) and roe deer (*Capreolus capreolus*) studied values.

Minimal vascular canal's diameter shows no statistically significant values, with slightly higher average values in roe deer (*Capreolus capreolus*) specimens. The statistical difference appears in case of the maximal diameter, where the largest average values are observed in the sheep series (*Ovis aries*), followed by the goat (*Capra hircus*) and then the roe deer (*Capreolus capreolus*) values. On the basis of the POST-HOC tests, the highest statistically notable differences are visible in case of sheep (*Ovis aries*) vs. goat (*Capra hircus*) series.

For circularity the average values vary — significant statistical differences being noted for sheep:goat and goat:roe deer values.

For the primary osteons, the value of the area shows, as far as average values are concerned, the highest values in roe deer series (*Capreolus capreolus*), followed by the sheep (*Ovis aries*) and then goat (*Capra hircus*) series. ANOVA instead gives us lower f-values than the f-crif values, leading to the fact that the series actually show no significant difference of variance. In case of the perimeter of the same structures, the average values give no significant difference among the groups. The ANOVA test shows instead values indicating that there are significant differences among some of the series, with an f higher than f crit values. The POST-HOC tests indicate a significant pair from this perspective — the *capra-Capreolus* pair. A similar situation encountered in the case of the minimum diameter of the primary osteons, indicating the same pair as being the most significant pair (*capra-Capreolus* pair). For the minimum primary osteonal diameter the statistical analysis shows no major differences. The circularity for these units shows some differences, with the highest values in case of the roe deer (*Capreolus capreolus*) specimens and the lowest in sheep (*Ovis aries*). The POST-HOC tests show that the most significant differences are found between the *ovis-Capreolus* pair.

Fractal and lacunarity assessment

Fractal analysis. For the humerus, the values for goat sample (*Capra hircus humerus*) expressed as average ± standard deviation, were the highest ones. For the metacarpal bone, the values for ch01 mcla (*Capra hircus*) samples were the highest ones (Table 4).

The comparison between the groups of data showed a significant statistical difference between the 3 species, both for metacarpus (one-way ANOVA, $F = 27.24$, $df = 2$, $p < 0.05$) and humerus bone

Table 5. Inferential statistics for fractals and lacunarity

Comparison	Mean difference	q	Significance level (p)
Humerus — Fractal dimension, inferential statistics Tukey-Kramer Multiple Comparison Test			
cc04hlp vs. ch02hla	0.01019	3.611	< 0.05*
cc04hlp vs. oa2011hla	0.01441	5.547	< 0.001***
ch02hla vs. oa2011hla	0.02460	10.257	< 0.001***
Humerus — Lacunarity, inferential statistics Tukey-Kramer Multiple Comparison Test			
cc04hlp vs. ch02hla	0.04989	5.772	< 0.001***
cc04hlp vs. oa2011hla	0.05271	6.626	< 0.001***
ch02hla vs. oa2011hla	0.002823	0.3844	> 0.05 (NS)
Metacarpus — Fractal dimension, inferential statistics Tukey-Kramer Multiple Comparison Test			
cc02mcla vs. ch01mcla	0.04392	10.403	< 0.001***
Cc02mcla vs. oa2012mclp	0.02862	6.719	< 0.001***
Ch01mcla vs. oa2012mclp	0.01530	4.692	< 0.01**
Metacarpus — Lacunarity, inferential statistics Tukey-Kramer Multiple Comparison Test			
cc02mcla vs. ch01mcla	0.0002474	0.02072	> 0.05 (NS)
Cc02mcla vs. oa2012mclp	0.1435	11.908	< 0.001***
Ch01mcla vs. oa2012mclp	0.1432	15.530	< 0.001***

*significant; **very significant; ***extremely significant; NS — not significant

samples (one-way ANOVA, $F = 27.25$, $df = 2$, $p < 0.05$). This significant statistical difference was also proved by the multiple comparison tests (Table 5).

The comparison of oa2012mclp (*Ovis aries* metacarpal) fractal dimension against the oa2011hla (*Ovis aries* humerus) fractal dimension showed that there is no significant statistical difference (unpaired t test with Welch correction, $t = 1.768$, $df = 133$, $p > 0.05$) (Table 5).

Lacunarity analysis. In case of humerus, the highest lacunarity level was obtained, for the oa-2012hla bone samples (*Ovis aries* — sheep) expressed as average ± standard deviation, followed closely by the ch02hla (*Capra hircus* — humerus goat) bone samples. In the case of metacarpus, the highest lacunarity level was obtained for the oa2012mclp (*Ovis aries*) bone samples. Regarding the cc02mcla (*Capreolus* metacarpal bone) and cho1mcla (*Capra hircus* metacarpal) bone samples, the average values were very close.

The lacunarity values proved to be significantly different for both humerus and metacarpal bone samples (one-way ANOVA, $F = 70.20$, $df = 2$, $p < 0.05$; one-way ANOVA, $F = 12.16$, $df = 2$, $p < 0.05$). When compared to each other, an insignificant difference was found between ch02hla and oa2011hla bone samples, in case of humerus ($p > 0.05$), and between cc02mcla and ch01mcla bone samples in case of metacarpus ($p > 0.05$).

The comparison of cc02mcla metacarpal bone lacunarity against the cc04hlp humerus bone lacunarity showed that there is no significant statistical difference (unpaired t test with Welch correction, $t = 0.5905$, $df = 88$, $p > 0.05$) (Table 5).

DISCUSSION

As a result of the assessments series performed by us, we can state that the simple micromorphological assessment (the classical approach) gives us just a general view over the investigated specimen. There is no clear distinction between the characteristics of 3 species, as the predominance of one of the laminar/fibrous components cannot be correctly stated, while all 3 specimens share a common feature — relative predominance of the fibrous tissue arranged as fibro-lamellar complex mostly in reticular form, lamellar mostly with primary longitudinal osteons with reticular canals, irregular Haversian bone tissue

comprising few, scattered osteons, mainly with no clear organisation or short rows of circular osteons — most frequently 3–4 osteons in a row-osteon banding. This feature seems to be common for all 3 species, most probably as a general feature for the small ruminants and more generally, for the large group of ruminants. One little remark has to be made when discussing about the roe deer (*Capreolus capreolus*) specimens, in the respect of the identification of the radial type (1f4) of the fibro-lamellar complex, a feature not mentioned by other authors.

When counting the micromorphology metrical data, the most significant elements are the characteristics of the secondary osteons. The cited literature mentions values for the area of the secondary osteon in case of sheep (*Ovis aries*), ranging from 21034–21553 μ^2 [22], to 10568–12461 μ^2 [9, 10]. Our obtained values show an average of 16513 μ^2, much closer to the ones given by Martiniakova [22] than the ones measured by Dittmann [9, 10]. The values for goat are situated in the 17880–17612 μ^2 range. Our values for capra are 16354 μ^2 (average), value that is less than 5% smaller than the reference values. The values obtained by us for the roe deer (*Capreolus capreolus*) are 18325 μ^2, values that seem to be higher than the ones found in sheep (*Ovis aries*) and goat (*Capra hircus*) in our investigation, but not concordant to the ones given by Dittmann [9, 10]. As a consequence of the fact that the statistical analysis shows no significant differences (ANOVA/POST-HOC), the basic mathematical difference may be attributed to the individual biological variability or to the limited choice of sampling.

The maximal diameters for the secondary osteons for sheep (*Ovis aries*) and goat (*Capra hircus*) are in accordance with the ones given by Dittmann [9, 10], showing very little difference in case of goat specimens (168/176 μ^2). The values provided by Mariniakova's study seem to be a little distant from these series, being almost 20% higher than the mentioned ones [10, 21, 22]. The statistical analysis shows, in spite of the strict mathematical values for the means, that the statistical difference can be set clearly between the group of *ovis* vs. *capra* in this case, the values for roe deer (*Capreolus capreolus*) being placed close to the ones of sheep (*Ovis aries*).

As the values collected in case of the minimum osteonal diameter are not very different (statistically) in all 3 species, the discussion about this item is not approached.

For the vascular's canal area, our determined values for sheep (*Ovis aries*) are close to the ones given by Dittmann (363 μ^2 vs. 396 μ^2) [9, 10]. The values for goat (*Capra hircus*) are also very similar to the ones mentioned by the same author (326.3 μ^2 vs. 321.2 μ^2). The values calculated by us for the roe deer (*Capreolus capreolus*) specimens are situated around 305 μ^2. According to our results, the most significant series of values refer to the pair *ovis* vs. *Capreolus*, whose values are the most unlike and, most probably, prone to metrical differentiation.

The vascular canal's maximal diameter, which showed for our values significant statistical differences for the *ovis* vs. *capra* pair, presents figures that are very similar with the ones provided by Dittmann both in sheep (*Ovis aries*) (25.35–25.8 μ^2) and goat (*Capra hircus*) (18.75–23 μ^2) [9, 10]. Martiniakova offers much higher values (25%) for *Ovis* in one of her papers [20] and quite similar values in another paper (21.6 μ^2) [21]. The values for roe deer (*Capreolus capreolus*) do not differ significantly from the series of sheep (*Ovis aries*).

The minimal diameter for the vascular canal shows no statistical differences for our 3 samples, so the question for this item is not approached.

We do not have the comparative values for the primary osteonal area, perimeter, minimal and maximal diameters. As a consequence, the only statements that we make are the ones strictly connected to our determined values. The statistical analysis reveals the fact that the area and perimeter for the secondary osteons is not a reliable element, also due to the subjectivity of the measurement, identification and the ample series of values. One element that seems to offer some distinguishing features is the minimal primary osteon diameter (*capra* vs. *Capreolus*), but under these circumstances we find hard to think that these values can be a differential feature.

Studies regarding the fractal characteristics of the bone tissue were conducted on histological slides [1, 14] or on radiographic, computed tomography and magnetic resonance images [32], but apparently not on the manually ground bone tissue samples. This bone processing method was able to maintain the structural architecture for further analysis. Both fractal analysis and lacunarity were able to give an objective point of view regarding the bone architecture features differentiation between the studied species. In comparison with the morphometry assessment, fractal analysis and lacunarity proved to offer a better insight on the differentiation of the bone architecture of the

studied species and also between different bones of an individual. Further extensive research is needed on a larger number of samples to guarantee these techniques (fractal analysis and lacunarity) to determine the degree of differentiation of the bone structure.

CONCLUSIONS

1. The overall morphology (qualitative assessment) of 3 species does not present clear distinguishing features that can be used for a firm diagnostic at the level of species.
2. There is a set of new histomorphometric data concerning roe deer (*Capreolus capreolus*) compact bone (none existing at this time to the best of our knowledge).
3. The metrical analysis provides some initial directions and possibilities for a metrical assessment and separation of the osseous material, especially when it comes to the elements of the secondary osteonal components. More precisely, the statistical canonical analysis (POST-HOC, Tukey-Kramer) provides some clues in the case of the separation of at least 2 species (maximal osteonal diameter or the area of the vascular canal, maximal diameter of the vascular canal, vascular canal area).
4. There is no clear metrical distinction established on the basis of the metrical data among all 3 studied species.
5. An absolutely new type of analysis performed on the digital images of ground bone samples — the fractal analysis and lacunarity — show a high potential in terms of bone structure differentiation between individuals and between an individual's anatomical areas. The fractal analysis seems to offer differential keys for all of the 3 studied species, regardless of the investigated bone (metacarpal and humerus), so continuing the studies in this direction seems a logical choice.
6. These new findings should greatly facilitate a new approach both in archaeozoological studies or forensic investigations and contribute to the development of new methods for these sciences.

ACKNOWLEDGEMENTS

This study was supported by the POS-DRU/ /89/1.5/S/62371 (European Social Fund) research grant. I would also like to thank my colleagues for the given support, especially to those from the Department of Veterinary Pathology and Legal Medicine of the Faculty of Veterinary Medicine of Cluj for the help in realising the histological specimens and the basic image examination procedures and also to Dr. Saddha Cuijpers (University of Amsterdam, the Netherlands) for the kind help and support provided in the early phase of this project.

REFERENCES

1. Byron CD (2006) Role of the osteoclast in cranial suture waveform patterning. Anatom Record, Part A: 552–563.
2. Cattaneo C, Porta D, Gibelli D, Gambe C (2009) Histological determination of the human origin of bone fragments. J Forensic Sciences, 54: 531–533.
3. Crowder C, Stout S (2011) Bone histology: an anthropological perspective [Internet]. CRC Press. Available from: http://books.google.ro/books?id=nS7P8-2UpUEC.
4. Cuijpers AGFM (2006) Histological identification of bone fragments in archaeology: telling humans apart from horses and cattle. Intern J Osteoarchaeol, 16: 456–480.
5. Cuijpers AGFM (2009) Distinguishing between the bone fragments of medium-sized mammals and children. A histological identification method for archaeology. Antropol Anz, 67: 181–203.
6. Cuijpers AGFM (2009) Species identification in archaeology [Internet]. PhD, aprox 150. Available from: www.ubvu.uv.nl/dissertations.
7. Cuijpers AGFM, Lauwerier RCGM (2008) Differentiating between bone fragments from horses and cattle: a histological identification method for archaeology. Environmental Archaeol, 13: 163–173.
8. Dammers K (2002) Using osteohistology for ageing and sexing. 9th ICAZ Conference-Recent Advances in Ageing and Sexing Animal Bones.
9. Dittmann K (2003) Histomorphometrische Untersuchung der Knochen mikrostruktur von Primaten und haustieren mit them Ziel der Speziesidentifikaton unter Berucksichtigung von Domestikationeffecten. Anthrop Anz, 61: 175–188.
10. Dittmann K, Grupe G, Manhart H, Peters J, Strott N (2008) Histomorphometry of mammalian and avian compact bone. Department Biologie, Facultat fur Biologie, Bereich Biodiversitatsforschung, Martinsreid, Germany.
11. Enlow DH, Brown SO (1956) A comparative histological study of fossil and recent bone tissues. Part II. Texas J Science, 8: 405–412.
12. Enlow DH, Brown SO (1957) A Comparative histological study of fossil and recent bone tissues. Part II. Texas J Science, 9: 186–214.
13. Enlow DH, Brown SO (1958) A Comparative histological study of fossil and recent bone tissues. Part III. Texas J Science, 10: 187–230.
14. Fazzalari NL, Parkinson NL (1996) Fractal dimension and the architecture of the trabecular bone. J Pathology, 178: 100–105.
15. Jowsey J (1996) Studies of Haversian systems in man and some animals. J Anat, 100: 857–864.
16. Landini G (2011) Fractals in microscopy [Internet]. J Microscopy, 241: 1–8. Available from: http://dx.doi.org/10.1111/j.1365-2818.2010.03454.x.

17. Lasota-Moskalewska A, Moskalewski S (1982) Microscopic comparrison of bones from medieval domestic and wild pigs. Ossa, 7: 173–178.
18. Maat GJR, Van Den Bos RPM, Aarents JMJ (2001) Manual preparation of ground sections for the microscopy of natural bone tissue: update and modification of frost's "rapid manual method". Intern J Osteoarchaeol, 11: 366–374.
19. Mainland I, Schutkowski H, Thomson AF (2007) Macro- and micromorphological features of lifestyle differences in pigs and wild boar. Anthopozoologica, 42: 89–105.
20. Martiniakova M, Grosskopf B, Omelka R, Dammers K, Vondrakova M, Bauerova M (2006) Histological study of compact bone tissue in some mammals: a method for species identification. Intern J Osteoarchaeol, 17: 82–90.
21. Martiniakova M, Grosskopf B, Omelka R, Vondrakova M, Bauerova M (2006) Differences among species in compact bone tissue microstructure of mammalian skeleton: use of a discriminat function analysis for species identification. J Forensic Sci, 51: 1235–1239.
22. Martiniakova M, Grosskopf B, Omelka R, Vondrakova M, Bauerova M (2006) Histological analysis of ovine compact bone tissue. J Vet Med Sci, 69: 409–411.
23. Mori R, Kodaka T, Soeta S, Sato J, Kakino J, Hamato S, Takaki H, Naito Y (2005) Preliminary Study of histological comparison on the growth pattern of long-bone cortex in young calf, pig and sheep. J Vet Med Sci, 67: 1223–1229.
24. Morris ZH (2007) Quantitative and spatial analysis of the microscopic bone structures od deer (Odocoileus virginianus) dog (Canis familiaris) and pig (Sus scrofa domestica). Department of Geography and Anthopology, Toronto.
25. Otton T, Da Silveira FRCS, Niterói R, Melo RHC, Lima RCF (2009) On Using lacunarity for diagnosis of breast diseases considering thermal images. In: Systems, Signals and Image Processing, 2009. IWSSIP 2009. 16th International Conference on Date of Conference: 18–20 June 2009.
26. Paral V, Witter K, Tonar Z (2007) Microscopic examination of ground sections- a simple method for distinguishing between bone and antler? Intern J Osteoarchaeol, 17: 627–634.
27. Parkinson IH, Fazzalari NL (2000) Methodological principles for fractal analysis of trabecular bone [Internet]. J Microscopy, 198: 134–142. Available from: http://dx.doi.org/10.1046/j.1365-2818.2000.00684.x.
28. Rajtova V, Briancin J, Kokardova M (1995) Lamellar bone structure in small ruminats. Folia Veterinaria, 3–4: 123–126.
29. Senesi N, Wilkinson KJ (2008) Biophysical chemistry of fractal structures and processes in environmental systems. Wiley, Chicester.
30. Yasara F, Akgunlub F (2008) Evaluating mandibular cortical index quantitatively. Eur J Dentistry, 2: 283–290.
31. Zaia A, Eleonori R, Maponi P, Rossi R, Murri R (2005) Medical Imaging and osteoporosis: fractal's lacunarity analysis of trabecular bone in MR images. Proceedings of the 18th IEEE Symposium on Computer-Based Medical Systems (CBMS'05).
32. Zaia A, Eleonori R, Maponi P, Rossi R, Murri R (2006) MR imaging and osteoporosis: fractal lacunarity analysis of trabecular bone. [Internet]. IEEE transactions on information technology in biomedicine. A publication of the IEEE Engineering in Medicine and Biology Society, 10: 484–489. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16871715.