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ABSTRACTS

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INVITED LECTURES

YCR@NIUM: GEOMETRIC MODELLING AND 3D RECONSTRUCTION OF THE SKULL

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Head injury is one of the main causes of death and morbidity in both industrialised and developing countries. Epidemiological studies estimated that brain injuries account for 15% of the total fatalities, and disabilities represent the main causes of death in young adults. However, in spite of the high speed of 6-7 m/s with a deceleration of 1000 g that woodpeckers have when they drum tree trunks, they do not experience any head traumatisms. This is due their skeletal cranium structure. The objective of this study was understand the cranium biomechanics behaviours. We were particularly interested in human craniums in order to predict possible cranial fractures. To carry out this analysis we abstracted the geometry of the human cranium, taking into account the more biomechanically representative structures. With abstraction, we created a 3D geometrically systematized representation of the most significant details of the skull. We proposed and 3D impressed a simplified skull model which could be mounted and disassembled. It is a novel simplified 3D skull model to predict cranial fracture patterns.

LANDMARK BASED ANALYSIS OF DEFORMATIONS AND MOTION

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Geometric morphometric methods have been widely applied in studies of the evolution, development and ontogenetic transformation of forms. They have been less vigorously applied in studies of sequential shape changes or transformations of form. Such sequential changes are found among structures that show metameric variation, or within a single structure as a result of sequential mechanical loading generating motion. In these cases we are concerned with the analysis of trajectories of transformation of the shape and size of landmark configurations over one or more steps. In the simplest case, such as the deformation occurring because of a single loading, the transformation is linear and can be described by a single vector. This is very similar to the study of allometry. In cases that are more complex, each step of the sequence of transformation is characterised by a different vector. Both cases involve the comparison of changes in size and shape and it makes sense in some circumstances not to separate these but to analyse them jointly in a size and shape space. It has previously been proposed that sequential transformations such as occur in locomotion are sensibly approached through the analysis of size and shape changes among sequential 'frames' or instances of a sequence. However, the study of sequential transformations using individual frames and the machinery of geometric morphometrics pose some practical and theoretical problems. These will be considered in this presentation as will pragmatic solutions and novel approaches that circumvent them.

MORPHOLOGICAL INTEGRATION, MODULARITY AND GEOMETRIC MORPHOMETRICS: AN UPDATE ON RECENT DEVELOPMENTS AND A LOOK AHEAD

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Morphological integration is a ubiquitous feature of organisms and their parts and has important implications for a wide range of biological studies. An increasing number of studies in recent years have analysed morphological integration and its logical counterpart, modularity, with the methods of geometric morphometrics. Yet, three have also been criticisms of analytical methods used in this area. This talk will examine recent achievements and challenges. There are technical challenges for morphometric studies of integration and modularity. Recent debate has focused on the effects of limited sample sizes and large numbers of variables on the RV coefficient that is used widely as a measure of covariation. It is less widely appreciated that other methods, such as principal component analysis and partial least squares, are also subject to similar biases and related statistical problems. The nature of morphological integration and modularity and their implications for evolution raise a range of biological questions that are still unsolved. Advances in this area can come from the use of new and unusual study systems, which may offer novel opportunities to gain insight into

biological systems from morphometric data.

FROM GENOTYPE TO PHENOTYPE THROUGH MORPHOMETRICS

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Shape is one of the most prominent expressions of an organism's phenotype. The analysis of shape has been key in biological studies to reveal the evolution, development and functional adaptation of morphological traits. However, even when shape is the result of gene expression and regulation, and the response of phenotypic traits to selection directly relies on the available genetic variation; the study of the phenotype has traditionally remained disconnected from the study of the genotype. Here I will provide examples to illustrate how Geometric Morphometrics (GM) can be used to associate morphology with the underlying genotype in different organs such as the skull, the brain, the face and the limbs, using both human and experimental mouse data. Combined with 3D imaging, molecular, demographic and computational tools, GM can shed light into the genetic-phenotypic correspondence in biological structures. Therefore, GM can have relevant applications in developmental biology and biomedical research. Thanks to the "morphometric revolution", GM has been successfully applied to all sorts of living and fossil organisms, from animals to plants and cells, from hard to soft tissue structures, and from embryonic to adult stages. Why not genes?

LECTURES

INVESTIGATING THE SEX RELATED GEOMETRIC VARIATION OF THE HUMAN CRANIUM

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Evaluating the expression of sexual dimorphism of the human cranium has been a long established task for physical anthropologists. Traditional methods of visual observation and metrics have been available for qualitative and quantitative analysis of the shape of the cranium revealing certain patterns of shape variation due to sexual dimorphism. Technological advancements have led to the wide application of geometric morphometrics in skeletal analysis over the recent years. However, the focus regarding sex discrimination remains largely on well-known patterns of sexually dimorphic expression. The present study assumes no priors of known patterns and adopts a computerized iterative approach on sex discrimination. Our sample consists of 176 crania from the Athens human skeletal reference collection, which were digitized with Microscribe 3D and 80 ectocranial landmarks were recorded for each individual of known sex. Custom software has been developed to calculate linear discriminant analysis and correct classification of all 3160 possible combinations of euclidean distances between the landmarks. Our results revealed 13 distances that yielded classification accuracy in excess of 85% with the most prominent being the distance between right Ovale mediale and right Mastoidale reaching 87.36% correct classification. Further multivariate analysis showed that a specific combination of 6 distances among 8 landmarks yielded 90.53% correct classification, whereas 25 distances among 26 landmarks reached 94.63%. Our study revealed specific geometric properties of the cranium with high expression of sexual dimorphism that have not been reported before. Furthermore, we demonstrated landmark based geometric morphometrics can provide high precision discrimination even on fragmentary remains.

THE BÉZIER CURVE MODEL APPLIED TO HUMAN EVOLUTION

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Most craniometric form and shape change studies in Palaeoanthropology are based on multivariate statistical analyses of distances between cranial landmarks or semi-landmarks. This predominant morphometric approach fails to take into account information of biological significance such as changes in curvature between homologous cranial points. Closed contour curve fitting techniques such as Fourrier and Eigenshape may be used to that effect. However, there exists a more suitable contour approximation method with a unique ability to extract parameters which allow to render accurately form and shape in a way that fits perfectly the original data: the Bézier curve model. I will ex-

plain the Bézier curve approximation method which is well suited to open contours and satisfies the requirements of curve-fitting accuracy and form reproduction: in particular its fundamental mathematical characteristics, its most important properties which makes this model suitable for the analysis of form and shape changes, the behavior of the curve related to its control polygon, and its limitations. The model will be illustrated with an application to the contentious issue of archaic Homo sapiens morpho-species recognition (also erroneously known as Homo heidelbergensis) with a fossil sample showing substantial cranial variability and including fossils, among others, such as Atapuerca SH and Petralona from Europe, Broken Hill 1, Saldanha, Eliye Springs, Omo Kibish 2, LH 18 and Djebel Irhoud 1 Singa from Africa, and Dali and Ngandong specimens from Asia. Finally, we will demonstrate how, thanks to its unique properties of form and shape rendering, one can implement dynamic graphic evolutionary simulations.

ECOMORPHOLOGICAL STUDY APPLYING FOURIER SHAPE ANALYSIS TO THE GENERA CRICETODON AND HISPANOMYS (RODENTIA, MAMMALIA)

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Rodents are one of the most useful group of mammals to characterize environmental changes. Because of its richness in the fossil record and the short stratigraphical distribution of most of its species, they are a useful tool to apply in paleoecological and biostratigraphical distributions. In this case, we applied Fourier Shape Analysis on the first upper molar (M1) of species of two genera of cricetids (Rodentia, Mammalia): *Cricetodon* and *Hispanomys*. For the first time, we used morphological information and outline data to analyse the ecomorphological changes in these genera through time. To apply it, we digitalized the contour of 242 specimens of M1 of 15 different species of both genera. To evaluate the morphospace, we performed a PCoA over a dataset including the Fourier Coordinates and the morphological information of the first molar of each species. To evaluate how the morphospace has changed through time, we have plotted the scores of the two first components and the data of the last appearance datum. Both genera have a temporal range lasts 7 My (Middle Miocene – Upper Miocene). During this period, two main climatic events were detected: the Miocene Climatic Optimum (warm temperatures) and the Middle Miocene Climate Transition (decrease of temperature). In the graph *Cricetodon* has less morphological disparity than *Hispanomys*. The increase of the morphological variability on the molar morphology (reflected by the wider morphospace of *Hispanomys*) could be explained by the changes in the dietary ecology caused by the aridification of the environment.

ANATOMICAL DIFFERENCES BETWEEN THE DISTAL RADIUS ARTICULAR SURFACE IN HUMANS AND CHIMPANZEES

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The distal radius articular surface is one of the most important components of the wrist joint. This articular surface presents different functional demands according to the different types of locomotion in hominoid primates, especially between biped humans and knuckle-walking chimpanzees and gorillas. In this study, a comparative morphological analysis of the distal radius articular surface of humans and chimpanzees was conducted in an attempt to determine the anatomical differences related to their different type of locomotion. The analysis is based on the articular surface of the distal radius topography and geometric morphometric methods. The analysis is characterized by a 3D point-cloud mesh set across the distal radius with the digit surface command in *Geomorph* Geometric Morphometrics package. A total of 4.800 points were used as surface pseudo-landmarks to build a cloud mesh indicative of the articular surface of the distal radius topography and comparisons of articular surface shapes among specimens of different species was made. We present a Geometric Morphometrics analysis of 51 distal radius (31 belonging to *Homo sapiens* and 20 belonging to *Pan troglodytes*). The results, based on the study of two actual known series of *Homo sapiens* and Pan *troglodytes*, showed morphological significant differences regarding the anatomy of the articular surface of the distal radius, derived from its

locomotion system.

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MORPHOLOGICAL VARIATION AND DISPARITY OF THE PALATAL REGION IN MODERN CROCODILES (EUSUCHIA, CROCODYLIFORMES)

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A progressive developmental process of the secondary palate is one of the most significant criteria in the interpretation of the evolutionary history of Crocodyliformes. Besides, the palate plays a key-role in the mechanical performance of the skull of more derived crocodyliforms. As geometric morphometric studies on modern crocodile skulls have focused on the dorsum, less attention has been paid to their ventral structures. Hence, an approach is proposed for exploring the poorly known patterns of morphological variation and disparity in the palate region of Eusuchia. In order to achieve this objectives 86 skulls were examined, comprising most of the extant species of crocodiles and several fossils, including non-Crocodylia eusuchians (Hylaeochampsidae and Allodaposuchidae). A new set of 20 landmarks was developed for the right half of the skull and was divided in two parts, the rostrum and postrostrum, to examine them individually. Principal Component Analyses (PCA) were performed to inspect patterns of morphological variability, and disparity analyses were conducted to outline the distribution of disparity among the principal eusuchian lineages. The results of these PCA show that the relative width and length of the snout are the main sources of morphological variability, but other structures as the incisive foramen, suborbital fenestrae or the internal nares are also highly variable. Alligatoridae, Crocodylidae, Gavialidae and Hylaeochampsidae form distinct groups in these PCA, reflecting different palatal morphologies. Furthermore, Allodaposuchidae palatal shape overlaps with that of Alligatoridae or Crocodylidae. The disparity analyses depict that hylaeochampsids and gharials have the most disparate morphologies within Eusuchia.

ANALYSIS OF DENTAL CROWN SHAPE VARIABILITY IN HUNTER-GATHERER AND AGRICULTURALIST AFRICAN POPULATIONS USING SURFACE PSEUDOLANDMARKS IN GEOMORPH R

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The rate of dental wear has been used to characterize patterns of tooth and dietary habits in human populations. However, the lack of precise age-at-death in skeletal collections questions the reliability of dental wear comparisons among populations. The aim of this research is to quantify dental crown shape using 3D Geometric Morphometrics (Geomorph in R) and test its reliability in making comparisons among populations. A total of 413 first and second molars (both upper and lower) belonging to nine African hunter-gatherer, pastoralist, agriculturalist, and fishing human populations were scanned with a 3D DAVID scanner. A configuration of 8 homologous landmarks was used to build a 4,800 points template that was projected onto the analyzed teeth. A PCA was used to explore the among-groups variability in dental crown shape. For the lower first molar, populations consuming plant foods from agricultural activities, such as Bantu and Maasai, showed greater cusp height reduction than the hunter-gatherers and pastoralists San, Pygmy, and Khoe. For the lower second molar, the Khoe, San and Zulu showed higher dental wear rates than the Maasai, Pygmy and Nilotic. By contrast, the upper dentition showed greater variation of dental morphology, rather than wear. For the upper dentition, the first molar showed moderate dental wear in most groups, whereas for the second molar the Hutu, Zulu, Pare/Gonja and San showed squared crown shapes, while the Maasai, Bantu and Pygmy showed a less developed disto-lingual cusp.

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ONTOGENY OF THE LOWER JAW IN DASYPUS NOVEMCINCTUS (CINGULATA, XENARTHRA)

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Armadillos (Dasypodidade, Cingulata) are the most diverse group of living xenarthrans, represented by 21 species and 9 genera. The genus *Dasypus* is the only extant representative of the subfamily Dasyponinae, the oldest of the subfamilies of this group (it separated from the rest of the group about 40 million years ago). *Dasypus novemcinctus*, the nine-banded armadillo, is the armadillo with a larger distribution, ranging from Argentina to USA. No studies have been developed on the ontogeny of *D. novemcinctus* (or other armadillos species) masticatory apparatus. Moreover, in morphological analyses on armadillos, adult maturity is usually inferred by the size of the specimen and the degree of fusion of the sutures, which can be misleading. In order to understand the ontogenetic changes of the lower jaw of this species, I have analysed 67 mandibles of *D. novemcinctus* using 2D landmarks to record their shape. Linear regression and Principal Component Analysis were developed on the shape variables (residuals of superimposition by Procrustes Generalized Analysis). Surprisingly, only 10 % of the shape variation was related to differences in size, and both subspecies included (*fenestratus* and *mexicanus*) showed the same ontogenetic pattern. The rest of the variation was not related to any of the other factors studied (geographic area, sex and subespecies). Static allometry was not significant. *D. novemcinctus* lower jaw shape does not change very much during grow, and adult size does not affect its shape.

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APPLICATION OF DOUBLE GENERALIZED LINEAR MODELS IN THE STUDY OF SKULL ASYMMETRY: A QUANTITATIVE GENETICS APPROACH

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Shape asymmetry has been proposed to be a consequence of different developmental and ecological perturbations, which alter the perfect symmetry intended in the DNA. However, the genetics of each individual can also vary the robustness against these perturbations and therefore, the asymmetry. Unfortunately, important features about the genetic origin of skull shape variation remain elusive. Here we present a method to detect genetic markers involved in the generation of fluctuating and/or directional asymmetry, measured using geometric morphometric techniques. A double generalized linear model is used in combination with both simple linear models and linear mixed models. This method allows first the detection of alleles related to changes in mean shape (i. e. directional asymmetry) and then the alleles related to changes in variance (i. e. fluctuating asymmetry). Directional asymmetry has been detected often in morphometric studies but its biological meaning has not been entirely elucidated. The identification of genotypes involved in its generation gives some hints about the evolutionary forces driving this shape feature. The genomic regions associated to fluctuating asymmetry may have a role in the robustness of the organisms to environmental perturbations, important also for medical applications. Finally, we present an example of the application of this method in a genome-wide association study of a population of 692 outbred Carworth Farms White mice used in previous studies.

PRECISE PHENOTYPING REVEALS EARLY LIMB DEFECTS IN APERT SYNDROME

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Relatively little is known of the mechanism underlying limb defects in Apert syndrome, a relatively rare congenital disease caused by one of two missense mutations in Fibroblast growth factor receptor 2 (FGFR2) and characterized by profound craniofacial malformations. Although bony syndactyly clinically differentiates Apert from other craniosynostosis syndromes, further research on the associated limb defects is rare in part because mouse models for Apert syndrome do not present obvious limb anomalies. To overcome this gap we

explored early limb development in the *Fgfr2*$^{+/P253R}$ Apert syndrome mouse model that carries the mutation associated with the most severe human limb malformations in Apert syndrome. Combining methods for visualizing gene expression patterns (WMISH) with mesoscopic 3D imaging (OPT) and quantitative shape analysis we compared limb phenotypes and associated gene expression patterns in mice between embryonic days 10.5 and 11.5. Our results challenge previous reports of no limb defects in *Fgfr2*$^{+/P253R}$Apert syndrome mice, showing significant differences between unaffected and mutant littermates that can be traced back to the earliest stages of limb development. These differences involve the size and shape of forelimbs and hindlimbs, as well as the expression pattern of *Dusp6,* a gene that is relevant for limb morphogenesis. Interestingly, the covariation pattern between limb morphology and gene expression domains was not disrupted in mutant Apert syndrome mice. Our method demonstrates that precise embryonic phenotyping can help identify the timing and nature of developmental processes that are altered by genetic mutations and further our understanding of the origins of abnormal morphogenesis.

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TESTING THE USE OF 2D GEOMETRIC MORPHOMETRICS ON THE STUDY OF CAVE BEAR TEETH

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There is a great diversity of studies in which geometric morphometrics has been applied to the analysis of the shape of the cranium and mandible of ursids, both in 2D and 3D. In the field of dentition, its application has been less frequent, even though this has been widely studied by means of classical biometrics. Nonetheless, the study of occlusal dental surfaces by applying geometric morphology in 2D is not new in the case of ursids. The present communication describes the methodology that has started to be used in applying geometric morphometrics to the study of ursid teeth. It details the process of photographing the occlusal surface of upper P4s and the most important steps to be taken in order to avoid increasing error in the subsequent analysis. To carry out such an analysis, we chose an upper P4 from a cave bear (*Ursus spelaeus*) from Ermitons Cave (Sales de Llierca, Garrotxa), dating from the Upper Pleistocene. This was photographed repeatedly and analysed with the free software TPSdig2 and MorphoJ, with a view to ascertaining how the method affects the variability appearing in the analysis. The results of a first test show how the orientation of the lighting in the photograph can influence the precision of the landmarks situated on the apex of the cusps, especially when these present a very rounded appearance as a consequence of enamel wear. The P-values for various Procrustes ANOVA obtained from a comparison of diverse randomly formed groups indicate that the sample does not tend to show significant differences, although in fact the influence of certain variables may cause these results to fluctuate enormously. The P-values for the six Procrustes ANOVA performed fell between 0.9875 and 0.4034. However, it is hoped that this difference decreases as the size of the sample increases, since the analysis initially only comprised 30 individuals.

ONTOGENY OF THE TIBIA DURING PUBERTY, GEOMETRIC MORPHOMETRIC ANALYSIS APPLIED TO AN CURRENT SPANISH POPULATION

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The objective of this study is to analyse morphological growth changes of the tibia in boys and girls. Six hundred digital telemetries from the left tibia in anterior view obtained from the Sant Joan de Déu Hospital database were analysed. The material corresponds to 300 boys and 300 girls aged between 9 and 18 years. The tibia shape variation was quantified by 19 two-dimensional landmarks located in both epiphyses and in the middle of the shaft. Morphological changes between both sexes as well as between the different ages were analysed by geometric morphometrics. Results indicated that the tibia varied significantly in size and shape with age in both sexes. In general terms these changes are characterized by an increase in tibia robustness, size modifications in

the epiphyses and a lateral torsion of the proximal half of the shaft. We observed significant increases in: the tibial tuberosity in relation to the shaft, the medial intercondylar spine in relation to the malleolus, the articular surface of the lateral condyle in relation to the articular surface of the medial condyle, and the robustness of the malleolus in relation to the thickness of the distal epiphysis. Sexual size differences clearly appear after 13 years of age, boys being bigger than girls. However, tibia growth trajectories in both sexes are quite similar, mainly after the growth spurt. This differs from previous studies of the femur. The timing, morphology and growth trajectories provided on the tibia can be very helpful in anthropological, paleoanthropological and evolution studies.

MANDIBULAR SEXUAL DIMORPHISM IN JUVENILE INDIVIDUALS WITH DIFFERENT FACIAL GROWTH PATTERNS

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One important problem in human osteoarcheology is the difficulty of sex estimation in immature skeletons. Explanations of sexual dimorphism pattern of the mandible and its large variation are diverse and the biological determinants by which differences between males and females are reached remains elusive. In light of the previous works, here we analyze how sex-specific features distinguish male and female subadult mandibles throughout the spectrum of growth facial patterns of variation (mesofacial, dolichofacial and brachifacial) using the computerized method of geometric morphometric. Two-dimensional (2D) landmarks describing mandibular morphology were collected from 275 lateral X-ray of Spanish children between 8 and 14 years old. Growth facial patterns (meso-, dolicho-, and brachyfacial) were distinguished following standard orthodontic criteria of Ricketts et al. (1982). The size (centroid size) and shape (Procrustes shape coordinates) were analyzed separately. The shape differences revealed by the PCA are visualized and explored using PC plots, wireframe, and rendered models using Morpho-J software. Multifactorial ANOVAs was performed to set the effect of growth in the shape change between growth facial patterns. The patterns of sexual dimorphism were evaluated with a generalized linear model with interaction term. Our results show that the growth facial patterns are associated with different mandibular morphologies. Sex-specific mandibular traits behave in a different way across mesofacial, dolichofacial and brachifacial subadults individuals. These results support previous studies suggesting that an assessment of vertical facial patterns of the individuals is needed before to attempt a sexual diagnosis of the mandible.

ASSESSING ALTERED SKELETAL DEVELOPMENT IN DOWN SYNDROME

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Ts65Dn mice are a good model to study the skeletal alterations associated with Down syndrome (DS), since both humans and mice with this partial trisomy show similar altered development, i.e. reduced bone mineral density (BMD) and modified bone structure. Overexpression of *DYRK1A*, a gene that plays a critical role in brain and bone development, may contribute to the bone defects associated with DS. We hypothesize that a prenatal treatment with the green tea flavonol epigallocatechin gallate (EGCG), an effective inhibitor of DYRK1A kinase activity, may normalize DYRK1A activity and improve some skeletal defects in DS. To test this effect, we acquired *in vivo* micro computed tomographies at three different stages of postnatal development (postnatal days 3, 14 and 29) in Ts65Dn mice and wild type littermates, to compare untreated mice with mice treated with EGCG. We estimated BMD in the humerus and performed volumetric and 3D morphometric analyses of the upper limb bones to compare the skeletal development within and among groups. Results of the volumetric analyses and the Principal Component Analysis (PCA), based on the Procrustes shape coordinates, showed that Ts65Dn mice

are significantly different than wild type mice. EGCG treatment showed effects on bone development but further research is needed to optimize the dosage of the treatment. With life expectancy increasing, individuals with DS present higher incidences of osteoporosis. Bone health has thus become an important medical issue for individuals with DS. Interestingly, EGCG treatment could be an important tool to prevent bone loss in DS, starting in early development.

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USING GEOMETRIC MORPHOMETRICS TO ANALYSE THE HUMAN ACETABULAR SHAPE FOR FORENSIC PURPOSES

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Due to the well documented relationship between the human acetabular morphology and ageing, detailed knowledge of its specific anatomy can be widely applicable to forensic anthropology. This study aims to examine detailed morphological variability in the adult human acetabulum related to sex and age. To conduct this work, 682 documented individuals (327 ♀, 355 ♂) aged between 15 to 101 years old were analysed. The material examined comes from three different documented skeletal collections from the Iberian Peninsula. Each acetabulum was photographed and two landmarks and thirty-two sliding semi-landmarks, were digitised. Two-dimensional geometric morphometric methods were applied to quantify acetabular size and shape variation associated with sex and age. Results indicated that size, sex and age significantly influence acetabular shape variation. Additionally, three main age-related modifications to the acetabular shape were documented: acetabular notch narrowing, outer acetabular profile modification, with extension into the postero-superior direction and reduction in the inferior direction, and acetabular fossa reduction. Interestingly, these age-related changes are shared by both sexes and appear to be related to bone production linked with age along the whole border of the lunate surface. Specifically, age seems to affect both acetabular horns, the acetabular rim and the outer edge of the acetabular fossa, respectively. Furthermore, morphometric data demonstrated the clover-leaf shape of the outer edge of the acetabular fossa in both males and females. In agreement with and supporting previous research on this subject, these results enhance our understanding of the acetabular shape and assist in refining and complementing current age-estimation methods.

EXPLORING THE EFFECTS OF EPIGALLOCATECHIN-3-GALLATE (EGCG) IN DOWN SYNDROME'S CHILDREN FACIAL MORPHOLOGY USING 3D IMAGES

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Down syndrome (DS) is a genetic disorder caused by trisomy of chromosome 21 that is associated with cognitive impairment, facial dysmorphologies, immunodefiency and congenital heart disease. *DYRK1A* (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A) is a dosage sensitive gene localized in the DS critical region (DSCR), which is purported to contain genes responsible for many features of DS, including craniofacial dysmorphology. In this study we hypothesize that treatment with epigallocatechin-3-gallate (EGCG), a green tea flavonoid and a specific inhibitor of DYRK1A kinase activity may normalize DYRK1A expression and therefore reduce the severity of the facial dysmorphologies. To test the effects of EGCG treatment in humans we compared facial morphology of children aged 0 to 18 years that were grouped in three different catego-

ries: children without DS, children with DS, and children with DS who are following an EGCG treatment. We acquired three-dimensional facial images using a multi-camera system and characterized facial shape by recording 3D coordinates from 21 anatomical landmarks and 50 semi landmarks. Results of a Principal Component analysis (PCA) based on the slided Procrustes coordinates showed that children with DS are differentiated from euploid children. Children with DS treated with EGCG tend to present a milder facial phenotype, closer to euploid children. These results suggest that EGCG treatment may be an effective way to ameliorate some craniofacial dysmorphologies associated with DS.

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RELATIONSHIP BETWEEN MATURATION OF DISTAL HUMERUS METAPHYSIS AND SKELETAL AGE

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There are numerous published methods and standards for estimating age in inmature individuals by analysing dental and/or skeletal remains. There are several issues pertaining to dental methodologies that hinder an accurate age estimation, such as post-mortem tooth loss or completion of dental development. In these cases, age estimation should be based on the development of other systems, such as the post-cranial skeleton. The most used maturational post-cranial methods are based on the development of the knee, wrist and/or vertebrae. However, as shown in previous work, the humeral proximal metaphysis shape is also a good estimator skeletal age. The purpose of our research was to test if the shape of the distal of the humerus will produce a similar result to the proximal end; thus providing another tool to estimate skeletal age of immature individuals. We measured the metaphysial shape of the humerus geometric morphometrics to quantitatively capture the shape changes throughtout humeral maturation. Our sample consisted of 24 skeletal dista humeral metaphyseis belonging to 11 skeletal individuals aged by dental methodologies and 13 individuals of known age. All humerii were scanned with the NextEngine's Desktop 3D Scanner and the ScanStudio software. In these virtual models a total of 27 landmarks were digitized using the 3D Landmark software. A principal component analysis (PCA) was conducted using MorphoJ software, in order to assess morphometric differences between the distal metaphyses of the humerii. Our preliminary results show that there is a shape pattern related to both dental and chronological age. These results along with those obtained from the proximal metaphysies suggest that humeral maturation could be particularly useful for estimating the age of immature skeletons for forensic, physical and evolutionary anthropology. A better understanding of the humeral metaphysis development will provide more relevant information to aid in comprehending the mechanisms of the shoulder and elbow joints.

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POSTER PRESENTATION ON GEOMETRIC MORPHOMETRICS

COMPARATIVE STUDY OF MANDIBLE FORM DURING THE POSTNATAL ONTOGENY IN SEMIAQUATIC AND FOSSORIAL WATER VOLES

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The knowledge of the ontogenetic trajectories in closely related species with distinct habits can help to understand how important the function can be as a causal factor of morphological differentiation. The mandible is a complex structure and in several hypogeal rodent species it is a potential target for analysis of fossorial habits due to its relevance in the digging activity. In this context, mandible form variation in two water vole taxa, the semiaquatic *Arvicola sapidus* and the fossorial *Arvicola scherman*, was studied. Since in latter species the mandible is an essential structure for digging, differences in the ontogenetic trajectories and in the patterns of integration and modularity between both taxa can be expected. The left mandible of

118 specimens of *A. sapidus* and of 153 individuals of *A. scherman* belonging to six classes of relative age were analyzed by geometric morphometrics. Fifteen bidimensional landmarks where placed in digital images of the external side of the mandible with tpsDig2. The analyses were conducted in MorphoJ and R (geomorph package). In all cases intersexual differences were not significant. According to multivariate regression of shape against size, the ontogenetic trajectories of both taxa appeared to be more similar than expected by chance. However, the ontogenetic trajectories during adulthood are further apart from juvenile stages. Interspecific differences in the modularity and integration patterns were more pronounced in adult specimens. Results suggest an association between the patterns of postnatal growth of the mandible and the mechanical stress derived from the digging activity in fossorial water voles.

EXPLORING THE MAMMALIAN MIDDLE EAR EVOLUTION WITH ANATOMICAL NETWORK ANALYSIS (ANNA)

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The evolution of the middle ear bones of mammals from the jaw bones of early synapsids was a major step in mammalian evolution. Many studies have discussed the processes related to this event, singling out the growth of the dentary bone and the increase in size of the masticatory musculature as the triggers of the exaptation of jaw bones to their new auditory function. This would explain, for example, the correlation in synapsids between the dentary growth and the reduction of post-dentary bones reported by morphometric studies. We have studied the evolution of the middle ear bones of mammals using Anatomical Network Analysis (AnNA) in a broad phylogenetic framework, spanning from the first synapsids to modern mammals. AnNA allowed us to focus particularly on the changes in jaw morphology, modularity, and changes in complexity underlying the evolution of the middle ear. Our results show how the bones involved in mastication and audition have been morphologically reorganized: varying in their topological connections, undertaking losses and fusions, and forming new patterns of connections. Such reorganizations are related to key steps in the evolution of the middle ear, for example, the emergence of the temporal fenestra, the dentary growth, the double jaw joint formation, and the detachment of jawbones from the dentary. We paid special attention to the ossification of the Meckel's cartilage; an event that allowed post-dentary bones to detach from the jaw, integrating them within the otic capsule as new middle ear bones, causing key changes to the anatomical modularity and complexity of the skull.

THE EFFECT OF EGCG TREATMENT ON BRAIN MORPHOLOGY IN DOWN SYNDROME MOUSE MODELS

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Down syndrome (DS) is a genetic disorder caused by trisomy of chromosome 21 that leads to cognitive impairment and brain dysmorphologies. This phenotype may be due to genes located in the DS Critical Region, such as the *DYRK1A* gene encoding a kinase involved in neurogenesis and brain development. A potent DYRK1A kinase activity inhibitor is epigallocatechin-3-gallate (EGCG). Pharmacological treatment using this green tea flavonoid can normalize DYRK1A kinase activity in the hippocampus and improve the cognitive deficits in DS young adults and trisomic TS65Dn mice. As *DYRK1A* is mostly expressed during embryonic development, we hypothesize that prenatal EGCG treatment could also potentially rescue brain dysmorphologies associated with DS. To assess the normal and disease-altered brain morphological changes in DS, we bred 9 litters of Ts65Dn mice and collected *in vivo* MRI data from trisomic and wild type mice at postnatal days 3, 14 and 29. Half of the litters were treated with 30 mg/kg day of

EGCG. We segmented the images and estimated the volume of the whole brain, the four brain ventricles and the cerebellum. We also recorded the 3D coordinates of landmarks defining the shape of the brain. Morphometric analyses, based on Principal Component analyses of the Procrustes coordinates, showed significant differences between trisomic and wild type mice. Ts65Dn mice showed marked ventriculomegaly, whereas trisomic mice treated with EGCG showed normal ventricles that were not significantly different from wild type mice. Overall, this provides evidence that prenatal EGCG treatment can rescue some brain dysmorphologies in DS mouse models.

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THE SUBACROMIAL SPACE IN MAN AND KUCKLE-WALKER PRIMATES

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In the present study we have analyzed by 2D geometric morphometric the anatomy of the subacromial space in man and two species of knuckle -walker primates, chimpanzees (*Pan troglodytes*) and gorillas (*Gorilla gorilla*). Our principal aim is to observe morphological differences that can be related to the different forms of locomotion of the species studied. The analysis was done in lateral view photographs of the subacromial space of 20 humans, 20 chimpanzees and 10 gorillas. The human scapulae came from the Unit of Anatomy and Embryology of the University of Barcelona and the scapulae of chimpanzees and gorillas came from the Museum of Anatomy of the University of Valladolid and from the Museum of Zoology of Barcelona. We observed significant differences in landmark disposition between bipedal humans and knuckle-walkers chimpanzees and gorillas, with no significant differences between the genus *Pan* and *Gorilla*. The main differences observed in the anatomy of the subacromial space between humans and knuckle-walkers included the size of the acromion, the orientation of the coracoid process of the scapula and the relation between the tip of the acromion and the coracoid process. In conclusion, we state that bipedalism and knuckle-walking present significant anatomical differences in the subacromial space that can provide information on the high prevalence of degenerative pathologies in the human shoulder.

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MODULARITY AND COVARIATION IN HUMAN MAXILLARY DENTITION

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Morphological integration refers to the phenotypic interdependence of different biological structures reflecting common functional demands. Modularity exists when integration is concentrated within parts of a structure or modules. Thereby, integration and modularity concern the degree of covariation between modules. Recent studies on dental integration between maxillary teeth in non-human primates with large diastema identified several levels of phenotypic modularity. However, little is known about dental covariance in modern humans. Here, we performed a geometric morphometric (GM) analysis of upper-tooth row shape (I1-M2) variation in adult volunteers (n=68). The dental covariation patterns among key and distal members of morphogenetic fields were analyzed to test their degree of modular integration. Shape variation was extracted from a landmark configuration digitized on 2D-digital images from *in vivo* high-resolution replicas, using generalized least-square Procrustes superimposition. Two-block partial least-squares (2B-PLS) analysis and vector correlation (Rv) coefficient were used to quantify the total amount of cross-covariance among teeth types within the arch. We found a framework of anterior and post-canine modules. Nonetheless, a submodularity within the post-canine dentition suggests a pattern of quantitative genetic independence. The premolar field displays stronger integration (Rv = 0.5; P < 0.001) and more covariance (80%) among the shapes of premolar teeth. In-

stead, molars account for a smaller portion of the total shape integration (Rv = 0.3; $P < 0.001$) and covariation (50%). Our findings indicate that post-canine dentition develops a remarkable range of shape variation, showing higher levels of pleiotropic effects than expected for dental morphogenetic field theory.

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POSTER PRESENTATION ON CLASSICAL MORPHOMETRICS

SEX AND AGE DIFFERENCES OF BODY HEIGHT, WEIGHT AND SOMATOTYPE IN MACEDONIAN ADOLESCENTS

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The purpose of this study was to evaluate sex and age-specific differences of body height, weight and somatotype in Macedonian adolescents. In this study 859 Macedonian adolescent students (447 males and 412 females), at age of 11 to 14 years were included from primary schools in R. Macedonia. Body height, weight, elbow and knee diameter, triceps, subscapular, supraspinale and calf skinfold, arm and calf circumferences were measured using standard equipment and measurement technique to assess the somatotype according to Heat–Carter somatotyping method. Testing of sex and age-specific differences was done with analysis of variance. Differences for $p < 0.05$ were considered to be statistically significant. In all age groups males were more mesomorphic than females. Sex-specific differences were found for endomorph component at the age of 14 years in favour of females, and age differences were found only in males at the age of 12 years ($p<0.05$). Sex-specific differences for mesomorph component at the age of 14 years were found in favour of males. In both males and females at the age of 11 to 13 years dominates two somatotypes mesomorphic endomorh and balanced ectomorph. Our findings suggest that found values might be used in clinical practise for better understanding of changes in body composition and sex differences of somatotypes in Macedonian adolescents.

ANATOMICAL VARIATIONS IN THE SHAPE OF BASILAR ARTERY

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The posterior circulation of the brain constitutes the vertebrobasilar system and its branches, which are responsible for about 30% of the brain blood supply. Many variations are seen in the basilar artery, majority of them in position, origin and shape of the artery. The aim of this study was to investigate the variations in the shape of the basilar artery with CT angiography. We examined radiographs of 103 patients who had CT angiography undertaken for a variety of clinical reasons, performed as a part of their medical treatment at the University Clinic for Radiology in Skopje, R. Macedonia. The study population included 58 male and 45 females, age range from 25-82, mean age 58.4 years. In this study we found three types of variations in the shape of basilar artery, such as arched course, S-shaped course, and the straight course type. The straight course was the most frequent, and it was found in 52.42% of the patients in this study. Arched course was present in 40.77% and S-shaped course was present in 6.79% of the patients. Knowledge of the variations of the basilar artery will increase the success of the surgical procedures and radiological procedures used in interventional radiology in the area of basilar artery.

MORPHOLOGIC CHARACTERISTICS OF SACRA ASSOCIATED WITH TRANSITORY LUMBOSACRAL STATE – LUMBARIZATION IN THE LIGHT OF THEIR POSSIBLE BIOMECHANICAL IMPACT

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Lumbarization is not a frequent transitional state that affects the biomechanics of load transmission at the lumbosacral junction. The aim of the study was to identify morphostructural characteristics of lumbarized sacra and analyse them in the light of their possible biomechanical impact. In order to achieve these objectives 55 dried human adult sacra were examined and divided in 2 groups: the first group consisted of 30 normal sacra and the second consisted of 25 sacra with partial lumbarization of the first segment. Overall linear dimensions of the sacral body and base including the surface areas of their auricular surfaces were measured with the help of a digital sliding caliper, Doctor's tape (Micropore) and a digital planimeter. Students t test was used for comparison between normal and lumbarized sacra. Results indicated that in general the overall dimensions in lumbarized sacra were significantly smaller compared to the normal sacra. Lumbarized sacra exhibited modified sacral base, with ventral small sloping articular part that bore at its sides low positioned auricular surfaces and dorsal bigger non-articular part with more pronounced wedging in comparison to the normal sacra. Additionally the roughen, non-articular areas for ligamentous attachments were lower positioned, at the sides of the second and the third sacral segment. In conclusion the evaluated morphological alterations in lumbarized sacra influence the biomechanics and reduce the effectiveness of the osteoarticular and ligamentous apparatus of the lumbosacral junction and the sacroiliac joints to maintain their stability.

THE MORPHOLOGICAL CHARACTERISTICS OF THE PERFORATING BRANCHES OF THE ANTERIOR CEREBRAL ARTERY

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The morphological characteristics of the perforating branches of the anterior cerebral artery were examined in 133 human cadavers formalin fixed brains in which cerebrovascular pathology was excluded. The samples were of both sexes and the age ranged between 23 and 68 years. We investigated frequency, topography and morphometry (diameter and length of these vessels) using an injection technique with an injection medium consisting of mixture of gelatin and blue Indian ink and microdissection under a surgical microscope. The branches of the anterior cerebral artery were clearly defined by the contrast colour. Data were statistically analyzed descriptively. Average values, standard deviations and range were computed for quantitative variables like diameters and lengths of blood vessels. There were two to ten perforating branches of anterior cerebral artery, most of them (80%) originated from the first 5 mm of the beginning of the anterior cerebral artery. The average distance of the first perforating branch from the terminal bifurcation of the internal carotid artery was 2.4 mm, range 0.4-4 mm. The average diameter of the small perforating branches was 138 μm, range 80-200 μm and the average diameter of the large perforating branches was 352 μm, range 200 -600 μm. The knowledge of the microanatomical and morphological characteristics of the perforating branches of the anterior cerebral artery is clinically relevant for neurosurgeons when performing surgeries in this area.

CRITERIA FOR DETECTION OF GROWTH AND NUTRITIONAL STATUS IN CHILDREN FROM R.MACEDONIA

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Growth monitoring and promotion of optional growth are essential components of primary health care for children. Serial measurements of weight, height/length, for all children, and measurements of circular and transversal parameters compared with growth of a large sample population help to confirm a child's healthy growth and development. The aim of the study is to evaluate the development and sex-specific differences of some anthropometrical parameters which serve as indicators of growth. In order to achieve this objective 200 healthy 3 years old preschool children from Macedonian nationality were analysed. Thirteen anthropometric parameters defining longitudinal, circular and transversal dimensionality of the skeleton were measured, using standard technique. In addition, we calculated the following indices: weight-for

–age; height-for-age and body mass index (BMI). Skin –folds (triceps, scapula and thigh) were also measured. This variables were analysed with descriptive statistics, using (arithmetic mean ± standard deviation), along with ranges expressed in percentiles. Testing of sex-differences was done with ANOVA. During the analysis, sex-specific differences for almost all anthropometric parameters were detected, but they were not significant except for BMI. Girls showed higher values than boys regarding height, weight but for BMI boys showed significant higher values than girls. Values at the 50^{th} percentile in girls were 16.8 kg for body weight (BW), 102 cm for body height (BH) and 16.5 kg/m² for BMI. The values of these parameters in boys were 18 kg for body weight (BW), 96.93 cm for body height (BH) and 17.5 for kg/m² for BMI. The values for skin fold for triceps were higher in girls (13.1 ± 3.5) instead of boys (12.2 ± 3.3). These results are useful as criteria for assessing and detecting deviations in growth and nutritional status in preschool children.

ANTHROPOMETRICAL INDEXES AS NUTRITIONAL INDICATORS IN 6 YEARS-OLD MACEDONIAN CHILDREN

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In order to prevent and reduce the increasing trend of obesity and its consequences, anthropometric characteristics of growth and nutritional status in children have to be constantly monitored. The main objective of this study is to evaluate sex-specific differences of anthropometrical indexes that are used as nutritional status. In order to achieve these objectives 228 healthy children (114 boys, 114 girls) aged 6 from Macedonian nationality were analysed. Body weight, height, mid upper arm circumferences-MUAC, skinfolds thickness triceps–SFTr and subscapular-SFSc were measured using standard equipment and following the International Biological Programme. In addition, and according to standard formulas we calculated: weight-for-age (BW), height-for-age (BH), body mass index-for-age (BMI), mid upper arm circumferences-for age (MUAC), and skinfolds thickness-for-age (SFTr and SFSc). These variables were analysed with descriptive statistics (mean ± standard deviation) along with ranges expressed in percentiles. Testing of sex differences was done with analysis of variance for large, independent samples-ANOVA. In general, results showed sex-specific differences in the examined parameters (BW, BH, BMI) in favour of the boys. On the other hand, skinfolds thickness (SFTr and SFSc) were significantly higher in girls. Values of the 50th percentile in boys were as follows: 24 kg for BW, 119.6 cm for BH, 16.52 kg/m2 for BMI, 16.5 cm for MUAC, 4.35 mm for SFSc, and 7.2 mm for SFTr. The values of these parameters in girls were: 262 kg for BW,118. cm for BH,15.94 kg/m2 for BMI, 16.7 cm for MUAC, 5mm for SFSc and 7.85 for SFTr. These results can be useful as criteria for the assessment and detection of deviations in the nutritional status in children aged 6 from Macedonia.