

## REVIEW

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**Regarding:** Dissertation work for the award of the Doctor of Science degree in the field of higher education: 4. Natural sciences, mathematics, and informatics, professional direction: 4.1. Biological Sciences, Doctoral program: Anthropology

**Author:** Silvia Yanakieva Nikolova

**Topic:** "Medico-biological aspects of cranial sutures: microstructure, physiological closure, metopism"

**1. General presentation of the procedure and the researcher.** By order No. RD-09-37/27.07.2023 of the Director of IEMPAM-BAN, I am appointed as a member of the scientific jury in connection with the procedure for the defense of the dissertation work on the topic "Medico-biological aspects of cranial sutures: microstructure, physiological closure, metopism" for the acquisition of the "Doctor" degree in the field of higher education 4. Natural sciences, mathematics, and informatics 4.3. Biological Sciences, Doctoral Program Anthropology. The author of the dissertation is Silvia Yanakieva Nikolova - Associate Professor in the "Anthropology and Anatomy" section of IEMPAM-BANS. To prepare the review, I received the materials required by the Regulations for the conditions and procedures for acquiring scientific degrees and holding academic positions at IEMPAM-BAN.

Assoc. Prof. Silvia Yanakieva Nikolova, Ph.D. completed higher education at the Faculty of Biology of SU "Kliment Ohridski", where she acquired a bachelor's degree in "Biology and Chemistry" (2003) and a master's degree in General Anthropology (2005). In 2011, he defended his thesis for the acquisition of the ONS "Doctor" at IEMPAM-BAN.

From January 2009 to April 2011, he worked as a specialist - biologist at IEMPAM-BAN. From May 2011 to June 2012, she was an assistant, from July 2012 to January 2023, a chief assistant, and from 2023, an associate professor at IEMPAM-BAN.

**2. Relevance of the topic.** The dissertation is devoted to the study and elucidation of the processes of formation, functioning, and closure of the sutures of the cranial vault, which are critical for the normal morphogenesis of the skull. Unraveling the molecular signaling pathways and cellular mechanisms that govern these processes occupies a major part of research in the field.

**3. Knowing the problem.** The introduction, provided basic information about the stages and mechanisms of formation and closure of the cranial sutures of the cranial roof, as a prerequisite for the normal development of the cranial roof, as well as the creative use of the cited literary material, showing knowledge of the state of the problem treated in the dissertation.



243 standard pages and contains 71 figures and 28 tables. In 7 appendices, the used craniometric points, reference lines, and measured parameters are specified.

The **literature review** was done thoroughly, with a clear, multi-layered, and representative examination of the problem, which shows a good awareness of the doctoral student and the possibility of deriving a relevant goal and research tasks. The factors determining the morphogenesis of the cranial vault are outlined. Particular attention is paid to the formation of sutures and their physiological closure. The molecular and cellular mechanisms of the morphogenesis of the cranial sutures are discussed. Etiological and epidemiological factors influencing the development of craniosynostosis are presented. Metopism is presented as the frequency of occurrence, determining factors, and possible changes in cranial morphology. The relationship between anatomical variations and pathological conditions is discussed.

The present work **aims** to study the microstructure and physiological closure of the cranial sutures and to evaluate the specifics of the cranial morphology in metopism.

The **research material** includes 318 skulls of adult individuals, of which 159 are male and 159 female. The cranial series are collective and unite material from 8 necropolises. The material is properly selected, homogeneous, well documented, and precisely registered, guaranteeing the results' reliability.

**Research methodology.** Reliable and sufficient methods were used to achieve the set goal. The morphometric analyses were performed entirely in the virtual space after generating two-dimensional and three-dimensional images of the studied skulls. This allows obtaining an adequate answer to the tasks solved in the dissertation work. A Nikon XT H 225 industrial micro-computed tomography ( $\mu$ CT) system manufactured by Nikon Metrology was used to take digital radiographs and generate volumetric three-dimensional images of the skulls.

Statistical methods are appropriately selected, including classical statistical analyses, geometric morphometrics and artificial intelligence, machine learning, and data mining, which provide the information necessary for the study.

The **"Results"** section is presented in 129 pages. The results of the research are presented in a very informative way, enabling a multi-layered and diverse presentation of the role, significance, and interrelationships between the studied indicators. The obtained data are thoroughly analyzed and graphically demonstrated. The tables and figures are well selected and presented in a modern design and analytical approach.

Based on an established correlation between the physiological closure of the sagittal suture of the skull and the age of adult males, Associate Professor Nikolova developed an original descriptive scale for reporting the degree of closure of the suture at the level of each of the three layers. The observed delay in closure of the sagittal suture in metopic skulls suggests that the factors responsible for the persistence of the metopic suture do not act in isolation in the frontal region. Correlations were established between the existence of metopic sutures and the presence of specific changes in the morphology of the cranial vault, orbital, and nasolabial regions. A persistent metopic suture is often accompanied by decreased frontal sinus pneumatization. Supernumerary bones have been demonstrated in the cranial vault of metopic skulls, probably formed by fragmentation of normal ossification centers, as well as by the occurrence of additional centers in the cranial sutures and fontanelles. The possibility is noted that the



combination of all the marks characteristic of metopic skulls expresses a disturbance in intramembranous ossification, which is overexpressed in some types of skeletal dysplasia. Models developed and applied by the doctoral student for determining age at death by sagittal suture closure in metopic skulls are presented.

**The discussion** is characterized by completeness and depth in reflecting on the research findings in the context of the research objective and literature data.

**The conclusions** adequately reflect the results obtained, but a more synthesized presentation would highlight the more clearly formulated generalizations.

**The contributions** that the author noted can be described as follows:

1. Protocols have been developed to generate three-dimensional images of dry skulls by laser scanning and microcomputer tomography with the possibility of subsequent statistical analyses of morphometric data.
2. An innovative method was developed and a protocol was approved for staged scanning of objects larger than the area of the detector of the  $\mu$ CT system (Nikon XT H 225) with subsequent merging of the two series of images.
3. A virtual database of three-dimensional images of homogeneous metopic and control cranial series of modern Bulgarians was created.
4. A descriptive scale was created to report the degree of seam closure in a cross-section in each of the layers.
5. Configurations of points describing the morphology of both the entire skull and its parts are constructed.
6. Applied approaches from geometric morphometry for comparative analyses of size and shape in metopic and non-metopic cranial series, with and without additional variations in the occipital region.
7. Artificial intelligence methods have been developed to extract morphometric features to distinguish metopic from control skulls.

Evaluation of publications and personal contributions. Assoc. Prof. Nikolova has presented a total of 32 publications on the topic of the dissertation, of which one chapter in a monograph abroad, 16 publications in foreign journals with impact factor and impact rank, 3 publications in journals with only impact rank, 11 publications in refereed journals without impact factor. In 26 of the publications, she is the lead author, which expresses her contribution to the scientific works.

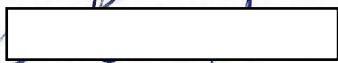
**The abstract** is structured properly, reflecting the content, main results, and contributions of the dissertation work.

**My recommendations** for the future use of the dissertation contributions and results are for their application in the further study and expansion of this promising issue, in which the obtained results are also published in independent scientific articles.

**Conclusion.** The dissertation work of Associate Professor Silvia Nikolova examines a problem that is of scientific and practical interest in the field of morphological and clinical sciences. Scientific results have been obtained, enriching knowledge on the processes of development and the cranial roof and cranial sutures. A large volume and difficult-to-perform work was carried out. This shows that she has theoretical knowledge and professional skills in the scientific specialty, as well as skills for conducting scientific research with a modern design.



In conclusion, I believe that the presented dissertation offers interesting findings and interpretations of the researched problem and Assoc. Prof. Nikolova deserves to be awarded the scientific degree "Doctor of Sciences" by the Extended Scientific Council of IEMPAM-BAS.



**Prof. Stefan Sivkov, MD, PhD**

15.09.2023