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ADAM MICKIEWICZ UNIVERSITY, POZNAŃ
Faculty of Biology
Institute of Human Biology and Evolution

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Prof. UAM dr hab. Marta Krenz-Niedbała
Adam Mickiewicz University in Poznan
Faculty of Biology
Institute of Human Biology and Evolution

Review of the doctoral dissertation of Ms. Joanna Heuchert, MSc
entitled „Radiomorphometric indices of the mandible in the assessment of bone mineral density in historical populations" written under the scientific supervision of Prof. dr hab. Sławomir Kozieł and auxiliary supervision of Dr Anna Spinek
at the Department of Anthropology
Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences

1. Formal foundations for preparing the review

The basis for this review originates from a letter authored by Prof. dr hab. Andrzej Gamian, Director of the Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wrocław, dated October 31, 2025 (RN-111/2025). The letter notified me of my invitation to serve as a reviewer for Ms. Joanna Heuchert's dissertation.

The formal foundation for this review is provided by the Law on Higher Education and Science of 2024, which outlines the procedures and requirements for conducting activities related to doctoral theses. According to the law, the doctoral dissertation presents the candidate's general theoretical knowledge in the discipline or disciplines and the ability to conduct independent scientific or artistic work. Furthermore, the subject of a doctoral dissertation is an original solution to a scientific problem, an original solution involving the application of the author's own research results in the economic or social sphere, or an original artistic achievement (Article 187 1-4, Journal of Laws of 2024, item 1571).

This doctoral thesis is situated within the discipline of the biological sciences. In the following sections, I assess the extent to which the dissertation meets the relevant requirements, with particular emphasis on its originality; its structural and formal qualities; the sources of information and research methods employed; its overall scholarly contribution as an original solution to a defined academic problem; and the Candidate's breadth of theoretical knowledge.

2. The evaluation of the structure and formal aspects of the dissertation

The dissertation spans a total of 141 pages, and comprises 13 well-organized, structured chapters. The content consists of *Summary* both in Polish and English, *List of abbreviations*, *Introduction* (24 pages), *Aims of the study* (1 page), *Materials and methods* (12 pages), *Results* (30 pages), *Discussion* (13 pages), *Conclusions* (3 pages), *References* (35 pages), *List of figures and tables*, and *Scientific achievements* of the Doctoral Candidate. The thesis includes 32 tables and 10 figures in the form of images and charts. The text is supplemented with information about the Author's research activity, i.e. peer-reviewed journal articles and conference presentations.

Each chapter is divided into sections and subsections. From a structural perspective, the clear division of the content into distinct parts enhances readability and facilitates comprehension. The Introduction chapter presents the research topic and its significance, together with an overview of the theoretical framework of bone biology and metabolism, as well as methods for assessing bone quality, including their challenges and limitations. This approach to organizing the content is somewhat unconventional. A more typical structure would place the theoretical background in a separate chapter or chapters, allowing the *Introduction* to guide the reader from general concepts to the specific research focus by establishing the research context, identifying gaps in current knowledge, defining the research problem and its significance, and outlining the specific research questions or objectives. The broad range of topics covered in the theoretical background has resulted in several sections being relatively short (for example, 4.7.1. Environmental factors affecting bone health in past populations), which limits the amount of information and depth with which the state of the art can be discussed. Nevertheless, the theoretical framework is logical and well organized, and the key concepts and arguments are clearly communicated.

The language used throughout the thesis is professional and precise, with technical terminology clearly defined and appropriately interpreted. Tables and figures are well

designed, clearly labeled, and properly referenced. The reference list is well organized in alphabetical order, and in-text citations are accurate and consistent.

In conclusion, with regard to its structure and formal aspects, the dissertation meets the requirements expected of a doctoral thesis.

3. Significance of the research problem raised in the reviewed dissertation

Bioarchaeology investigates bone health in past populations with the aim of gaining insights into nutrition, disease, activity patterns, reproductive behavior, and physiological stress. By examining how biocultural factors influenced skeletal development, strength, and fragility, this field sheds light on conditions such as osteoporosis, growth disturbances, and fractures, offering perspectives on historical health that extend beyond modern clinical assessments. Over the past decades, there has been heightened interest in examining long-term trends in human bone quantity and mechanical competence, such as a gradual decline in skeletal strength relative to body size, associated with increasing sedentary lifestyles. The research problem of bone gain and loss examined through human skeletal remains is up-to-date and significant not only in terms of reconstructing life conditions and challenges in the past, but also to provide a long-term, evolutionary and biocultural context for understanding skeletal health today. It can be achieved through exploring factors which influenced health of people living under different subsistence strategies, activity levels, and environments, since past populations serve as natural experiments that can help contextualize modern bone health challenges.

In conclusion, the research problem raised in the dissertation is both justified and important, because, despite growing interest in bone status, there is still need for non-invasive quantitative methods of bone mineral status assessment of different skeletal elements, considering fragmentary preservation of human remains from historical periods. On top of that, there are numerous clinical studies applying mandibular radiomorphometry, while this tool has been rarely used in research of past human populations. This study therefore addresses a well-defined and consequential research problem with the potential to contribute meaningfully to both scholarly knowledge and broader understanding.

4. Purpose and scope of work

The dissertation aims at examining the feasibility of mandibular radiomorphometric indices as a proxy for femoral bone mineral density, and the relationships between these two kinds of bone quality measures and biological and environmental factors of the historical

populations form Lower Silesia in Poland. This approach can potentially provide an additional tool for bone maintenance measurement, and reveal the factors influencing skeletal health.

The integration of bone health indicators with skeletal markers of oral health, including periodontitis, caries, and antemortem tooth loss, and childhood nutritional stress, i.e. enamel hypoplasia, is in line with life course perspective, and as such it provides insight into how early-life and proximate-life conditions affect adult skeletal health.

For future research design I would propose to include Fourier transform infrared (FTIR) spectroscopy analysis to quantify the degree of diagenetic changes in the examined human skeletal remains.

In my opinion, the study presents a well-defined and meaningful research aim, with an approach and scope that are appropriate for a doctoral dissertation.

5. Detailed assessment of particular chapters of the dissertation

Introduction

As noted above, the main concept of the study and the current state of knowledge could be more easily followed if the theoretical background of bone gain and loss were presented in a separate chapter. This could provide greater flexibility to expand specific aspects of the study and allow for a more in-depth discussion of these issues. Nevertheless, this is a minor concern.

Among the methods used to evaluate bone structure from radiographs, it may be beneficial to include fractal dimension (FD) analysis as a non-invasive approach for quantifying changes in trabecular bone architecture associated with mineral loss, especially in osteoporosis. Additionally, the use of more recent references could be considered in several instances—for example, when discussing the current state of calcium insufficiency (data cited from 2016), referring to "recent evidence" (Section 4.4.2, paragraph 3, reference from 2012), or stating that an issue has been widely examined (Section 4.8, paragraph 1, references from 2006 and 2010).

Generally, the scope of the information presented is comprehensive and well aligned with the topic of the dissertation. I particularly appreciate the inclusion of information addressing existing knowledge gaps in the study of the skeletal mineral status of the mandible, as well as the clearly articulated five research questions and their consistent reference throughout the *Results*, *Discussion*, and *Conclusions* sections.

Materials and methods

While the study seeks to incorporate environmental factors, the available information on the environments of the two skeletal series is somewhat limited. The archaeological context is outlined over approximately one and a half pages and focuses primarily on burial practices and general socio-economic background, and a reference to the general environment and diet is made in a single paragraph in Introduction, p. 17. Particularly, the discussion of the results might benefit from a broader contextual scope, potentially including additional aspects such as dietary structure, which is known to play an important role in mandibular bone loss and maintenance. In cases where site-specific dietary data are not available, relevant insights could be drawn from general archaeological and historical studies of comparable medieval settlements and early modern towns from Polish territories. Expanding the contextual framework in this way could further support and enrich the interpretation of the results.

With regard to sex assessment, I would appreciate clarification on how the study by Anastasiou and Chamberlain (2013) was applied, given that their work employed geometric morphometric methods to assess size and shape variation in the human sacroiliac joint in order to investigate sexual dimorphism. In addition, I would welcome clarification regarding the meaning of the aging approach described as age being estimated "as a complex value."

The methodology for investigating mandibular radiomorphometric indices is described in detail; however, for clarity and in keeping with the graphics that clearly depict the mandibular measurements, a similar form of visualization for the four-region densitometric measurements of the femur would be appreciated.

The description of the methodology for enamel hypoplasia and caries examination would benefit from additional detail, as it is unclear, for example, whether any inclusion or exclusion criteria were applied regarding the number or type of teeth retained for caries analysis. In Section 6.4.1, the Author cites Karkus (2018) for assessing periodontitis, though the method was originally proposed by Strohm and Alt (1998) and later modified by Karkus. In 6.4.2. section I would welcome the information which types of dental enamel hypoplasia have been examined, together with clarification of the meaning of the phrase "Only individuals with at least two teeth with hypoplastic defects were considered for the analysis" – did the Author mean, that only those with at least two teeth present were qualified for the study or that there must have been at least two teeth with EH to consider the individual

affected? I also would like to know which criteria by Buikstra and Ubelaker (1994) have been followed to record presence or absence of antemortem tooth loss.

The Doctoral Candidate employed a range of statistical methods to identify meaningful relationships among the examined variables. Parametric tests (Student's t-tests and ANOVA) were used for normally distributed data, while nonparametric tests (Chi-square test, Fisher's exact test, Mann–Whitney U test, and Kruskal–Wallis test) were applied to non-normally distributed or categorical variables. Effect sizes were reported, and post hoc tests were conducted where appropriate. Variables showing no sex-related differences were analyzed in combined male–female samples. Associations between radiomorphometric and densitometric measurements were assessed using correlation analyses, and adjustments for multiple comparisons were applied. Multiple linear regression models were constructed to evaluate the predictive value of radiomorphometric indices for BMD, with appropriate diagnostic checks performed to ensure that regression assumptions were met. Measurement reliability across different devices was evaluated using intraclass correlation coefficients and Bland–Altman analyses. In addition, absolute and relative technical measurement errors were calculated to quantify precision. All statistical analyses were performed using the R statistical computing environment. In my opinion, the statistical analysis has been reliably and accurately performed.

Overall, the Materials and Methods section is well developed and meets the standards expected of a research study.

Results

The *Results* chapter begins with descriptive statistics for demographics, radiomorphometric indices, BMD, and oral health indicators. The findings are then organized by type of relationship: sex- and age-related differences, associations between radiomorphometric and densitometric data, and links with oral health status, presented separately for the Milicz and Wrocław samples. These are followed by interpopulation comparisons of mandibular radiomorphometric indices, BMD, and oral health measures, starting with inter-equipment reliability. The title of Section 7.6, however, is somewhat misleading, as it refers only to bone status indices ("Interpopulation differences in the mandibular radiomorphometric indices and bone mineral density"), while the section also addresses oral health and multiple regression analyses examining predictors—biological profile, population, and radiomorphometric indices—of BMD. Despite the above mentioned

minor concern, I appreciate that the subsection titles explicitly reference the specific research questions.

For future manuscript design I would recommend minimizing redundancy between the text and the tables, such as the data on pages 60–62 compared with Tables 8–9, and including explanations of abbreviations in the table footnotes to improve clarity and readability.

On page 70, a trend is noted toward higher caries prevalence in young adults compared to middle adults. I suggest to consider whether this pattern can reflect the loss of molars with age, given that these teeth are particularly susceptible to decay.

Overall, this chapter is well organized and reflects the Author's effort to present the data in a clear and accessible manner.

Discussion

This chapter is organized around the specific research questions and is followed by methodological considerations, including the comparability of different approaches to assessing bone mineral status, the use of clinical versus bioarchaeological samples, and limitations inherent to the examined material. These limitations include broad chronological samples' attribution and measurement precision. The final subsection discusses implications for future research, highlighting the potential of panoramic radiography for bone status assessment, the advantages of further study of documented human osteological collections, and the application of micro-CT imaging. It also emphasizes the importance of integrating skeletal data with evidence on nutritional status, physical activity, and disease within broader socio-cultural and environmental contexts.

Overall, the Discussion is concise and clearly structured; however, its excessive brevity may leave the reader wishing for a more extensive contextualization of the results. The eight pages primarily summarize the Author's own findings, with relatively limited engagement with comparative studies. Expanding the discussion to include a broader range of contextual data and a more detailed comparison with existing research on bone mineral health could further strengthen the interpretations, and enhance the explanatory potential for the observed differences between the medieval and modern samples. For example, more detailed information on dietary structure in the two examined settlement types might help to better contextualize and support the observed patterns.

The Author refers to one study addressing mandibular radiomorphometric indices in a historical population, while additional relevant literature is available. Including further

studies—such as Savić Pavičin et al. (2024*), Diachronic Comparison of Three Historical Skeletal Series from Croatia with Regard to Mandibular Bone Quality* (Heritage 7[1]), published 31 December 2023 and López-Leyva et al. (2025*), Mandibular bone mass density in a medieval population and its relationship with stable isotopes $\delta^{13}C$ and $\delta^{15}N$* (Odontology 113:432–437), published online 06 July 2024—could enrich the comparative framework and provide additional perspectives.

My minor comment regards the absence of an observed correlation between periodontosis and bone mineral density, in contrast to findings reported in clinical studies. It may be helpful to consider whether this difference could be related to methodological variation, particularly in the assessment of periodontal disease in clinical versus bioarchaeological settings.

In conclusion, notwithstanding the remarks outlined above, the Discussion chapter generally meets the standards expected of a doctoral thesis.

Conclusions

The final chapter revisits the main findings, structured as responses to the individual research questions. It is commendable that the Author, on the basis of the moderate correlations observed between certain mandibular indices and femoral bone density, formulates a cautious conclusion regarding the utility of mandibular radiomorphometric indices in skeletal health assessment, emphasizing their role as a supportive rather than a self-sufficient method. I concur with the Doctoral Candidate that this approach represents a valuable asset for bioarchaeological research.

References

The dissertation is based on an extensive and carefully selected body of literature (N = 300), reflecting a thorough review process and a substantial scholarly effort on the part of the Doctoral Candidate. I would only recommend incorporating a limited number of more recent publications, as noted in my review above.

6. Scientific Achievements of the Doctoral Candidate.

The Doctoral Candidate is a promising researcher, as evidenced by two publications in 2024 in reputable journals: a meta-analysis and systematic review of mandibular radiomorphometric indices in *Osteoporosis International*, with M.Sc. Joanna Heuchert as first and corresponding author, and a research article in the *American Journal of Human Biology*.

A third manuscript, on a topic directly related to the dissertation and with the Candidate as first author, has been submitted to *Scientific Reports*. According to the Web of Science, the Candidate currently has an H-index of 2 and 22 citations.

7. Conclusion

In my assessment, the dissertation submitted for review constitutes rigorous research of both a theoretical and empirical character. Its relatively high degree of difficulty arises primarily from methodological challenges inherent to the skeletal sample, including limited sample sizes in certain subgroups and the use of different devices for the assessment of bone mineral status. The work demonstrates the Author's extensive knowledge and capacity to conduct complex and demanding research. The theoretical aspects are presented with conciseness and clarity, demonstrating the Candidate's ability to synthesize and communicate complex information effectively, while also highlighting the significance and complexity of the research topic, as well as gaps in the existing literature. The Author's judicious selection and adaptation of appropriate methods to address the research problem reflect a level of competence consistent with doctoral-level expectations. The Results chapter further demonstrates the Candidate's ability to analyze and present their own data with clarity and rigor. In my view, the main area for improvement lies in a deeper interpretation of the results, which would further strengthen the thesis.

To sum up, in my opinion, the doctoral dissertation constitutes an original solution to a scientific problem and demonstrates the Candidate's broad theoretical knowledge in the discipline of the biological sciences, as well as their ability to conduct independent scientific research. The research topic is cognitively engaging; the planned objectives were successfully achieved; the research questions were adequately addressed; and the conclusions were formulated in a proper and critical manner. Consequently, the aim of the dissertation was fully accomplished.

I hereby declare that the doctoral dissertation meets the requirements specified in the Article 187(1)-(4) 1-4 of the Act on Higher Education and Science (i.e. Journal of Laws of 2024, item 1571, as amended) and I request that the doctoral student be admitted to the further stages of the procedure for awarding a doctoral degree in the discipline of biological sciences.

Stwierdzam, że rozprawa doktorska spełnia warunki określone w art. 187 ust. 1-4 Ustawy *Prawo o szkolnictwie wyższym i nauce* (t.j. Dz. U. z 2024 r. poz. 1571 z późn. zm.). oraz

wnioskuję o dopuszczenie Doktorantki do dalszych etapów postępowania w sprawie nadania stopnia doktora w dyscyplinie nauki biologiczne.

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Podpis