ENHANCED KNOWLEDGE IN SCIENCES AND TECHNOLOGY
e-ISSN: 2773-6385

EKST

Vol. 6 No. 1 (2026) 717-724
https://publisher.uthm.edu.my/periodicals/index.php/ekst

Assessment on Skeleton Preservation Preparation Techniques of Rats

Haifa Hazwani Habir[1], Arney Sapaat[1]*

[1] *Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, UTHM Kampus Cawangan Pagoh, Hab Pendidikan Tinggi Pagoh, KM 1, Jalan Panchor, 84600, Pagoh, Muar, Johor, MALAYSIA.*

*Corresponding Author: arney@uthm.edu.my
DOI: https://doi.org/10.30880/ekst.2026.06.01.080

Article Info

Received: 4 January 2026
Accepted: 4 February 2026
Available online: 9 July 2026

Keywords

Skeleton Preservation, Rat Skeleton, Burial Method, Boiling Method, Chemical Method, Hydrogen Peroxide, Papain

Abstract

Small mammal bones are fragile and easily damaged and existing methods often produce inconsistent levels of cleanliness, discolouration and loss of structural detail, while also lacking clear information on time and cost efficiency. This study investigates and compares three preservation techniques for small mammal's skeletons: burial, boiling and chemical to address persistent problems in preparing rat skeletons for teaching and research. In response to these gaps, this study aimed to determine which preservation techniques are most effective in maintaining the structure of rat skeletons and to identify methods that are both time-effective and cost-effective. Nine protocols were tested: three burial variants (direct, in container, on tray), three boiling variants (plain water, enzyme-based detergent, papain) and three hydrogen peroxide concentrations (3%, 6%, 15%). Each protocol was evaluated for soft-tissue removal, bone colour, odour, skeletal integrity, processing time and material cost. Boiling with papain at 40–50 °C produced the best overall outcome, yielding bright white, fully defleshed bones within 45 minutes at moderate cost while preserving structural detail. Chemical maceration with 15% hydrogen peroxide gave rapid whitening in three days but at higher cost and with greater risk of surface etching whereas burial methods were the cheapest but required 67 days and were more difficult to manage. These finding suggest that boiling with papain is recommended as the most balanced technique for routine preparation of rat skeletons.

1. Introduction

Small mammal skeletal specimens are important role in biological sciences such as biodiversity research, anatomical studies, forensic investigations and museum curation where bones preserve morphological records for scientific references [1][2][3]. Their preservation allows long-term accessibility to anatomical and taxonomic information that can help regarding species identification, comparative study and evolutionary investigation [1][4]. Rats are common and popular as a model system for small mammals because of their ecological significance, their high adaptability and comparable anatomical features [5][6]. However, the small size and fragile nature of rat skeletons are constraints for skeleton preparation as bone breakage [7], incomplete removal of soft tissues and surface decay may occur when inappropriate preservation methods are used [8].

In laboratory and educational environments, inefficient skeleton preparation methods impose practical limitations. Traditional method such as burial, boiling and chemical method are widely differ in effectiveness, processing time, cost and impact on bone integrity [9][10]. The long processing times, unpleasant odours, high in chemical use and variability in quality preservation make some of these techniques less suitable for routine

teaching and repeated specimen handling [11][12]. These limitations emphasize the need for preservation techniques that are reliable, time efficient, cost effective which can keep all bones integrity for long period.

Various methods for cleaning and preserving skeletal material have been described in the literature. Burial methods depend on the natural decomposition and are usually low cost but time consuming and uncontrollable [9][10]. Meanwhile, for boiling methods allow rapid removal of soft tissues that can made it more efficient by adding detergents or enzymes. However, when the specimens expose to high temperatures which may eventually weaken the bone structure if not carefully controlled [11][12][13]. Next, for chemical method especially hydrogen peroxide is frequently used in bleaching and final cleaning. However, excessive concentration or duration of exposure can lead to surface etching and microstructural damage in small mammal bones [14][15].

Therefore, this study aims to systematically compare the skeleton preservation techniques using rat specimen as representative small mammal model. The objective of this study is to determine which preservation techniques are most effective in maintaining the structure of rat's skeleton and to identify which method that are both time effective and cost effective to preserve rat's skeleton. This study aims to give practical advice in choosing skeleton preparation methods in biological and anatomical research by addressing these objectives.

2. Materials and Method

2.1 Materials

This study utilized laboratory rat (Wistar rat) were employed as a model of a small mammal since they are used in many instances in an anatomy teaching and biological research. The number of rat specimens to be used was nine, which were prepared in preparation of nine preservation protocols. Preservation procedures were done by pre-dissecting all the specimens, thereby removing the skin and major organs.

2.2 Preservation Techniques

The preservation techniques evaluated in this study were categorised into three main approaches: burial, boiling and chemical methods.

2.2.1 Burial Method

For the burial method, three different burial approaches were used: direct burial in soil, burial in container and burial in tray. Samples were placed in a standardized depth and left to decompose in the soil with the help of natural processes until all soft tissue was removed through processes that could be replicated in the variety of skeletal preparation literature [9][10].

2.2.2 Boiling Method

For the boiling method, three different boiling approaches were used: boiling in plain water, boiling with enzymatic detergent and boiling with papain. The control conditions were to immerse the samples in water and heat them. Enzyme using boiling method, which involves application of enzyme in the process of boiling, is done at moderate temperatures, to result in higher tissue damage and lower bone damage as proposed by other previous studies [11][12][13].

2.2.3 Chemical Method

Three chemical concentrations approaches were employed in chemical method as 3, 6 and 15 percent. The reason why hydrogen peroxide was used is that it is readily applied in skeletal whitening and final cleaning [14][15]. After the treatment, all specimens were rinsed and air dried.

2.3 Data Collection and Analysis

Data to be used in this study were collected as systematic observation of all the skeleton preservation techniques and stages. All of the data were gathered and arranged in a structure table in the Microsoft Excel. Skeletal quality was assessed visually using three criteria: bone cleanliness, bone color, amount of time that was spent on total processing of the skeleton to obtain it, presence or absence of odor, amount of money spent on purchasing the material used. Bone cleanliness was rated on a descriptive scale ranging from incomplete defleshing to complete removal of soft tissue meanwhile for the bone color was evaluated from darkly to bright white appearance. Representative photographs were taken before and after treatment for documentation.

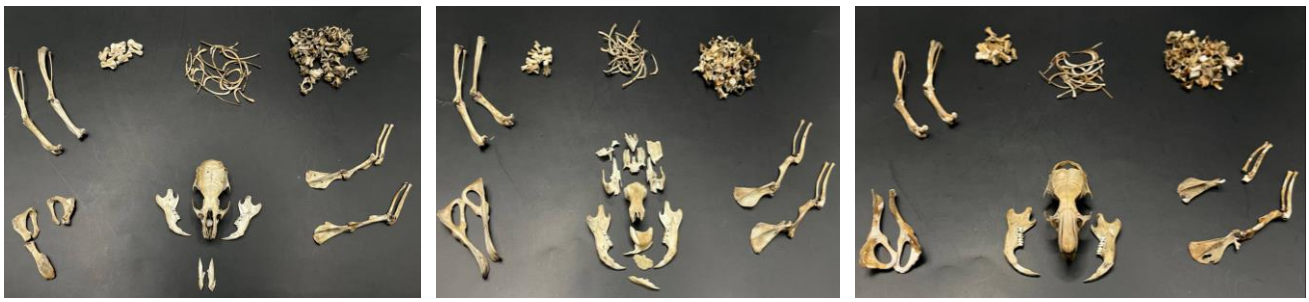
The assembled data was descriptively analysed in order to be in a position to compare the preservation methods. They were handled on Microsoft Excel to sort, filter and organise the data so as to effectively assess the processing time, cost and general skeletal condition that are related to each method of treatment. Such a combination of methodology guaranteed the systematic data management, minimized the risk of the

Enhanced Knowledge in Sciences and Technology Vol. 6 No. 1 (2026) 717-724

observational bias, and improved the visibility or the ability to replicate findings to determine the effectiveness or practical applicability of the non-evaluated approaches or methods of skeleton preservation.

3. Result and Discussion

The outcomes of the nine preservation protocols demonstrated clear differences in time efficiency, cost effectiveness and bone condition.



(a) (b) (c)

Fig. 1 Condition of rat skeleton that been: (a) Buried directly; (b) Buried on tray; (c) Buried in container

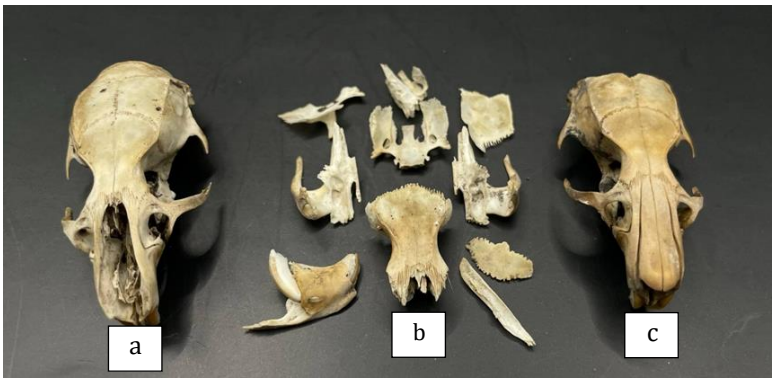


Fig. 2 The condition of rat's skull that been (a) Buried directly; (b) Buried on tray; (c) Buried in container

From figure 1, the burial method resulted in varying degrees of soft-tissue decomposition and skeletal cleanliness depending on the burial condition. Fig. 1 illustrates the overall condition of rat skeletons subjected to direct burial, burial on tray and burial in a container while in Fig. 2 shows that the rat's skull parts for each burial method. 67 days after burial, all burial treatments resulted in substantial removal of soft tissue validating the efficacy of natural decomposition in moist soil conditions.

Direct burial resulted in the cleanest skeleton with pale to off white appearance and very little remaining soft tissue among of all three treatments (see Fig. 1(a)). Meanwhile, burial on tray, the skeletonization was acceptable bun bone surfaces presented light brown stains (Fig. 1(b)). In contrast, specimens buried in containers resulted greater brown discolouration (Fig. 1(c)) especially on more robust skeletal elements. Skull specimens reflected similar trends with directly buried skulls appearing cleaner and lighter than those recovered from tray and container burials. All burial methods generated strong earthy and putrefactive odours at exhumation and required the same processing duration of 67 days while there is no cost for this burial method at RM 0.

Higher cleanliness of the directly buried specimens could be a result of free access to soil microorganisms and small insects without any barriers which could have allowed for the most effective natural decomposition. Highly moist lateritic soils also favour a rich microbial and invertebrate fauna leading to the decomposition of soft tissue during the burial [1][2]. In contrast, buried in container was also have barriers make the insect decomposer hard to get in to the specimens. Other than that, kept out drainage and prolonged the contact of bone with decomposition fluid causing greater absorption of iron oxides and humic substance resulting in deeper brown appearance on skeleton as reported in previous skeletal studies based on burials [3][4].

The tray burial enabled partial drainage and reduced staining when compared to container burial but the presence of rigid burial hardware increased the risk of damage of bones during excavation especially to fragile

cranial elements. This type of excavation related breakage has been reported in taphonomic and forensic recovery context where support structures complicate the retrieval [5]. Although burial methods were the most cost effective but is slow process to get the skeleton with pungent odors as well as excavation risks make it unsuitable for routine teaching labs, direct burial represents the most effective burial-based option when cost minimisation is prioritised and time constraints are less critical [1][4].

(a) (b) (c)

Fig. 3 Condition of rat skeleton that been: (a) Boil plain; (b) Boiling with vanish; (c) Boiling with papain

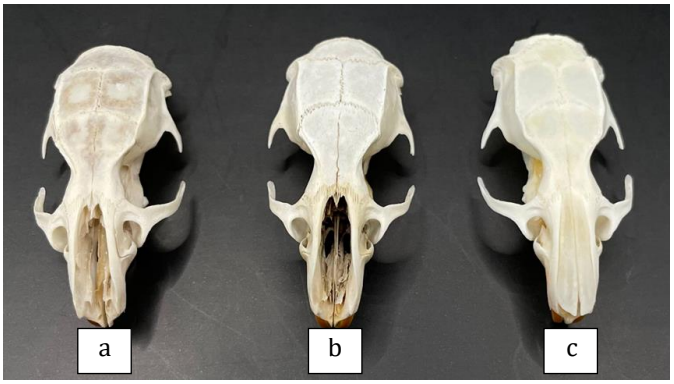


Fig. 4 The condition of rat's skull that been: (a) Boil plain; (b) Boil with vanish; (c) Boil with papain

Fig. 3 presents the condition of rat skeletons prepared using three boiling protocols namely plain boiling, boiling with enzyme-based detergent (Vanish) and boiling with papain while Fig. 4 illustrates the corresponding skull conditions. Clear differences were observed in cleaning efficiency, processing time, bone colour and soft tissue remnant in three treatments.

Plain boiling deboning at approximately 100 °C for 1 hour and 30 minutes partially defleshed the skull and some tissues were present in the cranial cavity and surrounding closely associated bones (Fig. 4(a)). The skeleton result in creamy white appearance rather than pure white appearance (Fig. 3(a)). The odor during the process resembles the smell of cooking broth. The material costs were also none because the polymer did not need to be dissolved by any chemical reagents.

The presence of boiling with vanish leads to the enhancement of separation of tissue in 1 hours. Cases of the soft tissues were almost free to removal and only had slight manual cleaning to do. The appearance of the bones was whiter than the plain boiling ones but retained low tone of cream colour (Fig. 3(b)). The odor during the process was dominated by detergent scent and the cost for this process was RM 6.26.

Papain boiling was done at specific temperature 40-50 °C less than 45 minutes were required to skeletonize the full body entirely. Bones were widely parted with soft tissue and appeared easily and bright white with a clean cranial cavity (Fig. 3(c)) (Fig. 4). The odor during the process was like burn like scent and the cost for this process approximately RM 10.

The incremental increase in cleaning efficacy that is observed between plain boiling on the one hand and enzyme-aided boiling on the other hand serves as an indicator of the acceleration of protein and lipid hydrolysis produced by enzymatic hydrolysis. Maceration with no additives is a commonly known uncomplicated, yet relatively poor approach, which can commonly leave behind tissue residues in small cavities and high chances of

Enhanced Knowledge in Sciences and Technology Vol. 6 No. 1 (2026) 717-724

collagen denaturation and bone weakness provided prolonged exposure occurs [6][7]. This is the reason why incomplete defleshing and creamy colour were found in the plain boiling protocol.

Tissue removal was made easier with the addition of enzyme-based detergent which is a combination of proteolytic enzymes and surfactants that disperse lipid thus, promoting tissue removal without the use of harsh mechanical cleaning methods. Related advantages of detergent maceration have been seen in anatomical and forensic experiments and enzyme-rich detergents were shown to give cleaner bones in less time than water alone and were less destructive than strong alkali solutions [8][9][10]. In this work, boiling with Vanish offered an effective trade-off between time advantage, cleanliness and reasonable price.

Boiling using papain gave the best overall results of the boiling techniques. Controlled sub-boiling temperatures-maintained enzyme activity and reduced heat-induced bone damage leading to rapid and complete defleshing with an outstanding visual appearance. The results are in line with the past reports showing that proteolytic enzymes like papain work well when used in optimum temperature ranges [9][11]. Therefore, papain boiling is the most efficient, skeletal preserving, and practical method of boiling with regard to laboratory use.

(a) (b) (c)

Fig. 5 Condition of rat skeleton that been: (a) 3% H_2O_2; (b) 6% H_2O_2; (c) 15% H_2O_2

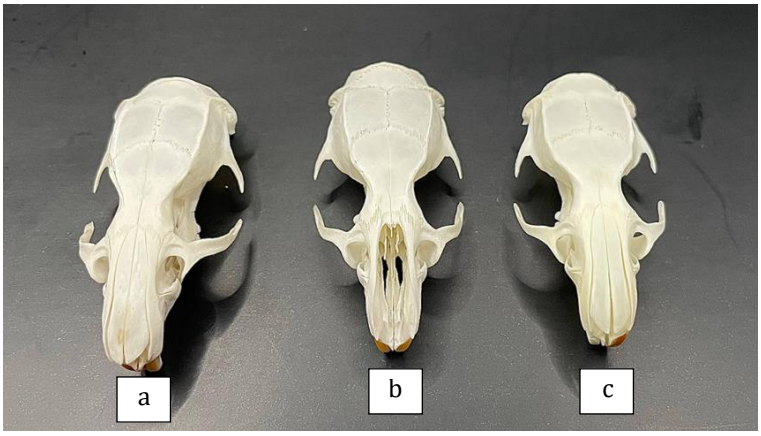


Fig. 6 The condition of rat's skull that been: (a) 3% H_2O_2; (b) 6% H_2O_2; (c) 15% H_2O_2

Fig. 5 depicts the state of the rat skeletons that were placed in 3%, 6%, and 15% of hydrogen peroxide where the Fig. 6 depicts the skeletal skulls. The peroxide concentration was found to differ significantly in regards to the effectiveness of soft-tissue removal, bone colour, odour intensity and processing time.

It took seven days to recuperate fully at 3% hydrogen peroxide. The soft tissues became soft progressively yet partly stuck, and needed further cleaning by hands. These bones were light cream in colour (Fig. 5(a)) which had residual staining that could be observed and pungent odor that remained strong throughout the treatment period. The estimated cost for this protocol was RM 9.90.

Hydrogen peroxide 6% treatment minimized the treatment time to four days. The majority of soft tissues were easily detached yielding cleaner bones in white-cream colour even though slight staining could still be

found in certain places (Fig. 5(b)). Odour remained strong during treatment, and the estimated cost increased to RM 11.90.

The 15% hydrogen peroxide procedure gave full defleshing in three days and yielded the best and lightest skeletal display of all the chemical treatments (Fig. 5(c)). Leftover tissues were nearly completely dissolved, did not require much manual cleaning (Fig. 6), and the odor was not as tenacious as in lower-concentration ones. However, this protocol incurred the highest cost at RM 15.50.

The concentration-related trend in the treatment of hydrogen peroxide is evidence of the oxidative power of the solution. At low concentration, hydrogen peroxide has a minimal impact as a potent bleaching solution compared to an effective macerating solution and such a solution clarifies the long processing time, residual soft tissue and lingering odor during the 3% protocol [3][6]. This validates past suggestions that low-concentration peroxide would be more effective in the end whitening process than an independent defleshing one.

Increasing the concentration to 6% improved both speed and cleanliness. That moderate concentrations accelerate oxidative breakdown of proteins and fats resulting in better defleshing and whitening without severe surface damage when exposure is carefully monitored [12]. Other than that, moderate concentrations can give satisfactory cleaning of small mammal skeletons with acceptable preservation of microstructures [10]. However, the residual staining and the strong smell level reveals that the action was incomplete across concentration levels which were higher.

The quickest and the most aesthetically pleasing results were obtained with the use of the 15% hydrogen peroxide treatment, which proved the high efficiency of the strong oxidising solutions in skeleton preparation. The same results have been obtained in comparative studies that have emphasized the benefits of high-concentration peroxide in the production of display-quality specimens over short periods of time [9][11]. Nevertheless, several authors have warned that high concentrations and prolonged exposure can cause surface etching, micro-cracking and loss of microscopic detail especially in small or thin bones [9][10]. These risks coupled with the uppermost reagent cost led to the nervous and discriminating utilization of high concentration peroxide relative to the purpose of reaction of the specimens.

On balance, the risk of time, cost and its long-term skeletal preservation, the selection of chemical concentration used to treat should be time efficient, visually pleasing, which is overall excellent, and inexpensive using hydrogen peroxide.

Table 1 Comparison of preservation methods by time and cost

Variables	Burial			Boiling			Chemical		
	Directly	**In container**	**On tray**	**Plain**	**With vanish**	**With papain**	**3%**	**6%**	**15%**
Time	67 days	67 days	67 days	$1\frac{1}{2}$ hours	1 hours	45 minutes	1 week	4 days	3 days
Cost (RM)	0	0	0	0	6.26	10.00	9.90	11.90	15.50

Table 1 shows a comparative overview of the processing time and cost of material of all the nine skeleton preservation protocols analysed in this paper. The methods of burial, such direct burial, burial in a container, and burial on tray, did not have any material cost, yet took the longest processing time of 67 days to obtain a satisfactory skeletal process. Being cost effective, long processing time is an important limitation that may severely restrict their use in routine laboratory and teaching tasks which need a quick collection of specimens.

Among boiling methods, plain boiling involved no chemical cost but required 1.5 hours and resulted in incomplete soft-tissue removal, reducing overall efficiency despite minimal expenditure. Boiling with enzyme-based detergent (Vanish) reduced processing time to 1 hour at a moderate cost of RM 6.26 and produced cleaner specimens. Boiling with papain yielded the shortest processing time of 45 minutes at a cost of RM 10.00 and produced the highest level of skeletal cleanliness, indicating the most favourable balance between time efficiency, cost, and preservation quality among boiling protocols.

Hydrogen peroxide chemical maceration was characterized by an apparent trade-off existing between concentration, processing time and cost. The 3% treatment required one week at a cost of RM 9.90 and produced limited cleaning efficiency. Increasing the concentration to 6% reduced processing time to four days at RM 11.90, while 15% achieved the fastest chemical processing time of three days but at the highest cost of RM 15.50 and with a greater risk of surface damage to delicate bones. In general, boiling with papain is the most effective trade-off concerning time and cost, as compared to the burial processes that are the least expensive but most time-consuming and the use of the chemical concentration method that is the fastest but relatively costly.

4. Conclusion

According to the output of this paper, the most efficient method of preserving skeletal functionality of rat specimen was found as boiling with papain, which gives a reasonable compromise between processing time, material cost, ability to remove soft-tissues and skeletal integrity. The technique regularly generated clean and well-defined bones with a short time and caused minimal structural damage hence it was very appropriate in routine laboratory, teaching and research work.

Further research is needed to improve the situation by enhancing reproducibility and increased application focusing on standardisation of papain-assisted boiling conditions especially enzyme concentration, temperature and exposure time to achieve inter-laboratory consistency and consistency in preservation [7]. There could also be a possibility of comparing it to other plant-based enzymes like bromelain to get an opportunity to process much faster and apply more environmental-sustainable ways to preserve it.

It is suggested that further research with the use of analytical methods like Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD) be conducted to determine how mineral composition, collagen integrity, and microstructural stability of the proteinous material undergoes various changes because of various preservation methods [6]. These evaluations would provide a supplement to macroscopic examinations and understand better the bone quality after treatment.

Moreover, papain-assisted boiling needs to be tested on other small vertebrates (mice, birds, amphibians) in order to ascertain species-species responses to the process of enzymatic processing. It is also advisable that long-term research on the colour stability, resistance to microbes, and brittle nature of the bones be used during the long storage, to assist museum curation and learning collections. Taken together, these activities would help to develop efficient, safe and sustainable skeletal preservation protocols.

Acknowledgement

The author would like to thank the Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia for its support.

Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Haifa Hazwani Habir, Arney Sapaat;* ***data collection:*** *Haifa Hazwani Habir;* ***analysis and interpretation of results:*** *Haifa Hazwani Habir, Arney Sapaat;* ***draft manuscript preparation:*** *Haifa Hazwani Habir, Arney Sapaat. All authors reviewed the results and approved the final version of the manuscript.*

References

[1] R. Anderson, Methods of Collecting and Preserving Vertebrate Animals. London, UK: Read Books Ltd., 2020.
[2] A. M. Christensen, N. V. Passalacqua, and E. J. Bartelink, Forensic Anthropology: Current Methods and Practice. London, UK: Academic Press, 2019.
[3] L. Backwell, J. B. Huchet, J. D. G. Harrison, and F. d'Errico, "Invertebrate modification of bone," in Manual of Forensic Taphonomy. Boca Raton, FL, USA: CRC Press, 2021, pp. 631–666.
[4] K. E. Galbreath et al., "Building an integrated infrastructure for exploring biodiversity: Field collections and archives of mammals and parasites," Journal of Mammalogy, vol. 100, no. 2, pp. 382–393, 2019.
[5] M. E. McCampbell et al., "Compensatory mortality explains rodent resilience to an invasive predator," Journal of Mammalogy, vol. 104, no. 5, pp. 967–978, 2023.
[6] L. M. Ceríaco and M. P. Marques, "Fluid-preserved zoological specimens in Portuguese natural history collections: A historical overview and implications for collection management," Natural History Collections and Museomics, vol. 2, pp. 1–69, 2025.
[7] C. A. Keyes, K. R. Giltrow, and T. J. Mahon, "A comparison of maceration methods for the preparation of infant skeletal remains for forensic anthropological analysis," International Journal of Legal Medicine, vol. 138, no. 3, pp. 1085–1092, 2024.
[8] Md. A. Z. Faruk and S. K. Das, "Detergent maceration: A convenient skeleton preparation technique for teaching and demonstration of veterinary anatomy," Journal of Applied Veterinary Sciences, vol. 7, no. 4, pp. 43–48, 2022.
[9] K. T. Onwuama, S. O. Salami, M. Ali, and J. O. Nzalak, "Effect of different methods of bone preparation on the skeleton of the African giant pouched rat (Cricetomys gambianus)," International Journal of Morphology, vol. 30, no. 2, pp. 425–431, 2012.

[10] A. I. Popp et al., "Effects of different traditional methods of cleaning skeletal material: Preliminary evaluation based on scanning electron microscopy," Forensic Science International: Reports, 2023.
[11] N. Jannat, R. Islam, and N. Sultana, "Preparing and presenting a pigeon skeleton for gross anatomical study using boiling maceration method," American Journal of Life Science and Innovation, vol. 2, no. 1, pp. 26–32, 2023.
[12] A. M. R. Bezerra, H. Celeste, and A. Soeiro, "The use of multi-enzymatic detergent for cleaning flesh from bones of small mammals preserved in fluid and frozen," Boletín Científico Centro de Museos Museo de Historia Natural, vol. 28, no. 2, pp. 87–101, 2024.
[13] R. C. Yakkundi, N. Matapathi, and V. Talawar, "A review of bone preparation techniques for anatomical studies," Journal of Ayurveda and Integrated Medical Sciences, vol. 8, no. 10, pp. 112–117, 2023.
[14] T. Chen et al., "Current surface modification strategies to improve the binding efficiency of emerging biomaterials with bone and soft tissue: A literature review," Journal of Prosthodontic Research, vol. 67, no. 3, pp. 337–347, 2022.
[15] J. W. Park et al., "Preparation of rat skeletal muscle homogenates for nitrate and nitrite measurements," Journal of Visualized Experiments, no. 173, e62427, 2021.