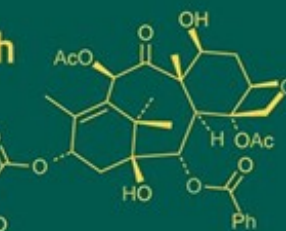
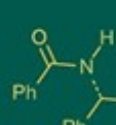


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An insight into different types of maceration and skeleton preparation techniques

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ABSTRACT

This article aims to provide an insight into various maceration and skeleton preparation techniques used for osteology instruction and research. The method involves a comprehensive review of procedures ranging from traditional burial and natural decomposition to contemporary approaches such as enzyme solutions, dermestid beetle colonies, and temperature-controlled baths. The main results highlight that method selection must be guided by specimen size, age, fat content, and anatomical delicacy to preserve the organic bone matrix and avoid damage like warping or chemical etching. The authors conclude that high-quality preparation is achieved by adapting the method to the specific specimen rather than prioritizing speed, and that the value of physical collections is further enhanced by integrating digital imaging and three-dimensional printing.

Keywords: Maceration, Skeleton preparation, Osteology, Dermestid beetles, Veterinary anatomy

Introduction

Skeletal material is essential for osteology instruction because it enables direct examination of anatomical features such as shape, orientation, joints, foramina, and specialized adaptations. Properly prepared skeletons serve diverse purposes, including education, anatomical comparison, surgical planning, forensic analysis, species identification, museum exhibitions, and research. In contrast to wet specimens, dry skeletons can be preserved for decades when properly cleaned, assembled, and stored. (Mandler *et al.*, 2026; Javid *et al.*, 2023; Savitri *et al.*, 2023; Kaliyamoorthy *et al.*, 2022; Singh, 2017) [1, 2, 3, 4, 5]

Skeleton preparation encompasses a series of procedures that begin with documenting the carcass and end with preserving or assembling the bones. During this process, skin, organs, muscles, and other soft tissues are removed while preserving critical anatomical structures. The primary challenge is removing soft tissue without compromising cartilage, collagen, delicate bone plates, or small, difficult-to-repair bones. Historically, skeletal preparation relied on burial or natural decomposition. Contemporary laboratories employ enzyme solutions, dermestid beetle colonies, temperature-controlled baths, advanced degreasing techniques, and digital imaging. These innovations allow practitioners to tailor preparation methods to the specimen's size, age, fat content, and intended application, rather than relying solely on traditional approaches. (Mandler *et al.*, 2026; Savitri *et al.*, 2023; Kirbach *et al.*, 2026) [1, 3, 6]

Basis of skeleton preparation

Bone consists of both mineral and organic components. The mineral fraction, primarily hydroxyapatite crystals, imparts strength, while the organic matrix, mainly type-I collagen, provides toughness and resistance to fracture. Excessively harsh cleaning may result in visually clean bones that subsequently flake, warp, or fracture due to collagen degradation. Therefore, preparation methods should prioritize preservation of bone integrity over processing speed. (Singh, 2017; Popp *et al.*, 2023; Bittner-Frank *et al.*, 2025) [5, 7, 8]

Alkalis saponify lipids and hydrolyze proteins, while soil organisms facilitate decomposition of buried remains. Dermestid larvae consume desiccated tissue. Microbial enzymes degrade proteins during maceration, with protease activity optimized at specific pH and temperature ranges. Heat denatures tissue, enabling manual removal. Each technique differentially impacts cartilage, ligaments, marrow fat, and bone, accounting for variability in specimen quality.

Characteristics for selection of a preparation method

An effective protocol should yield a specimen that is anatomically complete, structurally intact, free of residual tissue, dry, odorless, and stable during handling. In practice, the most advantageous method is one that achieves these outcomes while limiting permanent damage. (Mandler *et al.*, 2026; Savitri *et al.*, 2023; Kirbach *et al.*, 2026) [1, 3, 6]. Nasal conchae, auditory ossicles, sesamoid bones, hyoid components, and ju

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venile epiphyses are examples of delicate parts that should be preserved. There shouldn't be any heat cracks, warping, powdering, or chemical etching on the surface. To avoid subsequent yellowing and rancid odor, sufficient removal of marrow and cancellous fat is necessary. The procedure should enable the safe containment of biological dangers and the appropriate disposal of reagents or liquids. The final product should still be appropriate for labeling. Most preparation protocols adhere to a similar sequence, despite differences in specific techniques. Before cleaning, it is essential to document species, age category, sex, morphometric data, collection history, and any pathological findings. This information is irretrievable once the carcass has been deconstructed. (Mandler *et al.*, 2026; Kirbach *et al.*, 2026) [1, 6] Method selection is guided by the biological characteristics of the specimen and the intended outcome. For example, museum voucher specimens require greater preservation than temporary teaching bones, and small reptile skulls necessitate different handling compared to adult bovine limbs.

1. Body size and species: Larger carcasses require longer degreasing times, larger containers, and more thorough defleshing.
2. Age: Loss or deformation is more frequent in juvenile skeletons since they have more cartilage and unfused epiphyses.
3. Fat content: Obese animals, pigs, and marine mammals may need to be repeatedly treated with a solvent or detergent.
4. Anatomical delicacy: Insect or enzyme-based cleaning is beneficial for fish, birds, reptiles, and small mammals.
5. Time and infrastructure: Warm-water systems require consistent temperature management but are quicker than passive maceration.
6. Use: Different preservation standards apply to research samples, museum vouchers, forensic exhibits, and articulated teaching mounts.

7. Safety and waste management: the laboratory's ability to control odors, contagious materials, alkali, or flammable solvents must be taken into account.

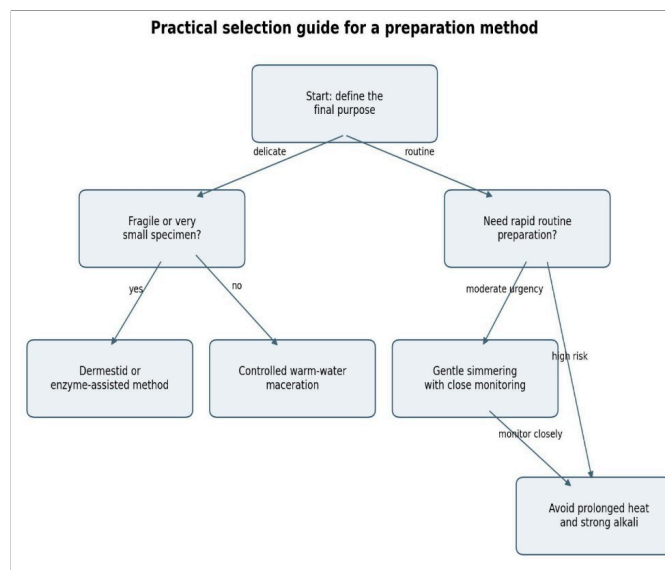


Figure 1. Simplified decision guide for selecting a cleaning method [original source]

Classification of cleaning techniques

In practice, categories often overlap. For example, a specimen may undergo manual defleshing, warm-water maceration, detergent degreasing, and peroxide whitening. Combining mild procedures is often safer than relying on a single severe treatment. (Mandler *et al.*, 2026; Savitri *et al.*, 2023; Kirbach *et al.*, 2026; Mahon *et al.*, 2021; Husch *et al.*, 2021; Rastekar *et al.*, 2026) [1, 3, 6, 9, 10, 11] (Figure 2)

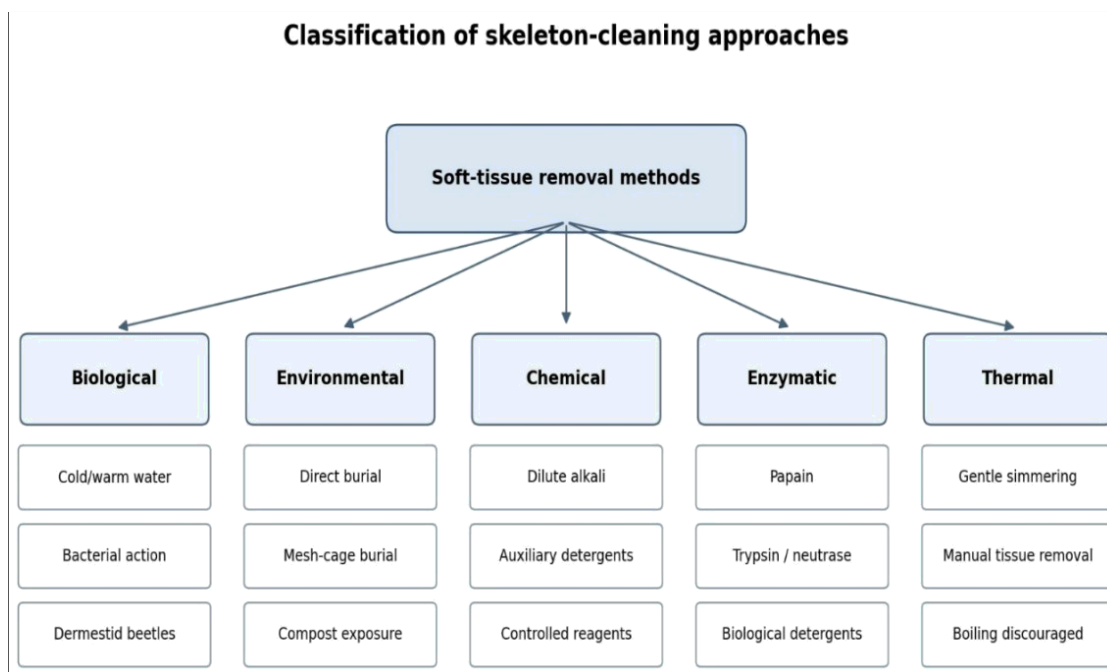


Figure 2. Major categories of skeleton-cleaning methods

Biosafety and environmental protection

Even when they appear to have decayed, carcasses can still contain zoonotic pathogens, and preparation processes may produce contaminated effluent, aerosols, splashes, or sharp pieces. Before skinning or maceration, a risk assessment should be completed. Without institutional approval and proper containment, materials suspected of rabies,

anthrax, brucellosis, or another regulated pathogen should not be brought into a regular teaching preparation area.

Personal protection:

- Closed shoes, a lab coat or apron, and impermeable gloves.
- Use protective goggles or a face shield when handling chemicals, splashing, or rinsing under high pressure.

- Respiratory protection in areas where there may be mould, aerosols, or strong decomposition odors.
- Supportive gloves that can withstand cuts when working with blades, wire, or sharp pieces of bone.

Chemical and fire safety:

- Only use concentrated peroxide and caustic alkali in a ventilated space with an eyewash station.
- Acetone and other solvents should be kept away from sources of ignition in authorized flammable liquid containers.
- Write the chemical name, concentration, date, and specimen identification on each bath label.
- Unless the institutional safety procedure specifies compatibility, avoid combining cleaning products.

Waste disposal:

Alkali waste, contaminated water, soft tissue, and solvent should all be kept apart. Chemical waste should be neutralized or collected by an authorized disposal system, whereas biological waste must adhere to institutional biomedical waste procedures. After all specimens, containers, equipment, and work surfaces have been thoroughly cleaned and sanitized.

Recent and emerging developments

Thermostatic And Recirculating Maceration Tanks

By keeping a steady temperature and eliminating odor, modern tanks increase uniformity. Marked sections and mesh baskets minimize the loss or mixing of tiny bones. Although automated systems can reduce processing times, they still need to be monitored to avoid overexposure. (Mandler *et al.*, 2026; Kirbach *et al.*, 2026; Bittner-Frank *et al.*, 2025) [1, 6, 8]

Table 1. Displays the Applications in Veterinary and Allied Sciences

Field	Use of prepared skeletal material
Veterinary education	Osteology practicals, joint orientation, surgical approaches and species comparison.

Field	Use of prepared skeletal material
Clinical and orthopedic research	Biomechanical testing, implant planning, fracture modelling and validation of imaging findings.
Wildlife conservation	Species identification, reference collections, biodiversity documentation and study of rare taxa.
Veterinary forensics	Assessment of trauma, age, species, anatomical origin and suspected animal abuse or wildlife crime.
Museums and outreach	Durable exhibits that demonstrate locomotor adaptation and comparative morphology.
Archaeology and zooarchaeology	Identification of animal remains, domestication patterns, diet and past human-animal relationships.
Taxonomy and morphometrics	Reference measurements, description of variation and comparison of skeletal characters.

Bioenzymatic Protocols

Selective tissue removal with lower chemical damage is made possible by fine-tuning the enzyme concentration, buffer composition, and incubation temperature. These methods are particularly useful for specimens that are small, delicate, or significant for study. (Mahon *et al.*, 2021; Husch *et al.*, 2021; Ramos-Bezerra *et al.*, 2024) [9, 10, 12]

Three-Dimensional Scanning And Printing

Students can rotate, magnify, and compare bones without repeatedly handling rare originals thanks to surface scanning and CT-derived replicas. For teaching, surgical practice, and public presentation, three-dimensional prints can replicate diseased or species-specific features. (Kapoor, 2024; Blackburn *et al.*, 2024; De Silveira *et al.*, 2021; Bakili *et al.*, 2025; Schirone *et al.*, 2025; Sakaue *et al.*, 2024) [13, 14, 15, 16, 17, 18]

Computer-Assisted Morphometry

Automated measurements and digital landmarking provide objective comparisons between species, age groupings, or illness conditions. Because trustworthy algorithms rely on precisely defined and thoroughly documented content, these technologies augment rather than replace the reference skeleton. (Kapoor, 2024; Blackburn *et al.*, 2024) [13, 14] (Figure 3.)



Conclusion

Figure 3. Representative images (A) 3D-printed anatomical bio models, in which students examined physical replicas of veterinary bones produced by additive manufacturing. (B) Real anatomical specimens obtained from the institutional osteological collection. (C) Two-dimensional educational materials, including textbooks, printed illustrations, and anatomical atlases (Sakaue *et al.*, 2024) [18].

High-quality skeleton preparation is achieved by adapting the method to the specific specimen, rather than prioritizing speed. For many mammalian specimens, passive or warm-water maceration yields reliable results. When preservation of delicate features is required, dermestid beetles and controlled enzymatic treatments are particularly effective. Aggressive chemical digestion presents significant risks to the organic bone matrix, while burial is economical but limits procedural control. Strong boiling and chlorine bleach should be avoided

for long-term specimens, although gentle simmering may be appropriate for robust routine material. (Mandler *et al.*, 2026; Kirbach *et al.*,

2026; Rastekar *et al.*, 2026; Pahl, 2020) [1, 6, 11, 19]

Table 2. Cumulative total of all types of processes.

Method	Typical duration	Preservation	Relative cost	Odour	Most appropriate use
Cold-water maceration	Several weeks to months	Excellent	Low	Very high	Fine structures and museum material
Warm-water maceration	About 1-3 weeks	Very good	Moderate	High	Routine teaching skeletons
Dermeistid colony	Days to weeks	Excellent	High initial care	Moderate	Birds, reptiles, skulls and research specimens
Burial / mesh burial	Months or longer	Variable	Very low	Low at surface	Large carcasses and field situations
Chemical digestion	Hours to days	Variable to poor if excessive	Moderate	Low	Rapid controlled preparation of robust material
Enzyme-assisted method	Days	Excellent	High	Low	Delicate and high-value specimens
Gentle simmering	Hours	Fair to good	Low	Moderate	Robust routine specimens
Boiling	Hours	Poor	Low	Moderate	Generally unsuitable for scientific collections

Cleaning represents only one phase of the overall process. The utility of the final specimen depends on comprehensive degreasing, meticulous whitening, gradual drying, accurate sorting, anatomically precise articulation, and permanent documentation. Given the involvement of tissue decomposition, hazardous chemicals, and flammable solvents, biosafety and waste management are equally critical. The educational and research value of physical collections is further enhanced by digital imaging, three-dimensional scanning, and printing, which nonetheless depend on well-prepared and thoroughly documented original specimens. (Kapoor, 2024; Blackburn *et al.*, 2024; Bakili *et al.*, 2025) [13, 14, 16]

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