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Preparing and Presenting a Pigeon Skeleton for Gross Anatomical Study Using Boiling Maceration Method: A Quick and Effective Method

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ABSTRACT

The study's main objective was to prepare and display the pigeon skeleton using a practical and effective approach. Two matured domestic pigeons (Columba livia), aged 8 months old were purchased from local market, Gazipur sadar, Bangladesh for this purpose. This experiment was carried out in the department of Anatomy and Histology, Faculty of Veterinary Medicine and Animal Science in Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, in January 2021. The birds were first killed by being injected with $MgSO_4$ directly into the hearts. After dead, they were peeled using a knife. 3% soda water solution was used to boil peeled animals for two hours. Having cooling, the bony samples were cleaned and dipped into 10% solution of bleaching water for 1 hour to avoid further growth of microorganism following washed by tap water. After drying in the sun for eight hours, the skeletal pieces were fully articulated for the skeletal frame. The whole processes were taken 2 days (33 hours). The boiling maceration method for preparation of skeleton is very effective because restrain the chance of losing small bones, requires less expenditure, time and labor. It is also visible that the bone's original color was preserved. Extracted bones were divided into two parts, axial and appendicular skeleton. Axial skeleton comprises the bones of skull, vertebrae such as-cervical, thoracic, lumbar and sacral vertebrae (fused to form synsacrum) and coccygeal vertebrae or caudal vertebrae then ribs, costal cartilages and breast bone or keel bone. In contrast, the appendicular skeleton comprises the forelimb or wing bone like scapula, coracoid, clavicle, humerus, radius-ulna and manus. On the other-hand hindlimb or pelvic limb comprises of pelvic girdle, femur, tibio-fibula and feet bone (metatarsal and digits). In adult pigeon no developed tarsal bone. Therefore, this is the most efficient way for quickly preparing and presenting skeleton for use in studies of the avian skeletal system.

INTRODUCTION

Skeletons serve as informative equipment in veterinary and medical education, exhibits in museums, hunting trophies, and an important part of scientific research materials (Kempa, 2016). A skeleton is usually a rigid supportive or protective structure or framework of an organism that supports the rest of the body and facilitates movement (Merrium-webster, 2021). The more delicate skeletal system of vertebrates is internal and is composed of mainly by connective tissues. This includes bone and the various fibrous substances that form the joints, connect bone to bone, bone to muscle, enclose muscle bundles and attach the internal organs to the supporting structure (Sidnie, 2019). This skeleton is divided into two parts for study purposes: axial and appendicular. In which axial skeleton formed by the bones of skull, vertebral column, ribs and sternum. On the other hand, the main component of appendicular skeleton are limbs (fore and hind limbs) (Ghosh, 2016). However, bird skeleton is less differing from the other vertebrates. Birds have a lightweight skeleton made of mostly thin and hollow bones. The keel-shaped sternum (breastbone) is where the powerful flight muscles attach to the body. Birds have a smaller total number of bones than mammals or reptiles.

The significance of bird skeleton preparation to ornithology study and biodiversity preservation. Avian skeleton preparation is basic need for gross anatomical study. The skeleton of birds is crucial for study on a variety of topics, including phylogenic studies, age and growth assessments, and functional morphology (Bemis *et al.*, 2004; Burke and Feduccia, 1997; Olson 1973). Skeleton are helpful for studying evolutionary morphology and identifying animals from archeological sites. Skeleton preparation serves a precious opportunity to gain anatomical knowledge of bones, muscles, tendons, nerves, etc (Takeshi, 2010). Skeleton collections are necessary for various forensic applications, including identifying animal and bird carcasses that have been illegally stolen or bones that have been used as evidence in other crimes (Olson, 2003). As a result, the necessity of bird skeleton preparation is increasing rapidly. For academic purposes different bird skeletons have also been prepared for anatomical and comparative studies. The main drawback of skeleton preparation is time consuming, with maceration often being the most time-consuming and difficult phase. Skeleton preparation is always a drawn-out, laborious, and delicate procedure requiring numerous processes. Several maceration methods, such as, burial maceration, cold water maceration, hot water maceration, insect maceration, chemical maceration, and enzymatic maceration are available (Raghavan, 1964; Gofur, 2010). Veterinary educational institutions do not undertake skeleton preparation due to procedural

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complexities (Mahapatra *et al*., 2018).

The main aim of this study was to describe the total time required for the skeleton preparation procedures as well as how quickly the axial and appendicular skeleton of bird species could be processed and assembled.The findings of this research will serve as a guide in the skeleton preparation of a bird species within a concise period of time and contribute to the knowledge for building up anatomy museum. Through anatomy (osteology), one learns about the bones in anatomical and physiological ways. This present study was undertaken for better understanding of avian anatomy by preparation of skeleton in a quick and effective methods so that we can treat the bird species in better ways.

MATERIALS AND METHODS

A total of 2 matured pigeons (Columba livia), aged 8 months old, were purchased from local market in Gazipur sadar and transported to the department of Anatomy and Histology, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. All the pigeon was possessed good health and devoid of any external deformities certified by the registered veterinarian. All kinds of precautions were taken during skeleton preparation such as use of rubber gloves during preparing a carcass and care was taken to avoid stick injuries according to Baker *et al.* (2003).

The materials used for skeleton preparation were: (1) Wires of various gauges (2) Drill machine (3) Soda (Sodium carbonate, NA_2CO_3) (4) 10% bleaching solution (5) Wooden platform (6) Good quality sticking materials (Fevicol) (7) Varnish.

The methods involved in skeleton preparation were: (1) Birds preparation (2) Skinning, evisceration and de-fleshing of carcass (3) Carcass maceration (4) Sequential arrangement of bone and bone drying (5) Construction of skeletal frame and bone varnishing.

Birds Preparation

Before slaughter all the pigeons were kept 6hours fasting condition. Then, $MgSO_4$ was injected directly into heart. The IACUC approved these euthanasia methods of killing animals for humane killing of animals and recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia, 2000 and in accordance with humane euthanasia defined by the Federal Animal Welfare Act (54 FR 36112-36163) (Source: IOWA)

Skinning, evisceration and de-fleshing of carcass

Skin was removed by cutting between the skin and muscle with a scalpel while pulling the skin. Then maximum amount of muscles, adipose tissue and all visceral organs were removed with the aid of scalpel, blades, forceps, scissors and knife up to the bones were visible.

Carcass maceration

The bones of the pigeon were heated by using normal heater in 3% solution of soda water (Sodium carbonate, NA_2CO_3) with anionic surfactant (detergent) to over 80ºC for 2 hours in a metal drum regarding to the concept from Baker *et al.* (2003) and Van Cleave (2010). Soda (Sodium carbonate, NA_2CO_3) water solution was used for proper and complete digestion of muscles (Gofur and Khan, 2010). Then it was kept 2 hours for cooling and after that the remaining fleshes, tissues and ligaments on the boiled bones were removed with knives. Then all the bones were rinsed with clean tap water and dip into 10% bleaching water solution for 1 hour to prevent further growth of microorganisms (Musa *et at*., 2015).

Sequential arrangement of bone and bone drying

Axial skeletal bones such as, skull, vertebrae, ribs and keel bone were arranged in sequential order and protected with the help of fine stainless steel wire. Appendicular skeletal bones such as, forelimb and hindlimb bones were also placed in order in a steel plate. Then all the bones were kept in sunlight for a period of 8 hours for complete sundry.

Construction of skeletal frame and bone varnishing

The skeletal frame was constructed in a wooden stage of 12-inch length and 10-inch breadth. All the bones were articulated in a proper way to form a bird skeleton.

Construction of axial skeleton

All the vertebrae such as, cervical, thoracic, lumbar and sacral vertebrae (fused to form synsacrum) and caudal vertebrae were articulated with the aid of fevicol glue in a sequential order. Then, occipital bone of skull and cranial part of the atlas were drilled and tied by using stainless steel wire. All pair of ribs were attached with their corresponding vertebrae to sternum by using glue according to the concept of Van Cleave (2010).

Construction of appendicular skeleton

The forelimb (wing) and hindlimb (leg) were articulated with the axial skeleton with the help of stainless steel wire. Firstly, wing skeleton bones namely, scapula, coracoid, clavicle, humerus, radius-ulna and manus were articulated sequentially by using adhesive glue. Then hindlimb bones namely, hip bone (consists of ilium, ischium and pin bone), femur, tibia -fibula and feet bone (metatarsal and digits) were tied up chronologically with the aid of fevicol glue.

The skeletal framework was positioned in a wooden platform of required dimension according to the size of the animal for display and gross anatomical study. Varnishing of bone was done by using combined mixture of thinner and varnish to give the bones shiny appearance.

RESULTS

The bones expelled from chemical maceration process

comprised of the axial and appendicular skeleton. All the bones appeared whitish and intact. The axial skeleton comprised of the bones of- skull, vertebrae such as-cervical, thoracic, lumbar and sacral vertebrae (fused to form synsacrum) and coccygeal vertebrae or caudal vertebrae, ribs, costal cartilages and breast bone or keel bone. On the other hand, the appendicular skeleton comprised of the forelimb or wing bone as like scapula, coracoid, clavicle, humerus, radius-ulna and manus, on the contrary hindlimb or leg bone comprises of femur, tibio-fibula and feet bone (metatarsal and digits).

The time needed by each of the steps of pigeon skeleton preparation technique is shown in Table 1.

Table 1: Steps involved with time requirement for preparation of pigeon skeleton.

Sl. No.	Steps involved for skeleton preparation	Time (Hours)
1	Birds preparation	7 hours
2	Skinning, evisceration and de-fleshing of carcass	3 hours
3	Carcass maceration	5 hours
4	Sequential arrangement of bone and bone drying	10 hours
5	Construction of skeletal frame and bone varnishing	8 hours

Description of pigeon skeletal system

There have been a number of modifications of the bones found in birds that enhance the ability to fly.

Axial Skeleton

This skeleton comprised of the bones of- skull, vertebrae such as-cervical, thoracic, lumbar and sacral vertebrae (fused to form synsacrum) and coccygeal vertebrae or caudal vertebrae, ribs, costal cartilages and breast bone or keel bone.

Skull

The skull is divided into two components: 1) Rounded cranium (neurocranium) and 2) Conical facial region (viscerocranium) (Figure 1). These two regions were easily identified by seeing two large orbits or openings into which the eyes fit. Two very thin bones, the sphenoid and ethmoid bones, together form the very thin septum that separates these orbits. Since the volume of the brain increases relatively little with respect to body size, smaller birds have a comparatively larger head than bigger species. Bones of the cranium are: i) Occipital bone (unpaired basioccipital bone, unpaired supraoccipital bone and exoccipital bones) ii) Sphenoid bone (unpaired basisphenoid bone, laterosphenoid bone and unpaired parasphenoid bone) iii) Squamosal bone iv) Parietal bone V) Frontal bone vi) Ethmoid bone and viii) Lacrimal bone Bones of the face are: The bones of the facial portion of skull are –i) Premaxillary bone ii) Nasal bone iii) Palatine bone iv) Maxillary bone v) Jugal/quadratojugal bone vi) Vomer (usually unpaired) vii)Pterygoid bone viii)Quadrate bone ix) Mandible (unpaired) and x) Hyobranchial apparatus (unpaired).

The conformation of the facial skeleton is influenced considerably by the shape and mobility of the beak. The rostral tip of the face is formed by the premaxillary bone having palatine process, frontal process and maxillary process (Figure 1A). The nasal bone is bounded by the frontal and maxillary processes of the premaxillary bone. The palatine bone forms the continuation of the incomplete hard palate. The relatively small maxillary bone joins the premaxillary bone to form the upper beak's short, caudal terminal portion.

The mandible is a laterally flattened bone that only contributes to the vertical dimensions of the head. Its six pairs of fused bones form an acutely angled, caudally open structure (Figure 1B).

The quadrate bone plays a key role in the movement of the maxillopalatine apparatus. It articulates with the mandible to form the principal joint of the lower jaw.

Vertebrae

The vertebral column has 38 bones and is divided into five segments – the cervical vertebrae, the thoracic vertebrae, the lumbar vertebrae, the sacral vertebrae and the coccygeal vertebrae. The vertebral column is often described by way of the vertebral formula that is:

C12, T7, L+S14 & C5 = 38

Cervical Vertebra

The cervical vertebral column is typically S-shaped in avian species. There are 12 cervical vertebrae in the pigeon (Figure 1D).

The first cervical vertebra (atlas) is a small and ring-shaped bone with a dorsal arch and a ventrally located body (Figure 1E). A recess on the cranial surface of the body, the condyloid fossa forms the articular surface for the occipital condyle of the occipital bone. The dorsal surface of the body bears the articular surface for the dens of the axis.

The second cervical vertebra (axis) is slightly larger than the atlas (Figure 1F). Its elongated body (corpus axis) articulates caudally with the third cervical vertebra. Its cranial surface possesses a small process; the dens forms a joint with the atlas. On subsequent vertebral bodies and the articular surface is saddle-shaped.

Thoracic, lumbar, sacral and caudal vertebrae

The seven thoracic vertebrae bear a pair of complete ribs consisting of a dorsal vertebral rib articulating with the vertebra and the ventral sternal rib, which in turn articulates with the breastbone (sternum). Each vertebral

rib bears a flat, backward-pointing spur, the uncinate process, which is characteristic of birds. Total number of lumbar and sacral vertebrae are 14 in number. The seventh thoracic, lumbar, sacral and first caudal vertebrae are closely fused to form the synsacrum, to which the pelvic girdle is attached (Figure 1G). Posterior to the synsacrum is a series of free fused caudal vertebrae form the pygostyle, which supports the tail feathers (Figure 1H).

Ribs

There are seven pairs of ribs articulate with the thoracic vertebrae. The first, second do not articulate with the sternum. The third to the sixth have two segments, the vertebral segment articulate with thoracic vertebra, and sternal segment articulate with breast bone or sternum. All except the first and last have uncinate (meaning hooked or bent) processes that project backward over the outer surface of the next rib and connect to it by a ligament, which adds strength to the thoracic cavity (Figure 1L).

Sternum

The sternum is an extensive bone looks like quadrilateral plate exhibiting a large ventrally directed crest known as keel or carina, which serves as the bony surface for the origin of the major flight muscles- pectorals and supracoracoideus (Figure 1M).

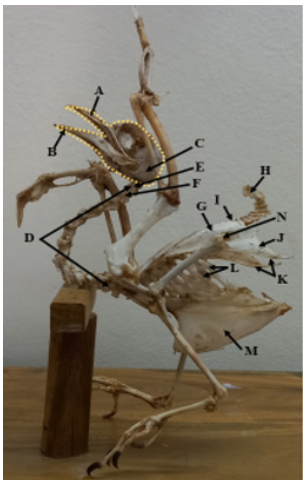

Figure 1: Diagram of a pigeon skeleton. Yellow border indicates the skull. (A) Premaxillary bone, (B) Mandible, (C) Occipital bone, (D) Cervical vertebrae, (E) Atlas, (F) Axis, (G) Synsacrum, (H) Pygostyle, (I) Ilium, (J) Ischium, (K) Pubis, (L) Ribs, (M) Keel or breast bone, and (N) Acetabulum.

Appendicular skeleton

Consists of forelimb or thoracic limb and hind limb or pelvic limb

The forelimb or thoracic limb

The skeleton of the forelimb or thoracic limb comprises the bones of the pectoral girdle and the bones of the wing. The fully developed avian pectoral girdle consists of the- scapula, coracoid, clavicle, furcula or wishbone (Figure 2).

The scapula is sabre-shaped, narrow, thin and slightly curved, unlike the shoulder blade of other animals (Figure 2.1). The glenoid process of the scapula laterally completes the articular surface for the head of the humerus. Craniomedially the scapula is joined to the clavicle. The long caudal extremity is slightly curved and lies approximately parallel to the vertebral column, extending almost to the ilium. The coracoid bone is the strongest bone in the pectoral girdle and rod-shaped bone that connects the cranial border of the sternum with the shoulder joint (Figure 2.3). The clavicle is a thin, curved rod like bone. Its proximal part connects with the cranial extremity of coracoid and scapula. An osseous union joins the two clavicles to form the 'wishbone' (furcula) (Figure 2.2). The bones of the wing consist of-humerus, radius-ulna and manus (carpus, metacarpus and digits).

Humerus, radius-ulna

The humerus is a largest wing bone with an ovoid head for articulation with the scapula, coracoid and clavicle (Figure 2A). The pneumatic foramen is located at the proximal end of the humerus allows the invasion of the clavicular air sac which pneumatizes the interior of this large bone. The forearm bone, ulna (Figure 2B) is thicker and longer but radius (Figure 2C) is thinner and shorter that lies laterally to the ulna. The large space between the ulna and radius is called the interosseus space.

Carpus, metacarpus, and digits (manus)

The manus or hand, consists of the carpus, metacarpus and the digits. The carpus of an adult pigeon having two bones, namely-the ulnar and radial carpal bone articulate with distal end of ulna and radius bone respectively (Figure 2D). The metacarpus is considered as a single bone that is produced by the union of three elements (alular, major and minor metacarpal bone). In the adult pigeon, these three elements are fused with the distal row of carpal bones, giving rise to the carpometacarpus (Figure 2E). At the proximal end of the carpometacarpus, the carpal trochlea articulates with the carpal bones.

The alular metacarpal bone bears the articular surface in

Figure 2: Diagram of a pigeon skeleton showing the forelimb. (A) Humerus, (B) Ulna, (C) Radius, (D) Carpals, (E) Metacarpals, (F) Digits, (1) Scapula, (2) Clavicle, and (3) Coracoid

the distal end for the alular digit. The major metacarpal and the smaller minor metacarpal bone fused distally and extended towards the digits. In the distal extremity, each metacarpal bone bears a surface for articulation with the phalanges. So, recognizable digits are three (3) in number (Figure 2F).

Pelvic Limb or Leg

The pelvic limb is divided into- pelvic girdle or hip bones and the leg. The pelvic girdle or hip bones consists of three bones - ilium, ischium and pubis or pin bones (Figure 1). The bones of the leg are- Femur or thighbone, tibio-fibula and pes (tarsus, metatarsus and digits or toes) (Figure 3).

The Pelvic Girdle

The ilium is large bone which fused to the synsacrum to provide strength and rigidity (Figure 1I). The ischium is much smaller and is continuous with the ilium (Figure 1J). An opening is present in ischium for the passes of sciatic nerve. The pubis is a narrow strip of bone that runs along the border of the ischeum to which it is joined for a short distance only. The free posterior end projects backward slightly beyond the ischeum to form the pin or pubic bones (Figure 1K). The ilium, ischium and pubis which all me*et at* a deep concavity called the acetabulum, into which the head of the femur articulate (Figure 1N).

The Leg

Consists of-femur or thighbone, tibiotarsus, fibula and pes (tarsus, metatarsus and digits or toes).

Femur

The femur is a typical cylindrical shape long bone with a slight cranial curvature (Figure 3A). The proximal extremity has a prominent head that articulate loosely into the acetabulum. The distal extremity has a deep pulley shaped articular surface for the patella (knee cap). Two convex condyles, namely, lateral and medial, are present in the distal end of femur. The lateral surface of the lateral condyles articulates with the head of fibula.

The tibia is a much longer bone than the fibula and is much thicker at the proximal end than it is at the distal end (Figure 3C). The proximal row of tarsal bones is fused to the distal end of the tibia. For that reason, it is known as tibiotarsus bone. The fibula is greatly reduced bone with a slender spicule (needle-like) structure and has a flattened head for attachment to the proximal head of the tibia (Figure 3B).

Pes (tarsus, metatarsus and digits or toes)

There is no independent tarsus bone in the adult pigeon. The matured metatarsus is a long bone formed by the union of the second, third and fourth metatarsal bones (Figure 3D). Most pigeon have four digits or toes – three facing forwards (Figure 3E) and one facing back (Figure 3F). The basic number of phalanges on the toes are two, three, four, and five, respectively, one more than the number of the toe.

Each toe having one claw. So, number of claw are eight (8). Claw is convex dorsally and concave ventrally (Figure 3G).

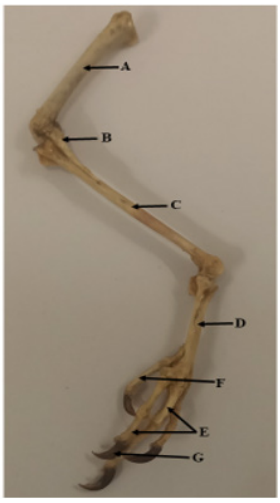

Figure 3: Diagram of a pigeon skeleton showing hindlimb. (A) Femur, (B) Fibula, (C) Tibia, (D) Metatarsal, (E) Forward digits, (F) Backward digits, and (G) Claw

DISCUSSION

Skeleton is the main framework of hard structures that supports and protects the soft structures of the body (Getty, 1975). It is composed of bones, cartilage and ligaments which maintain the body's shape amenability and locomotion (Ghosh, 2006). Skeleton preparation is one of the most important part for anatomical study.

In this study, we reveled total duration for the preparation of pigeon skeleton took 2 days which is short period compared to the 18 days-2 hours both in pigeon and squirrel, 5 months-6 days-6 hours in deer, and 10 months-11 days-12 hours-30 minutes in crocodile (Archana, 2018). Now a day, veterinary educational institutions do not take responsibility for the preparation of skeleton due to procedural complication because different methods of skeleton preparation need different time (Olson, 2003). Oliveira (2018) claims that biological maceration utilising fly larvae and cold water did not produce a good outcome, leading to fragile bone. In this experiment we used 2 matured pigeon aged 8 months old. According to the concept of Baker *et al.*, (2003) all kinds of care and precautions were taken to avoid injuries before skeleton preparation. After sacrificing animals, we did not use any fixative solution because it may affect the bone tissue (Takeshi, 2010). In order to quickly prepare the skeleton, skinning and defleshing was done as much as possible. Then, 3% solution of soda water (Sodium carbonate, NA2CO3) with anionic surfactant (detergent) was used for carcass maceration (Baker *et al.*, 2003 and Van Cleave, 2010). Gofur and Khan, (2010) stated that Soda (Sodium carbonate, NA2CO3) water solution is appropriate for proper and complete digestion of muscles. Carcass maceration can be done by remaining the carcass in a pit for 1-2 months, according to the size of the animals and birds (Raghavan, 1964). However, it is time consuming and have a chance to loose small bones. The odor and

discoloration of the skeleton are often the drawbacks of employing the fly larvae maceration method (Auricchio & Salomão, 2002). Then the bones of pigeon were boiled in 3% solution of soda water for 2 hours because too much boiling can crack the hard tissue (Takeshi, 2010). After boiling, removal of excess flesh as well as bleaching wash were done to avoid further decomposition of bone by microorganisms (Gofur and Khan, 2010). Thereafter, the bones were dried completely by sun rays. Afterwards, the bones were articulated sequentially to form a skeleton and this skeleton was positioned on a wooden stage with the help of stand and thick wire (Figure 1). Finally, the skeleton was labelled and housed in a display case (Musa *et al.*, 2015). The total number of bones evicted was 206. The axial and appendicular skeleton bones help in locomotion and protection of the brain, spinal cord and other internal organs of the body respectively (Sturtz, 2012). In this experiment, all the extracted bones of the skeletal system were appeared whitish and intact as reported by Kyle and Jesse (2018).

The structure of skeletal system of the pigeon is similar to previous descriptions on the domestic fowl (Getty, 1975; Konig, 2009). The axial skeleton comprised of the bones of- skull, vertebrae such as-cervical, thoracic, lumbar and sacral vertebrae (fused to form synsacrum) and coccygeal vertebrae or caudal vertebrae, ribs, costal cartilages and breast bone or keel bone. As opposed to, appendicular skeleton comprises of the bones of forelimb and hindlimb. Forelimb or thoracic limb bones are- scapula, coracoid, clavicle, humerus, radius-ulna, carpus, metacarpus and manus. Hindlimb bones are- pelvic girdle, femur, tibio-fibula, tarsal, metatarsal and toes. Description of the bone are similar according to the concept from konig, (2009).

In the present study, we followed the boiling maceration method for preparation of pigeon skeleton is swift and safe compared to the report of Kyle, (2018), whose method involved burying of the dead animal for 60 days and handling of decomposed carcass using dangerous chemicals such as chlorine bleach and fumes, which is time consuming and not safe.

CONCLUSION

Our major goal was to prepare the skeleton in a practical and economical way. This approach is also simpler, less costly, and time-saving while maintaining the integrity of all the bones. This technique required a total of 33 hours, or 2 days, to prepare the skeleton of a pigeon. This is the shortest time ever for skeleton preparation and study. However, this might change depending on the size of the animals and birds.

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