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Gross Description and Osteometrics of the Axial Skeleton (Ribs and Vertebrae) of *Eidolon Helvum* (African Fruit Bat)

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Summary: The *Eidolon helvum* is a straw coloured fruit bat, also known as the African fruit bat. This study details the anatomical peculiarities, gross description and morphometrics of the ribs. Fifteen adult bats were used for this study. The vertebral formula was $C_7T_{13\text{-}14}L_{3\text{-}4}S_7Cd_{3\text{-}4}$. Spinous process was rudimentary on thoracic vertebrae 1 to 7. The numbers of the ribs ranged from 13 to 14, with the longest being rib 7, and the shortest being rib 14. Twenty percent of the males and 50% of the females had 14 ribs; the male and one female had the 14^{th} rib being unilateral (present on the right side of male and left side of female). The first rib did not articulate directly with the sternum, but through a connecting triangular-shaped bone. The thoracic inlet and outlet were larger in males, relative to the females (inlet height – males 21.82 ± 2.68 mm, females 20.44 ± 4.91 mm; outlet height – males 36.46 ± 3.76 mm, females 33.23 ± 4.33 mm). The sternum was segmented, five in number and had a ventral elongation like the avian keel. Data obtained may find application in comparative and applied anatomy, and forensic medicine.

Keywords*:* *Eidolon helvum*, Axial skeleton, Osteology, Osteometrics, Triangular bone.

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INTRODUCTION

Bats are the only known mammals that can fly (Balthazary *et al*., 2007; Igado *et al*., 2015). The African straw coloured fruit bat belongs to the Order Chiroptera, Family Pteropodidae, Genus Eidolon, Species *Eidolon helvum* **(**Kerr, 1792**)**. It is the second largest and most widely distributed bat in Africa (DeFrees and Wilson, 1988; Juste *et al*, 2000, Igado *et al*, 2012). Bats are of economic importance, for example, they have been reported to cause damage to plants when feeding on them, thereby resulting in a loss to farmers, and they have also been implicated in viral disease transmission e.g. rabies (Crawford *et al*., 2002; Igado *et al*., 2012).

The skeleton of animals is an essential structure that serves as a landmark for identifying or obtaining access into the other structures or cavities of the animal system. For example, the intercostal space is used to identify the location of the heart, while the lumbar vertebrae serves as a useful landmark for the location of the kidneys. This usefulness of the skeleton makes it imperative to have detailed information about some skeletal structures of the *Eidolon helvum*. Currently, no literature documents the anatomical morphometrics of this particular bat nor of any other bat species. Careful electronic search using www.scholar.google.com and www.pubmed.com (July, 2017 – January, 2018) did not reveal any previous documentation of the morphometrics and detailed description of the osteology of the *Eidolon helvum* nor of other bat species.

This study aims to give a detailed morphological and morphometric description of parts of the axial skeleton (ribs and vertebrae) of the *Eidolon helvum*.

MATERIALS AND METHODS

Ethical approval for this work was obtained from the Ethical Committee, Faculty of Veterinary Medicine, ethical code no 12/13/03.

Experimental animals used for this study were 15 adult bats consisting 10 females and 5 males. Age was determined based on weight, according to DeFrees and Wilson (1988) and Richter and Cumming (2006), who estimated the average weight of adult bats to be 250 – 310 grams. The bats were captured at roosting and foraging sites with the use of mist nets and were transported to the Department of Veterinary Anatomy, University of Ibadan in metal cages and were thereafter euthanized using the chloroform chamber. All procedures for handling the animals complied with the Guidelines for the Care and Use of Experimental Animals (National Institute of Health – NIH, USA).

Bones were obtained by cold water maceration by soaking the specimens individually in plastic containers filled with 1% sodium hydroxide (NaOH) solution for about 5 – 7 days, until muscles and other attachments had been removed. Pictures were taken with the aid of a digital camera (Sony® Cyber-shot, DSC-HX400V, 50x optical zoom). All linear measurements were done with a digital vernier calliper. Except otherwise indicated, linear measurements were recorded in millimetres, and all measurements obtained from the right side in the case of paired bones. Parameters obtained (highlighted in Figures 1 – 9) included:

1. **Total number of ribs (Rn)** – counted from the first rib to the last floating rib.
2. **Total number of true ribs (Trn)** – number of all the ribs articulating directly with the sternum.
3. **Number of false ribs (Fsn)** – number of all ribs indirectly articulating with the sternum by the costal cartilage.
4. **Number of floating ribs (Fln)** – number of all ribs with no direct or indirect attachment to the sternum. The distal ends were unattached.
5. **Length of each rib (RL)** - measured from the head of the ribs where it articulates with the thoracic vertebra to the point of the sternum. Measured with the aid of a twine, the length of which was measured with a digital vernier calliper.
6. **Maximum rib width (MaRW)** – measurement of the widest part of each rib, close to the head.
7. **Minimum rib width (MnRW)** – measurement of the narrowest part of each rib, close to the sternum.
8. **Length of sternum (SL)** - measured from the cranial tip of the manubrium to the distal point of the xiphoid process.
9. **Thoracic inlet transverse diameter (TiTD)** - measured as the distance between the ventral rim of the first thoracic vertebra to the dorsal aspect of the manubrium.
10. **Thoracic outlet transverse diameter (ToTD)** - measured as the distance between the ventral rim of the last thoracic vertebra to the dorsal aspect of the xyphoid.
11. **Total number of vertebrae (VCn)** – counted from the first vertebral bone (atlas) to the last caudal (tail) bone.
12. **Number of cervical vertebrae (Cvn)** – counted from the first vertebral bone (atlas), to the vertebra preceding that which articulates with the first rib.
13. **Number of thoracic vertebrae (Txn)** – counted from the vertebra with the first rib attachment, to the vertebra with the last rib attachment.
14. **Number of lumbar vertebrae (Lmn)** - counted from the vertebra after the last thoracic, to the vertebra immediately preceding those that articulate with the pelvic and sacral bones.
15. **Number of sacral vertebral bones (Scn)** – counted from the first fused vertebra to the end of the pelvic bones where the vertebrae are no longer fused.
16. **Number of coccygeal vertebral bones (Cxn)** – all vertebrae after the sacrum/sacral vertebrae.

Statistical Analysis

This is calculated using Student 't' test (Graphpad prism statistical software, Version 5, La Jolla, CA, USA). Level of significance was $\alpha_{0.05}$.

RESULTS

The Vertebral Column

The vertebral formula was $C_7T_{13-14}L_{3-4}S_7Cd_{3-4}$. The atlas (Figure 1 A-D) was observed as the typical dorso-ventrally flattened mammalian atlas. The neural canal presented a roughly oval shape. The wings of the atlas as observed in this study appeared to be more pronounced, and projected more caudally than reported in cattle, equine and porcine, but similar to that observed in the dog by Getty (1975). Dorso-cranially (alar foramen), caudally, ventrally, and also on the dorso-cranial aspect of the neural canal, the body of the atlas presented bilateral foramina. The ventral and caudal foramina were connected, while the dorsal (alar foramen) and neural canal foramina were also connected, implying that these are entrance and exits for cervical spinal nerves. The central foramen showed a variation in size, due to the difference in shape of the bony ventral border (Figure 1C and D).

The axis (Figure 2) presented a large spinous process, which was directed dorso-caudally. Cranially, the dens/odontiod process was obvious; caudally, the

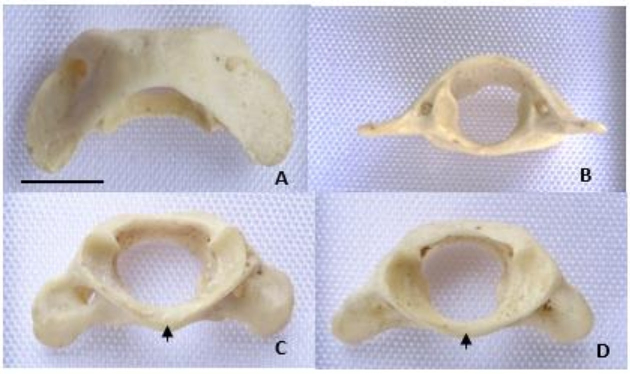

Figure 1: The atlas of the *Eidolon helvum*. A is the dorsal view; B – caudal view; C and D are the rostral view. Note the difference in the shape of the central foramen of C and D, due to the difference in shape of the ventral border of the foramen (arrow heads). Scale bar – 0.5 cm

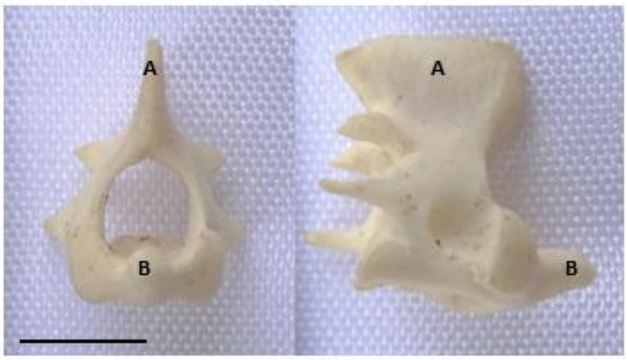

Figure 2: The axis of the *Eidolon helvum*. Left panel shows the cranial view, while the right panel shows the lateral view. Scale bar – 0.5 cm

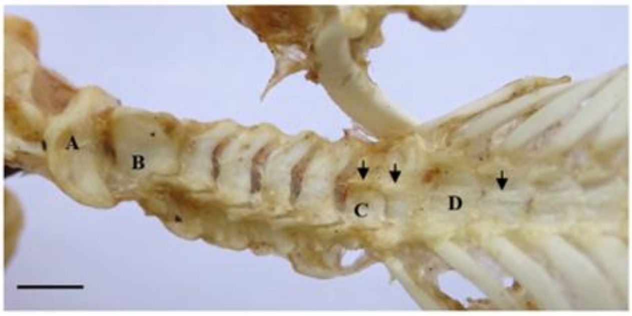

Figure 3: The cervical bones and some thoracic vertebral bones of the *Eidolon helvum*. Note the atlas (A), axis (B), the 7th cervical bone (C), the 2nd thoracic vertebra (D) and the rudimentary spinous processes (arrow heads). **Scale bar – 1 cm.**

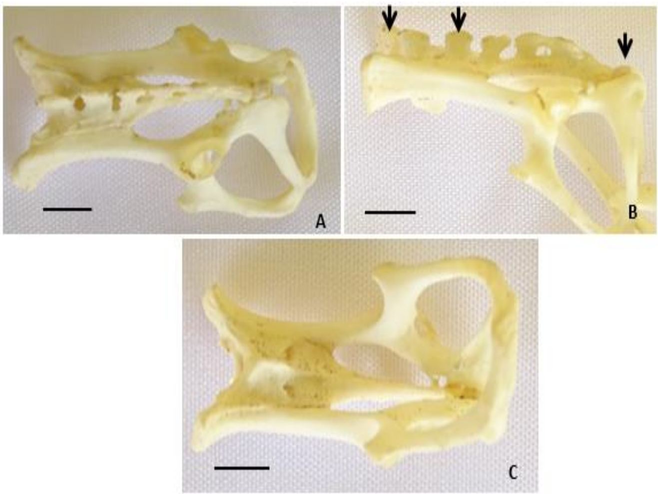

Figure 4: Pelvic girdle of the *Eidolon helvum*. Dorsal view (A), lateral view (B) and the ventral view (C). Note the spinous processes of the sacral bone (arrows). Scale bar – 0.5 cm.

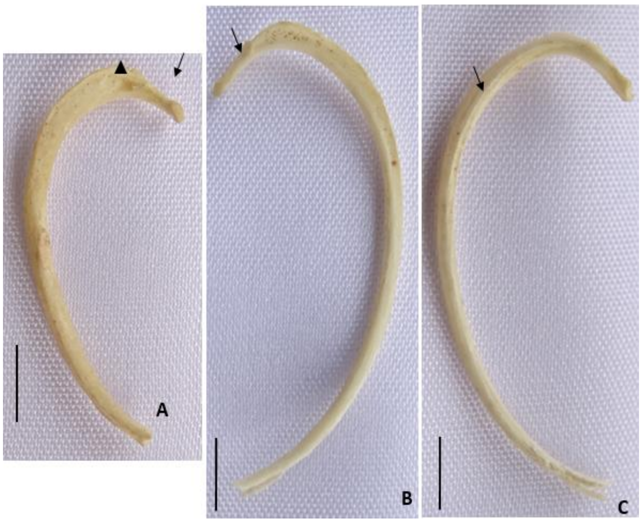

Figure 5: Ribs 2 (A) and 3 (B & C) of the *Eidolon helvum*. Panels 'A' and 'B' are the medial surfaces, while 'C' is the lateral surface. Arrow on 'A' indicates the head of the rib, on 'B' indicates the articular tubercle, while on 'C' indicates the ridge-like elevation on the lateral surface. Note the groove distal to the head on rib 2 (arrow head on 'A'). Scale bar 0.5 cm.

articular and transverse processes and laterally, the bilaterally placed vertebral foramen. The remaining cervical and 1st to 7th thoracic vertebral bones showed rudimentary spinous processes (Figure 3), until about the 15th vertebral bone (8th thoracic), where the spinous processes became more pronounced and became directed dorso-cranially till the last sacral bone.

In addition to the less prominent spinous processes of the thoracic vertebrae, the body appeared stouter, and constricted, relative to the cervical vertebrae. The transverse processes projected caudally and decreased in length as the bones progressed caudally.

The lumbar vertebrae had prominent dorso-cranially directed spinous processes and a body resembling the thoracic vertebrae, although the body appeared slightly longer (cranio-caudally) relative to the thoracic bones. The transverse processes of the lumbar vertebrae projected cranially and were observed to be less prominent.

The sacral bones were consistently 7 in number and were fused. They were fused laterally to the pelvic girdle, had no transverse processes but very prominent spinous processes. They extended almost the whole length of the pelvic girdle. They also possessed a prominent ventral process, along the midline. This ventral process was continuous, unlike the spinous processes which were separate (Figure 4).

The caudal bones were 3 to 4 in number, very small in dimensions relative to the other bones, decreased in size as they progressed caudad. Transverse processes were rudimentary, while spinous processes were not observed. The shape of the bodies was generally similar to the lumbar vertebrae

The Ribs

The ribs possessed a distinctly sickle-shaped appearance and were dorsoventrally flattened in the typical mammalian rib appearance. They had two extremities (proximal and distal), two surfaces (lateral and medial), and two borders (cranial and caudal).

The head of the rib was very distinct and projected cranially. The head was dorsoventrally flattened without any distinct tubercle. A very slight narrowing distal to the head resulted in what could be described as a 'neck'. This neck was not a consistent feature in all the ribs, appearing more distinct in the cranial ribs than the caudal ribs. Caudal to this neck and a little more medial was an articular surface, which articulated with the bodies of the thoracic vertebrae. The shaft was roughly cylindrical in shape, tapering to a thinner distal extremity to articulate with the cartilage at the costo-chondral junction (Figure 5).

The medial surface possessed a groove at the proximal part immediately distal to the head of the rib. The lateral surface was smooth, except for ribs 2 and 3, which had a ridge-like elevation (Figure 5A-C).

The first rib was very straight, lacking any curvature. Ribs 2 to 10 displayed the curvature

Figure 6: Dorsal view of vertebral column of female *E helvum*. Arrows indicate 1st & 14th ribs. Note the straightness of ribs 1, and 11 to 14. Scale bar – 1 cm.

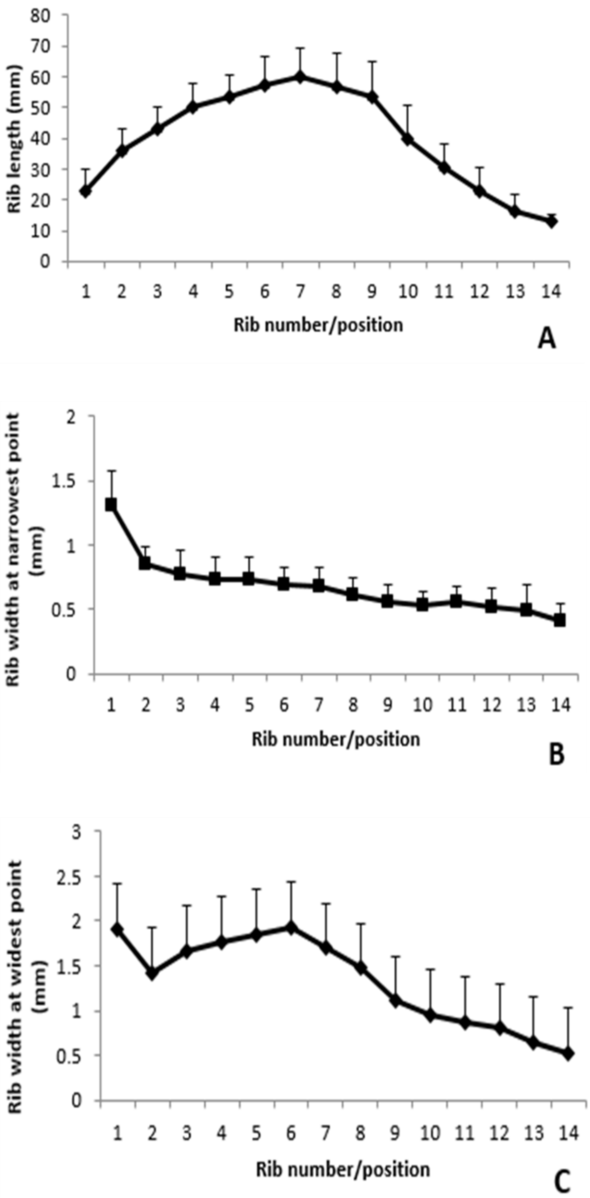

Figure 7: Graphs showing the progression of the lengths (A), width at narrowest point distally (B) and width of ribs at widest point proximally (C) of the ribs of the *Eidolon helvum*. Note that in 'A', highest length was recorded at rib 7; rib 1 was the thickest rib, while rib 14 was the thinnest and shortest rib (A-C).

aforementioned, while 11 to 14 were straight and projected more latero-caudally than latero-ventrally (Figure 6).

The numbers of the ribs ranged from 13 to 14. Of the five males sampled, only 1 (20%) had 14 ribs (unilateral, present on the right; left side had 13 ribs); of the females, 5 (50%) had 14 ribs, one was unilateral, present on the left. The true ribs, articulating with the sternum were consistently 7 in the males, while in the females, only 1 (10%) had 6 true ribs, 9 (90%) had 7 ribs. The false ribs had a common costal cartilage, joined together in the typical mammalian fashion. The length of the ribs increased until about the 7th to 9th rib, when the length gradually reduced. The lengths of the ribs varied greatly, ranging from 59.81 ± 9.54 mm (7th rib) to 13.21 ± 2.24 mm (14th rib). The values for the males (65.39 ± 4.42 mm – 7th rib) were higher than the

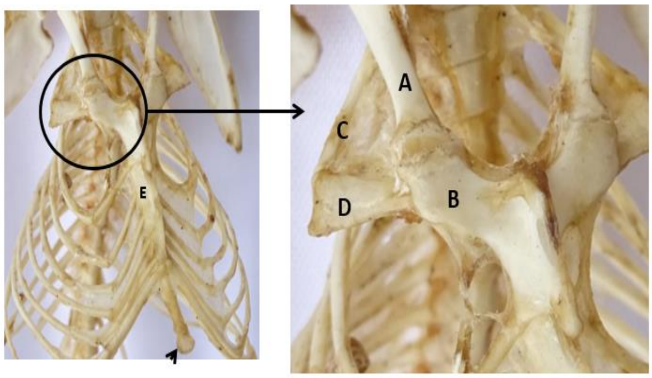

Figure 8: Ventral view of bony thorax of *E helvum*. Right panel is a magnification of highlighted region in the left panel. A – clavicle; B – sternum; C – first rib (R1); D – Triangular bone. Note the keel-like appearance of the sternum and its segmentation (E on left panel). Arrow head is the xyphoid. Note the presence of the costochondral cartilage joining the true ribs to the sternum.

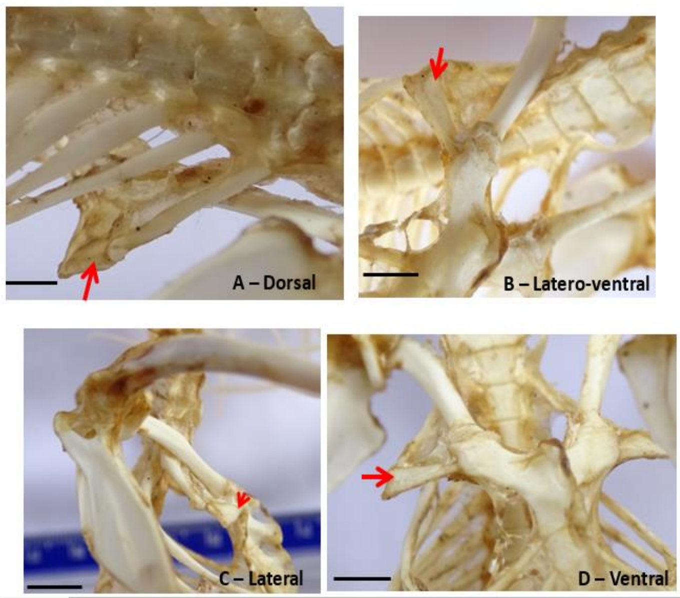

Figure 9: Views of bony thorax of the *E helvum*. The red arrows show the triangular bone from different views. Scale bar – 1 cm

females (57.03 ± 10.34 mm – 7th rib). (Figure 7A–C). The first rib did not articulate directly with the sternum. A small triangular-shaped bone acted as the connection between the first rib and the arm of the Y-shaped manubrium (Figures 8 and 9).

The Sternum

The sternum possessed a roughly keel-like shape, as observed in birds (Figure 8).

The manubrium was distinctly Y-shaped, due to the extensive jugular notch. The ventral surface (or tail of the 'Y') was bilaterally flattened and shaped like the keel-bone of the avian species. The two arms of the 'Y' were dorsoventrally flattened; and attached cranially to each arm was the clavicle; laterally was the small flat triangular bone, the narrow end of which articulated with the lateral aspect of the 'Y' (Figures 8 and 9). This triangular bone served as a link between the first rib and the sternum. The manubrium and the body of the sternum showed divisions at the keel region, which were consistently 5 in number in all the animals. The xyphoid was a thin slender, rod-like bone, pointed caudally (Figure 8). The mean length of the sternum was 31.32±3.90 mm, with the males having statistically significantly ($p > 0.05$) longer sternum (33.82±1.08 mm), relative to the females (30.07±4.24 mm).

The Thoracic Inlet and Outlet

The inlet displayed a roughly conical shape, was bounded dorsally by the thoracic vertebra, ventrally by the manubrium and laterally by the clavicle and the first rib. The outlet was roughly "heart-shaped", bounded dorsally by the last thoracic vertebrae, ventrally by the xyphoid and laterally by the ribs (11th ribs).

The transverse diameter of the thoracic inlet was 20.93 ± 4.19 mm, males – 21.82 ± 2.68 mm, females – 20.44 ± 4.91 mm. The transverse diameter of the thoracic outlet was 34.38 ± 4.30 mm, males – 36.46 ± 3.76 mm, females - 33.23 ± 4.33 mm. The inlet did not show statistically significant differences between the sexes, while the outlet showed statistically significant difference between the sexes.

DISCUSSION

The number of the vertebral bones, and therefore the vertebral formula varies from animal to animal. The vertebral formula of the *Eidolon helvum* observed in the current study was $C_7T_{13\text{-}14}L_{3\text{-}4}S_7Cd_{3\text{-}4}$. The number of cervical vertebrae observed in this study is consistent with what was previously reported in other mammals; the number of thoracic vertebrae was similar to that of ruminants (13); but the lumbar, sacral and caudal bones were dissimilar to previous reports in domestic mammals (Getty, 1975; Dyce *et al*., 2002). The lumbar, sacral and caudal vertebrae were reported to be respectively 6 -7, 4 and 20 – 23 in the porcine, 7, 3 and 20 – 23 in the carnivores, 6, 5 and 15 – 21 in the equine and 6, 5 and 18 and 20 in ruminants (Getty, 1975). However, previous reports in the mole rat showed a slight similarity in the number of the caudal bones (5 in number) (Özkan, 2007).

Characteristically, some of the identifying features of the mammalian thoracic vertebrae include the more pronounced spinous process and the stouter body (Getty, 1975; Rohen *et al*., 1998; Dyce *et al*., 2002; Netter, 2006). Although the thoracic vertebrae observed in this study had a stout body, the spinous processes were rudimentary, except for the last few. The reason for this is not clear, as the spinous processes serve as origin and insertion for muscles that incline the neck, arch the back and assist in extending the head and neck (Getty, 1975). In contrast to mammals, pictorial assessment of the thoracic vertebrae of avians from texts showed the lack of spinous processes (Getty, 1975; Dyce *et al*., 2002). It is therefore possible that since bats are mammals of flight and do not spend a long time walking or swimming like other mammals, the need for the pronounced spinous process was not necessary. This could also account for the more pronounced transverse and spinous processes in the lumbar vertebrae. Muscles attaching to these processes may provide additional strength and support when the bats hang upside down, as the lumbar vertebrae is closer to the pelvic girdle.

In mammals, the number of ribs varies with species – 13 pairs in ruminants and carnivores, and 18 to 19 pairs in equine (Getty, 1975). In the current study of bats, the numbers of the ribs ranged from 13 to 14 pairs in number. Both genders recorded 14 ribs, but the females had a higher incidence. The unilateral extra rib is similar to what is observed sometimes in the equine (Getty, 1975; Dyce *et al*., 2002). The widest point of the thoracic cavity could be said to occur around ribs 7 – 9, as these ribs had the longest values.

The shape of the rib is similar to previous reports in other mammals, although in this species of bats, the curve of the rib is more pronounced at the proximal end than at the shaft. The ribs serve as attachment for muscles of respiration; they protect the thoracic viscera and also define the lateral limits of the bony thoracic cavity (Getty, 1975; Dyce *et al*., 2002). The ridge-like elevation observed on the lateral surface of ribs 2 and 3 is similar to that observed in the mole rat, same rib number, even though these are not flying animals (Özkan, 2007). These ridges may possibly serve as an extra attachment for muscles, to aid in respiration while the animal is in flight.

Generally, the first rib is unique, being relatively straighter and stouter than the other ribs. In this study, it lacked any curvature, probably because the distance between the dorsal and ventral limits of the bony thorax at this point (the thoracic inlet) is the smallest region or point of the thorax. The curvature of the ribs allows for a greater space in the thoracic cavity. However, unlike other mammals (Getty, 1975; Popesko, 1977; Dyce *et al*., 2002; Netter, 2006; Panyutina *et al*., 2015), the first rib did not articulate directly with the sternum. A small triangular-shaped bone acted as the connection between the first rib and the arm of the Y-shaped manubrium. In view of the fact that careful electronic search did not reveal earlier documentation of this triangular bone connecting the sternum to the first rib, the authors proffer the name *Triangular bone of Igado*.

The jugular notch of the manubrium is observed in humans (Netter, 2006). However, the bat showed an extensive jugular notch, similar to the indentation of the furcular in the avians. The more extensive jugular notch probably provides a greater surface area for muscle attachment, as visual observation during dissection revealed an extensive pectoralis muscle (the major muscle of flight). The sternum was segmented as observed in mammals (Getty, 1975; Popesko, 1977; Netter, 2006), although the ventral aspect was avian-like, having a keel-like appearance.

In conclusion, this study highlights the features and peculiarities of the *Eidolon helvum* axial skeleton previously undocumented, and shows its similarities to the avian skeleton (the keel and reduced spinous processes of the thoracic vertebrae). Results obtained from this study may find application in the fields of applied wildlife medicine, archaeology and forensic medicine, while also providing basic anatomical data.

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