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Original Article

Anatomical and Normal 3D CT-Scan Study of the Vertebral Column and the Shell of the European Pond Turtle (*Emys orbicularis*)

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ABSTRACT

Limited information about the skeletal anatomical features of the European pond turtle (*Emys orbicularis*), one of the species of turtles in Iran, is available. Given that performing clinical examinations as well using imaging techniques require complete anatomical information of the animal, it is essential to study these features in various researches. This study was done to provide complete anatomical information of the vertebrae and different shell parts in European pond turtles, as well as their normal three-dimensional computed tomographic (3D CT)-Scan images in both flexed and extended neck positions. This study was performed on 10 European pond turtles. CT-Scan images were taken from each sample and in the 3D reconstruction of the images, different patterns were used. 8 cervical vertebrae, 10 dorsal vertebrae, 2 sacral vertebrae, and 25 caudal vertebrae were observed in European pond turtles. The cervical vertebrae were highly mobile and there were no cervical ribs. Due to the fusion of the dorsal vertebrae, there were no intervertebral foramina in this section, but very small lateral vertebral foramina were visible. These foramina were formed in the last four dorsal vertebrae at the fusion site and they were larger than the foramina of the cranial vertebrae. According to the results of this study, it can be concluded that the use of diagnostic techniques such as a 3D CT-Scan is very useful in the study of skeletons. The correct direction and position of the bones can be easily determined using this technique. Part of the turtle`s ability to contract the neck is due to the special structure of the articular processes of the last two cervical vertebrae and the first dorsal vertebra. One of the most important adaptations in the evolution of the special structure of the seventh and eighth vertebrae of the neck and the way they are articulated.

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Introduction

Reptiles, particularly turtles that inhabit both on land and in water, have made special adaptations. Many people keep turtles, such as pets. Therefore, attaining more knowledge about the turtles` anatomy is essential for therapeutic purposes.

The skeletal system serves as a framework in which dictates the position of the internal organs.[1] In diagnostic imaging methods, beside the importance of the direct study of the bones, the components of the skeletal system are used as a topographic guide of other organs.[1] Diagnostic imaging studies have not been performed on the European pond turtles (*Emys orbicularis*) before, but similar studies have been done on other species. For example, radiographs of the neck and the trunk of the *Caretta caretta* sea turtle have been examined, and a number of useful indicators have been detected to identify internal structures, including the bronchi, the coracoid bone, and the acetabulum.[2] In another study, radiographic anatomy of the limbs of the *Caretta caretta* sea turtle was studied, their normal radiographic views were assessed and the three-dimensional computed tomographic (3D CT)-Scan was used to demonstrate the anatomic features in more details.[3] Various studies have been performed on different organs of different turtle species.

So far, various studies have been conducted on different organs in different species of turtles in the world.[4] Among the simultaneous radiological and anatomical works, the following can be mentioned: In 2006, Valenti and his coworkers examined the radiographic images of the neck and the trunk of a *Caretta caretta* sea turtle and provided a number of useful indicators for internal organs, including the bronchi, the coracoid and the acetabulum bones.[3] In 2007, Valenti and his coworkers studied the anatomy of *Caretta caretta* turtle limbs and marked out their normal radiographic profiles. They also used 3D CT-Scan s for this matter.[3] In 2007, Valenti and his coworkers studied the CT-Scan of the vertebrae and the coelomic cavity of a red sea turtle and pointed out the position of the various organs of the coelomic cavity in relation to the carapace and the vertebrae. One of the most important indications was that the tracheal bifurcation in this species is more backward than the other turtles and this feature explains why these turtles can`t constrict their neck.[3] In 2012, Lyson and Joyce examined the topological relation between the scapula and the rib cage and realized that the shoulder girdle was placed inside the shell and in front of the rib cage.[5] In 2003, Sheil studied the morphology of the bones during the embryonic period in *Apolone spinifera* soft-shelled turtles and compared it with another species of turtles. The bones of adult turtles have also been examined in detail in this study.[6] In 2009, Sánchez-Villagra and his coworkers examined the morphology of the bones during the embryonic period of *Pelodiscus Sinensis* species which is a soft-shelled turtle and studied the patterns and the ossification sites, and found out differences in bone formation period between this species and the *Apolone spinifera*.[7] In 2005, Sheil and Greenbaum re-examined the formation period of different bones in the body of the *Chelydra serpentine*, and pointed out intergeneric differences based on previous studies of other species.[8] In 2007, Sánchez-Villagra and his coworkers examined the carpus and the tarsus bones in 25 species of adult side-necked turtles and found greater variation in carpus-tarsus morphology.[7] In 2009, Faberzi and his colleagues studied the organ development in *Pleurodiran* turtles and identified the components of the tarsus bone.[9] In 2010, Delfino and his colleagues studied the evolutionary status of differences in digit formulae between pig-nose and soft-shelled turtles.[10] In 2009, Wernberg and his coworkers examined the formation of embryonic organs in the *Emydura subglobosa* turtle chronologically and realized that the formation of some bones in this species take place earlier or later than the same bones in other species.[11] In 2005, Sheil studied the evolution of the bones of a crocodile species called *Macrochelys temminckii*, which is similar in appearance to a species of turtles called *Chelydra serpentine* and found out differences in morphology of the skull and the carapace in these two species.[12] In 2009, Nagashima and his colleagues examined the evolution of the body plane in turtles.[13]

Numerous studies have been performed on the skull bones in terms of morphology and embryonic development. These include the following: In 2007, Tulenko and Sheil examined the formation of the skull cartilage in *Trachemys scripta* and compared it with other turtles, which showed that the general pattern and formation of the cartilage was almost similar to other species with only slight differences between them.[14] In another study in 2009, Bona and Alcalde studied the formation of the cartilages and the skull skeleton in a species of turtles called *Phrynops hilarii.*[15]

In 2009, Bever examined the skull skeleton of a turtle species called *Pseudemys texana* and noted the differences between different species of turtles. The emphasis of this study was on increasing the taxonomic range with better descriptions of morphology and anatomy.[16]

Since the skeletal system of turtles is related to the dorsal and ventral parts of the shell, there are also studies on this structure`s morphology, its attachments to the bones, its ossification process and its formation steps during embryonic period.[17] In 1998, Ertan Taşkavak conducted a study on the Euphrates turtles, including a comparative study on the morphology of the external body parts which is a useful resource for zoologists.[18] In 1998, Taskawak examined the skull skeleton of the Euphrates turtles and identified the proximity of the different bones, pointing out some differences between species.[18] In 2014, Zehtabvar and his coworkers studied the computed tomographic anatomy and topography of the lower respiratory system of the european pond turtle.[19] In this study, in order to take the first steps in identifying and preserving the European pond turtles, the anatomy of their vertebrae and the shell and the images of their 3D CT-Scan s were analyzed and compared with the results of other studies.

European pond turtles are one of the species of turtles in Iran. Limited information about the anatomical features of this species is available. Given that for clinical examinations and diagnostic imaging purposes a complete anatomical knowledge of the examined animal is essential, it is necessary to study these featured in more detail.

Materials and Methods

Individuals

All experimental procedures were approved by the Faculty of Veterinary medicine, University of Tehran Local Ethics Committee (30704/6/5). The study was conducted on 10 adult European pond turtles (*Emys orbicularis*), 5 males and 5 females, with the mean weight of 450 ± 45.22 g. The turtles were transferred to the dissection room of the Faculty of Veterinary Medicine, University of Tehran. The samples were kept in reptile suitable conditions for one week so that they would get used to the new environment and return to their normal body state. During this period, the whole carcass of Kilka fish were used to feed the turtles. Identification keys provided in the references were used to determine the male and female turtles.[20]

In this species, the iris in males is reddish and orange, while in females it is almost yellow. The number of yellow spots on the head and the neck of males is less and smaller than in females. In addition, males have more depressed plastron compared to females.

Computed Tomography (CT) Scanning

Computed tomographic scanning was performed using a two slice CT- scan machine (Siemens Somatom Spirit II; Berlin, Germany). After transferring the samples to the small animal radiology department of the Faculty of Veterinary Medicine, University of Tehran, the samples were anesthetized with ketamine (25 mg/kg) and diazepam (1 mg/kg) through intramuscular injections.

Technical parameters for this imaging protocol were as follows: Rotation time, 1s; slice thickness, 1mm; reconstruction interval, 0.5–1 mm; pitch, 1; X-ray tube potential, 120 kV; and X-ray tube current, 130 mA. In the 3D reconstruction of the images, different patterns were used, which are mentioned in each of the 3D CT-Scan images in the results section of this paper.

Gross Anatomical Studies

By deepening the anesthesia, the samples were euthanized. After separation of carapace and plastron, the skin and all unnecessary soft tissues of this section were removed and bone structures were isolated for one week using insect method (*Tenebrio molitor* worm). The temperature of 21° C and relative humidity of 70% were maintained to provide an optimal environmental condition for insects. After dissecting the bones from other tissues, the images were taken with an Olympus SZX12 stereo microscope equipped with an ASP-CellPad E digital camera.

3D Modeling

For 3D modeling, the CT-Scan output information of each sample in DICOM format was transferred to 3D slicer 4.8.0 software and a 3D sample was prepared in obj format with a bone pattern. Then, the obj file was transferred to 3Ds max 2018 software, the process of clearing the incorrect cases detected by Slicer software was performed, the final editing 3D coat 4.8.15 software was done, and finally the 3D printing was performed.

Results

Vertebral Column

This turtle`s vertebrae were regional and these regions were separable. This species had 8 cervical vertebrae, 10 dorsal vertebrae, 2 sacral vertebrae and 25 caudal vertebrae. The cervical vertebrae were highly mobile and there were no cervical ribs. The dorsal vertebrae were immobile and fused together. The neural spines were fused and merged caudally to the carapace`s neural plates. The dorsal ribs were also fused together and united to the carapace`s pleural plates. The sacral region had two vertebrae which had two sacral ribs. The 25 caudal vertebrae were highly mobile (Figures 1 and 2).

Cervical Vertebrae

There were 8 mobile cervical vertebrae with a special shape. These vertebrae had distinct cranial and caudal articular processes. The first and the eighth vertebrae had significantly higher width than length and their shape was unique. The first vertebra was smaller than the rest of the vertebrae and had a special shape (Figures 3 and 4).

Atlas

It is consisted of two neural arches, a centrum and an intercentrum. The neural arches were fused together on the top and were seen as a whole. They were connected to the intercentrum and the centrum on the bottom. The intercentrum had a triangular shape which its base was on the cranial part and its apex on the caudal part. There was an articular surface on the cranial part of the intercentrum that its placement next to the articular surfaces of the arches formed the cranial articular cavity. This cavity had a foramen and the occipital condyles were placed in it. The centrum had a crest on its ventral surface. Its dorsal surface was slightly depressed, forming the ventral surface of the spinal cord. There was a notch on the caudal part that formed the caudal articular cavity and articulated with the cranial part of the Axis. There was a foramen on the either side of the centrum that perhaps can be called the lateral vertebral foramen. The arches had wing-like shapes that were drawn backwards. They had a caudal articular facet, which was almost vertical and articulated with the cranial articular process of the Axis. On the top part, where the two arches were connected, a longitudinal crest was seen (Figure 5).

Axis

The Axis was more elongated than the Atlas. Its width was high in the area of the caudal articular processes. From the dorsal view, the cranial articular processes were inclined craniolaterally and the caudal articular processes caudoventrally. The Axis consisted of three distinct parts: two neural arches that made up the dorsal section of the spinal cord and a centrum that made up the ventral part of the vertebra. There was a crest-like structure at the edge of these three sections, which can be called the spinous process. This process had a cranial protrusion and inclination. Each arch had a distinctive cranial articular process on its cranial part and a caudal articular process on its caudal part. The cranial articular processes were directed to cranial, lateral and ventral parts and articulated with the caudal articular facets of the Atlas. The caudal articular

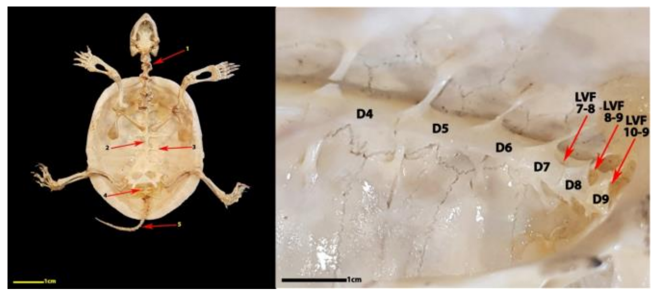

Figure 1. **Left*:*** Ventral view of the carapace and its associated skeletal structures in the European pond turtle (the plastron has been removed). **Right:** Ventral view of the dorsal vertebrae of the European pond turtle (caudal part of the carapace), the vertebrae`s number has been labeled. Cervical vertebrae, 2. Dorsal vertebrae, 3. Rib head, 4. Sacrum, 5. Caudal vertebrae, 6. D: Dorsal vertebrae, 7.LVF: Lateral vertebral foramen.

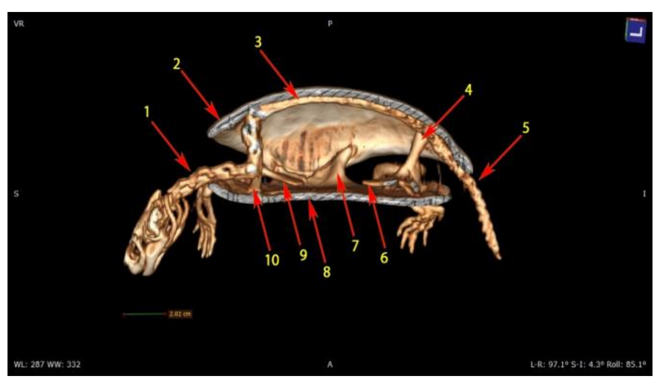

Figure 2*.* Lateral view of a sagittal section of the 3D reconstruction of the European pond turtle`s CT-Scan and the osseous-shaded-vp pattern. The head and the neck are outside the shell. Different parts of the image are labeled. 1. Vertebral column (cervical part), 2. Carapace, 3. Vertebral column (dorsal part), 4. Ilium, 5. Vertebral column (caudal part), 6. Pubic, 7. Bridge, 8. Plastron, 9. Coracoid, 10. Acromion.

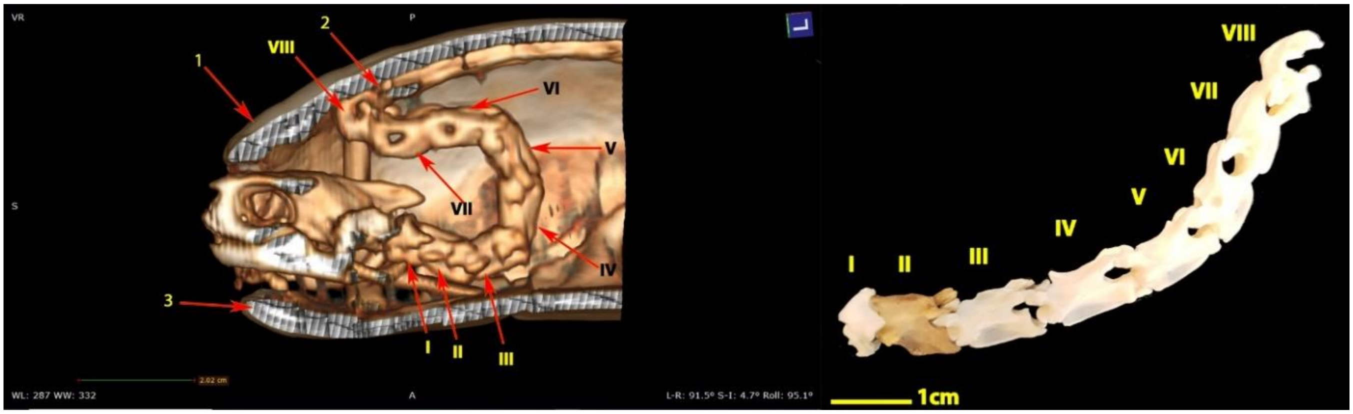

Figure 3. **Left:** lateral view of the sagittal section of the 3D reconstruction of European pond turtle`s CT-Scan and the osseous-shaded-vp pattern, with higher magnification. The hyoid device has been removed in the image. The cervical vertebrae are marked with Roman numbers. **Right:** lateral view of the European pond turtle`s cervical vertebrae, marked with Roman numbers. 1. Carapace, 2. 1st Dorsal vertebrae, 3. Plastron.

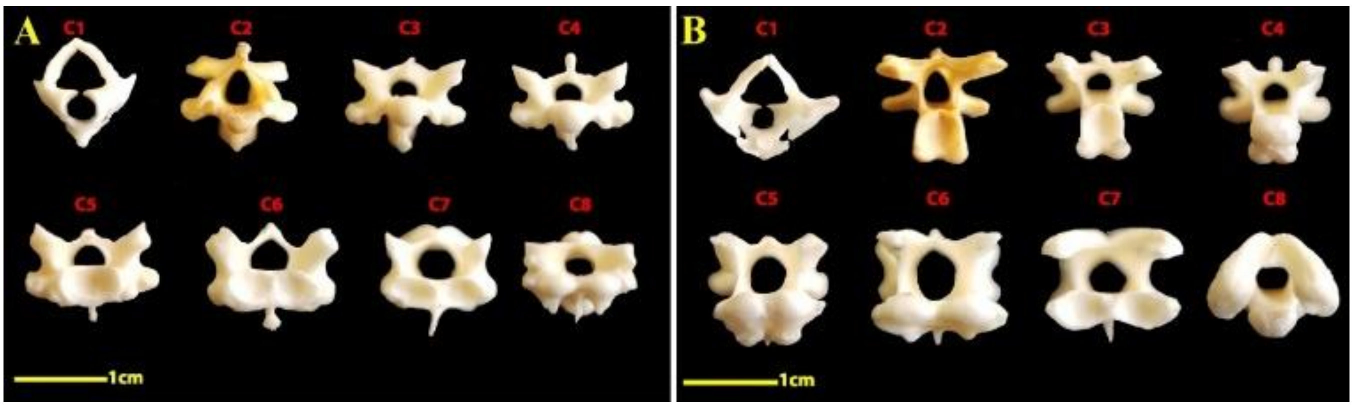

Figure 4. **A,** Cranial view of the cervical vertebrae of the European pond turtle. **B,** Caudal view of the cervical vertebrae of the European pond turtle. The number of each vertebra is displayed above it.

processes were positioned laterally and articulated with the cranial articular processes of the third vertebra. There was a longitudinal crest on the ventral surface of the centrum. On either side of the cranial part of the centrum, at the point where it was connected by the arch, there was a protrusion that can be considered a transverse process. The cranial articular surface of the centrum was protruded and the caudal articular surface was depressed. It should be noted that the centrums of the vertebrae in a row were articulated through the same protrusions and depressions (Figure 6).

The Third, the Fourth and the Fifth Cervical Vertebrae

These vertebrae were very similar. They consisted of two neural arches and a centrum. They had a distinctive crest on the ventral surface of the centrum. They had a spine on the dorsal part of their arches which elongated all through the arches. An intervertebral foramen was made through the vertebrae. They had a spine on the dorsal part of their arches which elongated all through the arches.

An intervertebral foramen was made through the vertebrae. This foramen was also formed between the second and third vertebrae. On the dorsal surface of the vertebrae, the cranial and caudal borders were also depressed, and when they were placed side by side, a rhombus-shaped gap was created between the vertebrae. The articular surface of the cranial articular processes was upward and for the caudal ones it was downward. The cranial articular surface of the centrum was round and prominent at the level of the third and fourth vertebrae, but was depressed at the level of the fifth vertebra. It should be noted that the caudal articular surface of the centrum was depressed at the third vertebra but was prominent and round at the fourth vertebra (Figure 7). In fact, the caudal and cranial articular surfaces of the centrum were both prominent at the fourth vertebra. At the fifth vertebra, the cranial articular surface of the centrum was depressed and the caudal articular surface was prominent; the prominent part was divided into two parts. The length of the vertebrae were increasing from the second to the fifth vertebra. The third vertebra`s spine was prominent in its middle part and the fourth vertebra at its cranial part. Also, at the fifth vertebra, the spine was prominent at its caudal part. In all the three vertebrae mentioned, the transverse processes were on the cranial part of the vertebrae.

The Sixth Cervical Vertebra

This vertebra was similar in appearance to the previous ones. There was a crest on the ventral surface of the centrum. The articular surface of the cranial articular processes was slightly positioned caudally. The articular surface of the cranial articular processes was slightly depressed but it was slightly arched at the caudal articular process. The cranial articular surface of the centrum was depressed and the caudal articular surface was prominent. The cranial and caudal articular processes were bifurcated (Figure 7).

The Seventh Cervical Vertebra

The overall shape of the seventh vertebra was similar to the previous vertebrae, only it was wider than them. There were crests on the arches and the caudal articular processes, but they did not have sharp edges. A spine was not seen on this vertebra. The articular surface of the cranial articular processes was caudally inclined. The caudal articular processes were downward and their articular surface was concaved (Figure 4). The articular process was arched. The ventral surface of the centrum had a crest. The cranial and caudal articular surfaces of the centrum were bifurcated and depressed. The transverse crest was located on the cranial part of the centrum (Figure 8).

The Eighth Cervical Vertebra

It had a completely unique shape. Its length was less than the previous ones. From the ventral view, the cranial part of the centrum was wide and its width was reducing caudally. On its ventral surface, there were two protrusions with a crest between them. The ventral crest of this vertebra was smaller than the previous vertebrae. The cranial articular surface had two protrusions. The centrum had a prominent articular

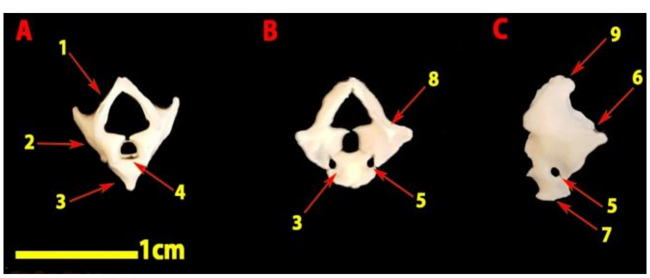

Figure 5. The Atlas of the European pond turtle in A, cranial, B, caudal, and C, lateral views. 1. Neural arch, 2. Intercentrum, 3. Centrum, 4. Cranial articular cavity, 5. Lateral vertebral foramen, 6. Apex of the neural arch, 7. Ventral crest, 8. Caudal articular facet, 9. Dorsal border of the neural arch.

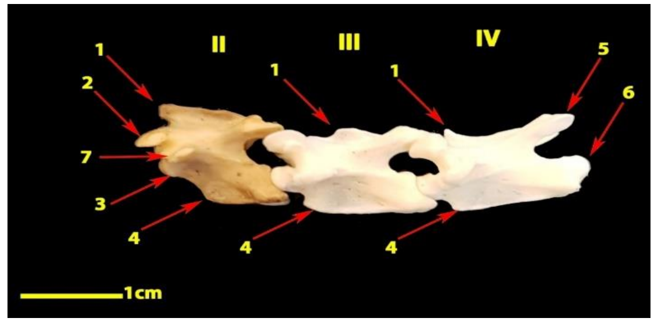

Figure 6. Lateral view of the articulation of the second, the third and the fourth cervical vertebrae of the European pond turtle. These vertebrae are marked with Roman numbers. 1. Spinous process, 2. Cranial articular process, 3. Centrum, 4. Ventral crest, 5. Caudal process, 6. Caudal part of the centrum, 7. Transverse process.

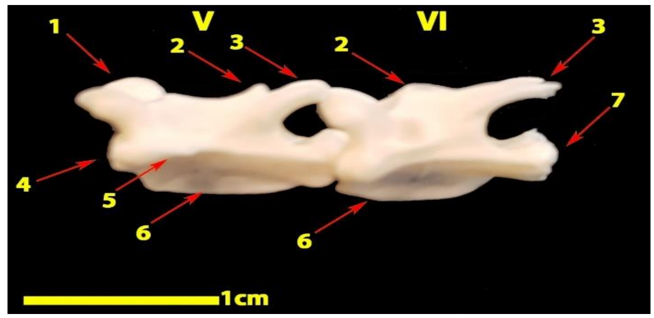

Figure 7. Lateral view of the articulation of the fifth and sixth cervical vertebrae of the European pond turtle. These vertebrae are marked with Roman numbers. 1. Cranial articular process, 2. Spinous process, 3. Caudal articular process, 4. Cranial part of the centrum, 5. Transverce process, 6. Ventral crest, 7. Caudal part of the centrum.

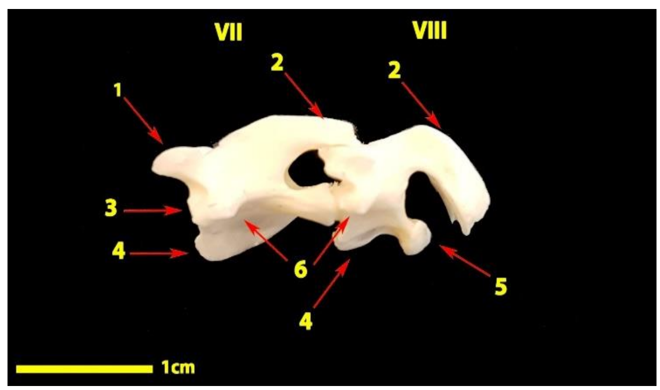

Figure 8. Lateral view of the articulation of the seventh and eighth cervical vertebrae of the European pond turtle. These vertebrae are marked with Roman numbers. 1. Cranial articular process, 2. Caudal articular process, 3. Cranial part of the centrum, 4. Ventral crest, 5. Caudal part of the centrum, 6. Transverse process

surface on the caudal part. The articular surface of the caudal articular processes was sharply depressed and pulled down. These surfaces were almost vertical. The articular surface of its cranial articular processes was somewhat caudally inclined (Figure 4). The size of the caudal articular processes was larger than that of the cranial. The caudal processes had more curve than the other vertebrae (Figure 8). There were crests on the arches and caudal articular processes, but they did not have sharp edges. No spine was found on this vertebra.

Carapace and the Related Vertebrae

The set of dorsal vertebrae, ribs and dermal bones formed a single bone called the carapace. The number of dorsal vertebrae was 10, to which a pair of ribs was attached (Figure 9). The first dorsal vertebra had a different geometric shape than the other vertebrae in order to articulate with the eighth cervical vertebra. The articular surface of the cranial articular processes was semicircular and articulated with the caudal articular processes of the eighth cervical vertebra. The cranial part of the first dorsal vertebra`s centrum had a joint for the caudal articular protrusion of the eighth cervical vertebra`s centrum. From the cranial part of the first dorsal vertebra`s centrum, two very fine bony rods were pulled toward the second rib, and finally attached to the cranial border of the second rib`s head. The rest of the dorsal vertebrae were similar in appearance. There were only differences in their length and width from the cranial part to the caudal part. The vertebrae`s centrum was visible only from the ventral view and from the caudal view the neural dermal plates were taken on them. The first rib`s head was attached to the first dorsal vertebra. The second through the fourth vertebrae were attached to two consecutive dorsal vertebrae and the fifth through tenth vertebrae were connected to dorsal vertebrae with the same number as the ribs. The ribs were connected to the vertebrae proximally and to the dermal plates distally. These were ribbed dermal plates. There were 8 ribbed dermal plates on each side. The first and second ribs` heads were connected to the first dermal plate of the ribs, the ninth and tenth ribs` heads were connected to the eighth dermal plate (Figure 10).

There were no intervertebral foramina between the dorsal vertebrae due to their fusion, but very small lateral vertebral foramina were observed. These foramina were formed in the last four dorsal vertebrae (the tenth, the ninth, the eighth, and the seventh vertebrae) at the fusion site of the vertebrae. These foramina were larger than the cranial ones (Figure 1). The foramina`s site at the cranial vertebrae were mostly above the centrum, instead of close to the vertebrae`s fusion sites.

Carapace`s dermal plates include a nuchal, 7 neural, 8 pairs of costal, 11 pairs of peripheral, a pygal, and a suprapygal fragments (Figure 10).

The sacrum consisted of two vertebrae. This structure had no connection with the carapace. The sacral vertebrae consisted of a centrum and two arches on either side. The arches had articular surfaces for the ilium (Figures 1 and 9).

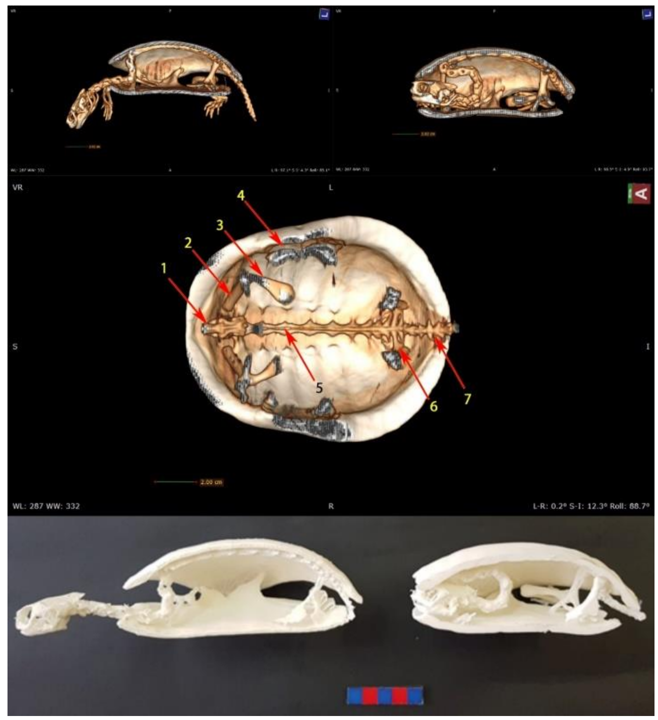

Figure 9. **Top*:*** Lateral view of a sagittal incision of the 3D reconstruction of the European pond turtle`s CT-Scan and the osseous-shaded-vp pattern. In the top left image, the head and the neck are outside the shell and in the top right image they are pulled back inside the shell. **Middle:** Ventral view of a dorsal incision of the 3D reconstruction of the European pond turtle`s CT-Scan and the osseous-shaded-vp pattern. Different sections are labeled in this image**. Bottom:** Lateral view of a sagittal incision of the 3D printing of the European pond turtle CT-Scan and the osseous-shaded-vp pattern. In the bottom left image the head and the neck are outside the shell and in the bottom right image they are puled backed inside the shell. 1. Vertebral column (cervical part), 2. Scapula, 3. Coracoid, 4. Bridge, 5. Vertebral column (Dorsal part), 6. Sacrum, 7. Vertebral column (caudal part).

The Caudal Vertebrae

The tail region consisted of 25 caudal vertebrae. These vertebrae had a centrum, two arches, and transverse processes on either side of the arches. The transverse processes became smaller in the last ones.

The length of the vertebrae gradually decreased towards the last ones. These vertebrae also had cranial and caudal articular processes on their arches through which the vertebrae were connected in series (Figure 1).

Plastron

The dermal plates that made up the plasteron included two right and left epiplastrons, two right and left cranial hypoplastrons, two left and right caudal hypoplastrons, and two right and left xyphiplastrons. Between the right and left epiplastrons was an entoplastron. The cranial and caudal hypoplasterons on each side were involved in the formation of the ridge between the carapace and the pleura (Figure 10). During the dissection of the soft tissues from the bones, it was observed that the clavicle bone was attached to the epiplasteron and entoplestron through ligaments. The cranial and caudal hypoplastrons on each side were involved in the formation of the ridge between the carapace and the plastron. It was also observed that the acromion bone was connected to the epiplasteron and entoplasteron through ligaments.

Discussion

Most anatomical studies on turtles` skeletal system have been performed on the developmental stages of the bones and the embryonic period. In these studies, the bone formation period, especially the skull bones, in the embryo of different species of turtles has been compared with each other. The studies have shown that in the embryonic period, bones with a dermal origin such as the postorbital, the maxilla, the trigeminal, etc. form earlier than bones with a cartilaginous origin such as the prootic, the supraoccipital and the opisthotic. In some of these studies, while examining the embryological development of the bones, the skeleton of an adult species of the same turtle was studied. This comparative study helped determining the final anatomical pattern and understanding the stages and morphological changes of the bones under study.[6] Few studies have simultaneously examined the anatomy and the radiology of turtle`s bones.[2,3] Some of these studies focused on the radiograph device`s settings and the required voltage for preparing graphs of the desired quality in sea turtles. According to the findings of these studies, for scanning the cranial third of the carapace`s length, it is better to increase the kilovoltage and decrease it for the caudal third. It has also been suggested that in reptiles, it is better to use mammographic films to observe in more details.[2] In the current study, due to the use of 3D CT-Scan technique, it is possible to determine the exact location of the bone structure and eliminate the radiography`s artifact due to the overlap of the bones. In 2003, Sheil examined the morphology of bones during the embryonic period in the *Spinifera Apolone*, a soft-shelled turtle, and compared it with another species of turtles. Adult turtles` bones were also closely examined in this study.[6] In 2007, Valente and his colleagues examined the radiographic anatomy of *Caretta caretta* turtle`s limbs and described normal radiographic features of the limbs. They also provided 3D CT-Scan s along with their radiographs.[3] In the current study, the anatomy and the skeleton 3D CT-Scan of the European pond turtles were simultaneously examined for the first time. 3D CT-Scan

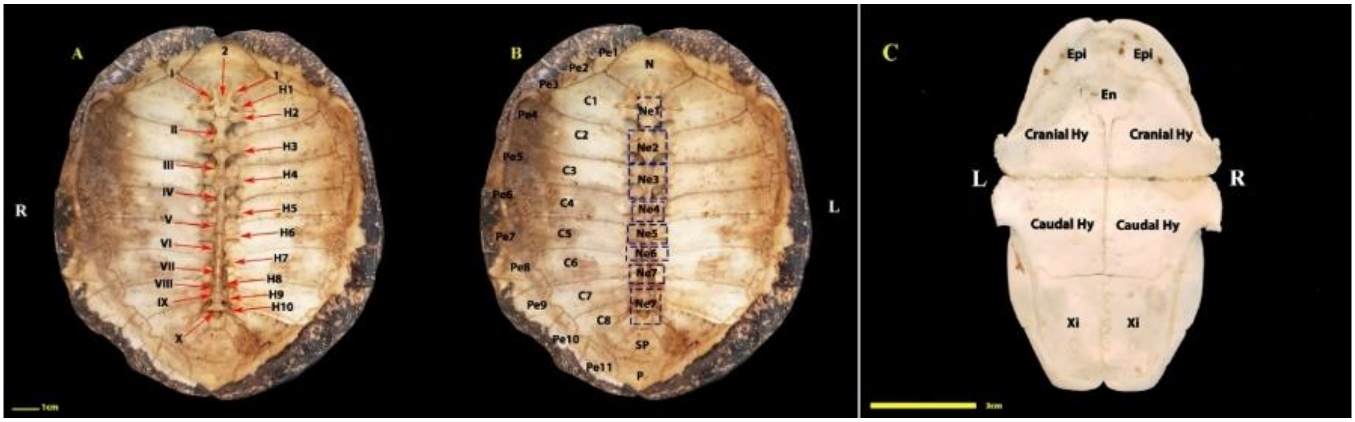

Figure 10. **A,** Ventral view of the European pond turtle`s carapace. Each dorsal vertebra is marked with a Roman number, the rib`s head is shown with the letter H and the corresponding number. **B,** Ventral view of the European pond turtle`s carapace and dermal plates. **C,** Dorsal view of the European pond turtle`s plastron and dermal plates. 1. Cranial articular process, 2. Cranial part of the centrum, N: Nuchal, Pe: Peripheral, P: Pygal, SP: Suprapygal, Ne: Neural, Epi: Epiplastron, En: Entoplastron, Hy: Hypoplastron, Xi: Xiphiplastron.

helped us determine the correct orientation, angle, and position of the bones. Due to the small size of the bones and the loose connection of some of them to each other, inevitably some of the joints break down during the dissection of soft tissues from the bones. For this reason, utilizing the CT-Scan imaging is very useful in assembling skeletons, especially in exotic species whose skeletons have not been thoroughly studied. The CT-Scan provides a good overview of the skeletons and it is a non-invasive and inexpensive method for confirming fractures in turtles.[2] On the other hand, CT-Scans have solved the problems that existed due to overlapping bones in radiographs and are used to diagnose fractures, dislocations, osteoporosis and neoplasms.[3] In the current study, by using The CT-Scans and the anatomical study of the bones simultaneously, the possibility of accurate naming, as well as comparing these two methods were provided.

In this study, a large joint surface was seen between the eighth cervical vertebra and the first dorsal vertebra. In other words, since the movement between these two vertebrae brings the head closer to or away from the body, the range of motion between these two vertebrae is high and the joint surface between these two vertebrae is semicircular and the contact surface between them is increased. On the other hand, in order to increase the range of motion between these two vertebrae, an arch has been created in the neck to allow the head to move as much as possible towards the shell. The articular surface of the cranial articular processes was inclined caudally from the cranial part of the surface to its caudal part. The caudal articular processes were inclined distally from the cranial vertebrae to the caudal vertebrae in order to attach to the cranial articular processes and had a concaved shape. As a result of these changes, an arch was created in the back of the neck. The cervical vertebrae of European pond turtles are very similar to those of *Apolone spinifera*, and both have eight highly mobile vertebrae.[12] However, in sea turtles, the first seven cervical vertebrae are mobile and the eighth one is attached to the carapace. Since sea turtles are unable to pull their heads toward their shell, they do not have a caudal neck arch. The length of their vertebrae is approximately equal, while in European pond turtles the length of the caudal cervical vertebrae was reduced compared to the cranial vertebrae.[4] It seems that the limited space for the placement of the caudal cervical vertebrae inside the shell`s chamber can be the reason for this reduction in length. Valente and his colleagues also studied radiographs of the neck and the trunk of the *Caretta caretta* sea turtle and applied a series of landmarks to identify internal organs, such as the bronchi, the coracoid bone and the acetabulum. They pointed out that by viewing radiographic images, it is possible to establish a connection between external and internal landmarks and to address the approximate location of the coelomic organs in relation to the dermal plates of the carapace and the vertebral column.[2] In 2007, Valente and his coworkers examined a CT-Scan of the vertebral column and the coelomic cavity of the Red Sea turtle called *Caretta Caretta*. These researchers used anatomical slices to better interpret the CT-Scan images. They also determined the position of different parts of the coelomic cavity in relation to the carapace and the vertebral column, in which facilitated interpreting other diagnostic techniques such as radiography and ultrasound, and performing biopsies and surgeries. They noted the position of the intervertebral and the lateral vertebral foramina in the Red Sea turtle, which are the same in European pond turtles.[3] In the current study, intervertebral foramina were observed in the cervical vertebrae and lateral vertebral foramina were observed in the dorsal vertebrae. Since the vertebral neural arches are connected in turtles, the nerves cannot emerge through the adjacent vertebral arches, resulting in the position of the intervertebral foramina in this group of reptiles as mentioned before.

Turtles` bone dermal plates include neural, pleural, and peripheral plates.[4] Trionychidea species have a very diverse number and shape of neural plates. As some researchers have stated, the number of these plates in *Pelodiscus sinens* is seven, eight or nine.[7] The number of these plates in all the samples of this study was seven. Morphological variation is seen in the neural plates of the Trionychidae species, as well as in the European pond turtles due to their positions. Peripheral plates are present in terrestrial and marine turtles, and as a result, the carapace in these species covers the entire dorsal and lateral surface of the viscera. Since the carapace is connected to the vertebral column, any damage to the carapace can lead to spinal cord injury and presence of neurological symptoms. Radiography is the best diagnostic method for these types of fractures in turtles. Radiographs taken in 2006 by Valente and his colleagues from the trunk of a *Caretta caretta* sea turtle showed a large amount of overlap, especially in the cranial part of the carapace, so that the nuchal bones, entoplastron and vertebrae in this section were inseparable.[2] Turtles do not have sternum; instead, they have a structure called the plastron on their ventral surface. The plastron is made up of 9 parts and each of these parts has a structural equivalent in other vertebrates. The epiplastron is equivalent to the clavicle bone, entoplastrone to the interclavicle, and other parts to gastralia. The Gastralia are ventral ribs located on the ventral surface of the abdominal cavity, between the sternum and the pelvis, and are found in some lizards and crocodiles.[4] In the anatomical studies of the current study, the ligament connections observed between the interclavicular bone and the components of the epiplastone and the entoplastone confirmed the evolutionary phase of these components in turning into the clavicular and the interclavicular bones in mammals.[4] In the anatomical studies of the current study, the ligament connections seen between the acromion bone and the components of epiplastone and entoplastone also confirmed the evolution of these components to the clavicle and the interclavicle bones in mammals.[4]

Turtles` skeleton has a special feature and their cervical and pelvic girdles are located inside the rib cage, while in other vertebrates these girdles are located outside the rib cage. This positioning is due to the presence of a structure called the shell in these animals, which incorporates all the viscera and the thoracic and pelvic girdles. Have the girdles been placed outside and gradually moved inside during the evolution of the new turtles? By studying the fossils of these animals, it has been determined that in early turtles, as well as the modern ones, the chest and pelvic girdles were placed inside the rib cage and there is no middle phase between them. This shift from outside to inside happened suddenly. Modern molecular genetics can explain this sudden change. Turtles have a set of Hox genes that influence how axial skeletal components are arranged. The shape and the position of the bones in turtles can also be considered evolutionarily. It is said that this position is similar in reptiles, amphibians and early mammals. In 2012, Lyson and his coworkers examined the relation between the scapula and the rib cage topologically and realized that the scapula in the turtles was placed vertically inside the shell and in front of the rib cage. This position is also seen in early amniotes such as amphibians, monotremes, and reptiles of the Lepidosauria group. They concluded that the evolutionary studies of shellfish should be compared with those of monotremes and reptiles which resemble turtles more closely instead of mice and chickens.[5]

According to the results of this study, it can be concluded that utilization of diagnostic assistive techniques such as the 3D CT-Scan in the study of the skeletons is very useful. By using this technique, it is easy to determine the correct direction and position of the bones. Using radiographic images and 3D CT-Scan s is very useful in assembling the skeletons, especially in exotic species whose skeletons have not been studied. Due to the fact that internal organs of the turtles are located inside the confined space of the shell, performing examinations on turtles is associated with limitations, so utilizing diagnostic imaging methods is very helpful in confirming various problems in turtles. Also, due to the availability of 3D CT-Scan images, in subsequent studies, the position of different organs of the coelomic cavity can be determined in relation to the skeleton to facilitate the interpretation of other diagnostic techniques such as radiography and ultrasound, so that biopsies and surgeries can be performed easier.

Due to the close relation between the bones and the muscles, and the effect of their tension on the shape and the formation of different processes on the bones, it is recommended to examine the muscles of this species. It is also suggested to compare the skeleton of this species with other freshwater-dependent species and determine the differences between them.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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