Turkish Journal of Bioscience and Collections
Volume 7, Issue 2, 2023
E-ISSN: 2601-4292

İSTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE

Evaluation of Siamese Twins of *Testudo graeca* Linnaeus, 1758

Ayfer Şirin[1], Serdar Düşen[1], Eyüp Başkale[1]

Abstract

Objective: In the present study, the morphological features of common tortoise (*Testudo graeca* Linnaeus, 1758) found in the Pamukkale ruins and determined to be two-headed Siamese twins, were investigated using computed tomography.

Material and Methods: The size and weight of the twin tortoise were measured and placed under care in a terrarium similar to their natural habitat. The anatomical features of the two headed Siamese twins were evaluated by three-dimensional volumetric computed tomography.

Results: The Siamese twins combined from the end of the shell have 4 anterior extremities and 2 posterior extremities. Even though tortoises were conjoined, they were reacting independently, but had to move together due to their abnormal shell structures. The Siamese twins weighed 13.2 grams when they were found. They survived 47 days in the terrarium. The tomography scanning showed that that one of the twins was fused from the 7th vertebrae and the other from the 8th vertebrae. Although the internal organ structure is not clear, it has been observed that they use a shared cloaca.

Conclusion: The anomalies rarely seen in reptiles can be caused by many factors, including environmental and genetic factors.

Keywords: Anatomy, Computed tomography, Common tortoise, Pamukkale ruins, Siamese twins

[1]Pamukkale University, Faculty of Science, Department of Biology, Denizli, Turkiye

ORCID: A.Ş. 0000-0002-7815-7652;
S.D. 0000-0002-9071-9320;
E.B. 0000-0003-4489-8687

Received: 12.02.2023
Revision Requested: 02.05.2023
Last Revision Received: 13.05.2023
Accepted: 15.05.2023
Published Online: 11.08.2023

Correspondence: Ayfer Şirin
asirin04@posta.pau.edu.tr

Citation: Sirin, A., Dusen, S, Baskale, E. (2023). Evaluation of Siamese Twins of *Testudo graeca* Linnaeus, 1758. *Turkish Journal of Bioscience and Collections*, *7*(2), 39–43.
https://doi.org/10.26650/tjbc.1250401

Introduction

Developmental anomalies in living organisms have always aroused interest. Conjoined twins or congenital duplications (congenital anomalies) were first described for the family Chelonidae in the 17th and 18th centuries (Edwards, 1751). Although the description of the species cannot be performed properly in those records, it is thought that it is a hawksbill sea turtle and it is stated that it has a single body with two separate heads. The heads connected to the body with the fusion of two necks and moved independently. Since the late 20th century, conjoined twins or congenital duplications have been recorded in some domestic animals (Hiraga & Dennis, 1993; Mostafa *et al*., 2005), salamanders (Pereira & Rocha, 2004), reptiles (Harkewicz, 2002) and marine mammals (Dabin *et al*., 2004). Likewise, examples of congenital twinning have been observed in some species of the family Testudinidae (Dimitropoulos, 1985; Eckert, 1990; Hildebrand, 1938; Molina *et al*., 1996; de Silva *et al*., 2020; Tucker & Funk; 1976; Tucker, 1996; Tucker & Janzen 1997; Yntema, 1970). The only record from Türkiye belongs to the Green Turtle

Chelonia mydas species detected on Samandağ beach, in Hatay (Sönmez *et al*., 2017).

The aim of this study is to investigate the morphological and anatomical characteristics of the Siamese twin common tortoise (*Testudo graeca*) specimen found in the Pamukkale ruins in Denizli using computed tomography (CT).

Materials and Methods

The Siamese twins were delivered to Pamukkale University, Animal Ecology Research Laboratory via the General Directorate of Nature Conservation and National Parks on November 10, 2021. There is no information about the hatching date of the tortoises. Straight carapace length (SCL) and straight carapace width (SCW) of the Siamese twins were measured (Başoğlu & Baran,1977; Tiar Saadi *et al*., 2022) with a digital calliper with a precision of 0.02 mm, and curved carapace length (CCL) and curved carapace width (CCW) were measured with a tape measure with a precision of 0.1 mm. The weight of the Siamese twins was weighed with a precision balance with a sensitivity of 0.01g (Fig. 1).

After a general health examination and external parasite control (Fig. 2) by a veterinarian, the tortoises were placed in a 25×40×30 cm terrarium prepared similarly to their natural habitat and placed under care. The terrarium was covered with bryophytes, natural grasses and leaves to create an optimal environment for the tortoises, all daily activities were observed every 24 hours, and changes were recorded. Since the tortoises are herbivores, they were fed with natural grasses, lettuce, cucumbers, etc. while they were in care. Drinking water and nutrients were provided without limitation and food was reachable at any time.

After the tortoises died, the specimen was preserved in alcohol, and three-dimensional images were taken using computed tomography (Vimago brand, high-resolution three-dimensional volumetric computed tomography) in a veterinary clinic.

Figure 1. Weight and carapace measurement of tortoises.

Figure 2. Cleaning and care of tortoises.

Results

Morphological observations: The individuals have 2 heads, 4 anterior extremities and 2 posterior extremities. The tortoises are referred to as right and left individuals from the dorsal view for ongoing definitions in this study (Fig. 3).

The right and left individuals conjoined were from the 8th marginal and supracaudal scutes of the right individual and from the 10th marginal and supracaudal scutes of the left individual. The supracaudal was unique and formed separately in each individual. It was calculated that the outer sites of the carapace of both individuals had 10 marginal plates, while we counted different numbers of marginal of the inner (connection) sites of the carapace (the right individual had 10 marginal plates and the left individual had 8). It was also observed that the right and left individuals had 4 costal plates on both sides and 5 vertebral plates, and these plates were not abnormal. On the other hand, both individuals had their own pair of gular, humeral, pectoral and abdominal scutes on the plastron. In detail, the posterior portion of the abdominal scute was deformed, and the conjoining started from this part. At the site of connection, the femoral and anal scutes fused in

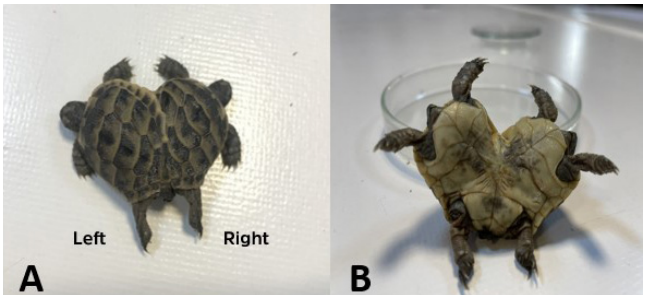

Figure 3. Dorsal (A) and ventral (B) views of tortoises.

both individuals, each with only one pair of these scutes. In addition, three ant heads with their mandibles belonging to the genus *Formica* were found attached to the skin of the tortoises. Two of them were located on the hind leg of the right individual, and the third one was located under the neck of the left individual.

Body measurements: The SCLs of the right and left individuals were 29.42 mm and 32.96 mm, while the SCWs were 25.75 mm and 28.55 mm, respectively. CCLs were 33.00 mm and 37.00 mm, while CCWs were 35.00 mm and 37.00 mm, respectively. The total body weight of both individuals was 13.2 g.

Observation on the thirtieth day at under care: Both individuals showed an enlargement in the width of the flat carapace due to growth, while their weights decreased by 0.1 g. The SCW measurements of the right individual increased from 25.75 mm to 30.16mm, and the left individual increased from 28.55 mm to 30.66 mm during this period. Therefore, the individual on the right grew by 4.41 mm and the individual on the left grew by 2.11 mm. The total weight of the two individuals decreased to 12.3 g until the first 15th day in terrarium conditions due to adaptation stress, and then it was measured as 13.1 g at the end of the first month.

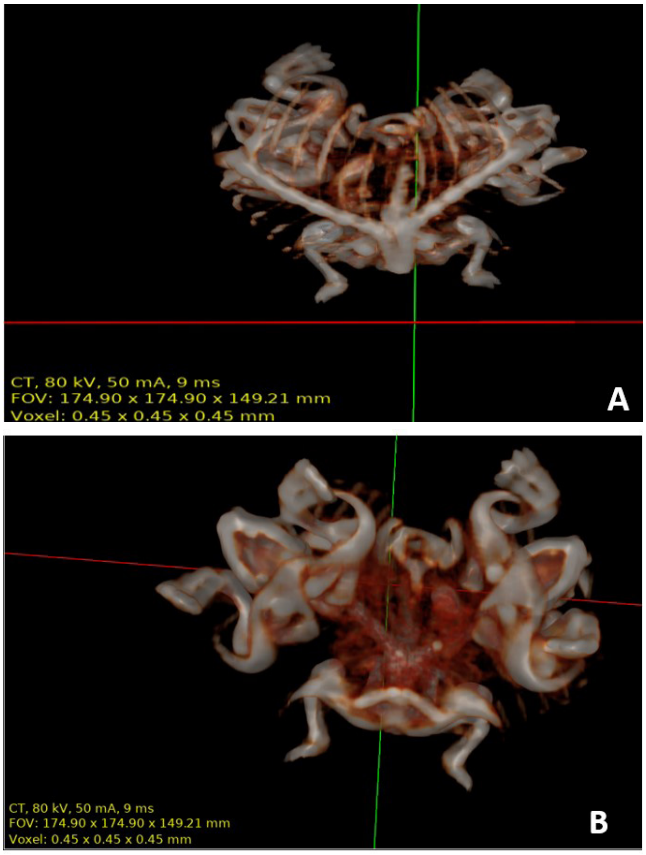

Figure 4. Dorsal CT image (A) and ventral CT image (B).

The results of computed tomography: It was observed that the skeleton in the anterior part of the two individuals was formed separately. In the posterior part of the individuals, it was determined that the right individual was connected from the 7th thoracic vertebra, and the left individual was connected from the 8th thoracic vertebra. They had common sacral and caudal vertebrae (Fig. 4). The tibia lengths of the individuals were measured as 6.9 mm and 5.16 mm, and the femur lengths were measured as 7.29 mm and 6.52 mm, respectively. The right individual was found to be longer than the left one.

Observation of some behaviour: Under care, the twin tortoises survived a total of 47 days in the terrarium, even though they showed self-feeding behaviour. Although the twins were able to respond independently, they were found to move together in one direction due to the conjoined and abnormal shell structure. The direction of movement is usually directed by the right individual that was stronger than the left individual. It was also recorded that both individuals moved towards the food separately.

Discussion

Anomalies in living organisms are not often observed due to the breeding and nesting behaviour of animals. General morphological descriptions of the two-headed tortoise specimens are available in the literature (Barbour, 1888; Cooper, 2009). However, there is no detailed information on internal anatomy. Due to their abnormal body structure, they are unlikely to survive in the wild, as they would have difficulty feeding in the natural environment and could be easily hunted. For instance, a twinning case was observed in *Geochelone elegans* in Sri Lanka. This tortoise in the national zoo did not eat any food given under observation and lived for only 12 days (de Silva *et al*., 2020), whereas the Siamese twin common tortoises in this study were capable of self-feeding and lived for 47 days in the terrarium.

Stockard (1921), Newman (1917, 1923) and others have noted that anomalies can occur and that temporary developmental arrest or regression at a critical stage of the twins' egg development can cause such differences. Interference with normal development or various natural factors can cause these anomalies. Stockard (1921), in his experiments cooling fish eggs during the cell division stages and limiting the oxygen demand, found that malformed embryos were formed as a result of irregular division.

Thus, it was concluded that environmental conditions such as sudden and sharp changes in temperature or oxygen deficiency in the habitat where the organisms spend their embryological period may cause these anomalies to occur (Stockard, 1921; Newman, 1923). It has also been suggested that various factors such as genetic defects and biotic and abiotic factors may play a role in the occurrence of these anomalies and malformations (Velo-Antón *et al*., 2011). It is very difficult to say anything about the cause of Siamese twinning because we have only one case. However, considering the factors mentioned above, the changes in the ecological environment during the incubation period and the effects of these changes on the embryonic development of the common tortoise, genetic factors and the combined effects of these factors may affect the formation of the Siamese twin. It would be beneficial to increase the number of studies on malformation or anomaly for more precise results and evaluations.

In conclusion, the general morphological characteristics of the Siamese twin common tortoises found in the Pamukkale ruins were determined in this study for the first time in the world. Especially up to the 21st century, the small number of individuals with anomalies in nature made it difficult to understand such anomalies. The study of developmental abnormalities in more reptile specimens will facilitate the understanding of such anomalies or malformations that may occur in the wild. Furthermore, when considering species conservation or area conservation strategies, an increase in the number of individuals with anomalies and malformations in a population will inform us about population or ecosystem health and enable early action to be taken.

Acknowledgements: We would like to thank the Ministry of Agriculture and Forestry, 5th Regional Directorate of Nature Conservation and National Parks, Denizli Nature Conservation and National Parks Branch Directorate and its staff for the transportation of the tortoises to Pamukkale University Animal Ecology Research Laboratory, and Veterinarian Caner Can and the Can Veterinary Clinic team for their support to the study.
Peer Review: Externally peer-reviewed.
Conflict of Interest: The authors have no conflict of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: Conception/Design of Study- A.Ş., E.B.; Data Acquisition- A.Ş., E.B.; Data Analysis/ Interpretation- A.Ş., E.B., S.D.; Drafting Manuscript- A.Ş., E.B.; Critical Revision of Manuscript- A.Ş., E.B., S.D.; Final Approval and Accountability- A.Ş., E.B., S.D.; Material and Technical Support- A.Ş., E.B., S.D.; Supervision- A.Ş., E.B., S.D.

References

Barbour, E. H. (1888). On a young tortoise, *Chrysemys picta*, with two heads.*The American Journal of Science* [third series.] Vol. XXXVI, No. 214.--OCT., 1888, pp. 227-230 & Plate V.

Başoğlu, M., & Baran, İ. (1977). *Türkiye Sürüngenleri Kısım I. Kaplumbağa ve Kertenkeleler*. Ege Üniversitesi Fen Fakültesi Kitaplar Serisi, 76, 272 pp.

Cooper, J. E. (2009). Conjoined ("Siamese") twins of the leopard tortoise (Geochelone pardalis), with a plea for documentation of such abnormalities in reptiles. *Journal of Herpetological Medicine and Surgery*, 19(3), 69-71.

Dabin, W., Cesarini, C., Clemenceau, I., Dhermain, F., Jauniaux, T., Van Canneyt, O., & Ridoux, V. (2004). Double-faced monster in the bottlenosed dolphin (*Tursiops truncatus*) found in the Mediterranean sea. *Veterinary Record: Journal of the British Veterinary Association*, 154(10).

De Silva, A., Bandaranayake, R., & Karunarathna, S. (2020) Note on a case of congenital twinning of the star tortoise (*Geochelone elegans*) in Sri Lanka, *Radiata* 29 (4).

Dimitropoulos, A. (1985). A case of living twins in a single egg of the Marginated tortoise, (*Testudo marginata* Schoepff, 1792) in a clutch taken from the wild. *Herptile* 10(3):79-80.

Eckert, K. L. (1990). Twinning in leatherback sea turtle (*Dermochelys coriacea*) embryos. *Journal of Herpetology*, 24(3), 317-320.

Edwards, G.. (1751). *A Natural History of Birds*, Part IV, p. 206.

Harkewicz, K. A. (2002). Prolapse of the penis in a pair of cojoined twin male Hermanns tortoises (*Testudo hermanni* spp.). In *Proceedings of the Association of Reptilian Amphibian Veterinarians Conference, Reno*.

Hildebrand, S. F. (1938). Twinning in turtles. *Journal of Heredity*, 29(7), 243-254.

Hiraga, T. & Dennis, S. M. (1993). Congenital duplication. *Veterinary clinics of North America: Food animal practice*, 9(1), 145-161.

Molina, F. B., Farias, E. C. & Gomes, N. M. B. (1996). Case of twinning in the d'ORBIGNY's slides, *Trachemys dorbigni* (Testudines, Emydidae). *Bulletin of Chicago Herpetological Society*, 31(8), 145-6.

Mostafa, M. B., Koma, K. L. M. K. & Acon, J. (2005). Conjoined twin piglets. *The Veterinary Record*, 156(24), 779.

Newman, H. H. (1917). The biology of twins (mammals). *University of Chicago Press*.

Newman, H. H. (1923). *The physiology of twinning*. University of Chicago Press, Chicago, 1923, 230 pp., 71 figs.

Pereira, R. & Rocha, S. (2004). *Chioglossa lusitanica* (golden-striped salamander): Dicephalic larva. *Herpetological Bulletin*, (87), 29-30.

Sönmez, B., Sert, M., Kayikçi, S., Bedirci, Ö., & Özdilek, Ş. Y. (2017). A two-headed green sea turtle (*Chelonia mydas*) hatchling on Samandağ beach, Turkey. *Russian Journal of Herpetology*, 24(2), 158-162.

Stockard, C. R. (1921). Developmental rate and structural expression: an experimental study of twins,'double monsters' and single deformities, and the interaction among embryonic organs during their origin and development. *American Journal of Anatomy*, 28(2), 115-277.

Tiar-Saadi, M., Tiar, G., Bouslama, Z., & Široký, P. (2022). Mechanisms Determining Body Size and Shape Difference in Algerian Spur-Thighed Tortoises (*Testudo graeca*). *Animals*, *12*(10), 1330.

Tucker, J. K. (1996). Two examples of twinning in the red-eared slider (*Trachemys scripta elegans*). *Bull. Chicago Herp. Soc.* 31, 41-43.

Tucker, J. K. & Janzen, F. J. (1997). Incidence of twinning in turtles. *Copeia*, 166-173.

Tucker, J. K., and R. S. Funk. 1976. Twinning in the Gulf Coast box turtle, *Terrapene carolina major*. *Florida Scientist* 39:238–239.

Velo-Antón, G. Becker, C. G. & Cordero-Rivera, A. (2011). Turtle carapace anomalies: the roles of genetic diversity and environment. *PLoS One*, 6(4), e18714.

Yntema, C. L. (1970). Twinning in the common snapping turtle, *Chelydra serpentina*. *The Anatomical Record*, 166(3), 491-497.