



# Twinning in Asian Giant Tortoise (*Manouria emys phayrei*) Embryos

Sushmita Kar<sup>1,3</sup>, Shailendra Singh<sup>1</sup>, C. Zupeni Tsanglai<sup>2</sup>, and Awadhesh Kumar<sup>3</sup>

<sup>1</sup>TSA Foundation India, D-1/317 Sector F, Jankipuram, Lucknow-226021, Uttar Pradesh, India (shailendrasingh.phd@gmail.com [corresponding author])

<sup>2</sup>Nagaland Zoological Park, Rangapahar, Dimapur-797112, Nagaland, India

<sup>3</sup>Northeastern Regional Institute of Science and Technology, Nirjuli-791109, Arunachal Pradesh, India

Turtles and tortoises are among the most highly threatened groups of vertebrates on Earth (Stanford et al. 2020). The genus *Manouria* (Testudinidae), considered the most primitive genus of living tortoises, contains two known species, *M. emys* and *M. impressa*. The Asian Giant Tortoise, *Manouria emys* (Schlegel and Müller 1840), is the largest tortoise of mainland Asia, with a maximum straight carapace length (SCL) to 600 mm and mass to 37 kg (Stanford et al. 2015). The range of the species, which is listed as Critically Endangered on the IUCN Red List (Choudhury et al. 2019) and on CITES Appendix II (CITES 2024), extends from Bangladesh and northeastern India through mid-elevation hilly areas of Myanmar and western and southern Thailand through Malaysia and Indonesia (including Sumatra and

Borneo). Numbers have declined over 80% in the past three generations with principal threats that include deforestation, subsistence collection, and poaching for the international pet trade (Auliya 2009; Stanford et al. 2015; Choudhury et al. 2019). In India, the species is protected nationally by inclusion in Schedule-I of the Indian Wildlife (Protection) Act, 1972. Two subspecies are currently recognized on the basis of distribution, coloration, plastral scute pattern, and maximum size (Aranyavalai 1996). *Manouria e. phayrei* occurs in India, Bangladesh, Myanmar, and western Thailand (Stanford et al. 2015), where it inhabits dense wet vegetation, often near streams, at elevations of 600–1,500 m asl.

*Manouria emys* has been held in conservation facilities across the world (e.g., Stanford et al. 2015; Tragesar 2018). It



**Figure 1.** The first pair of Asian Giant Tortoise (*Manouria emys phayrei*) twins at Nagaland Zoological Park, Dimapur, India, in the egg (left) and after being placed in a Teak-leaf cover for protection during continuous monitoring (right). Photographs by Sushmita Kar.

was first bred in the Honolulu Zoo in 1978 (McKeown 1990) and subsequently in the Fort Worth and Toledo Zoos in the USA and the Wassenaar Zoo in Den Haag, The Netherlands (McKeown 1990). Because the species' high fecundity and resilience are eminently suitable for conservation breeding, the Turtle Survival Alliance Foundation India in collaboration with the Nagaland Forest Department established the country's largest conservation-breeding facility for *M. e. phayrei* at Nagaland Zoological Park, Rangapahar, Dimapur, Nagaland, with the eventual goal of reintroducing tortoises into natural habitats (Singh et al. 2019; Singh et al. 2021).

The 176-ha Nagaland Zoological Park, located in Dimapur District of Nagaland (25.850150, 93.721064), is recognized by the Central Zoo Authority of India. The *Manouria emys* conservation-breeding enclosure, established in 2018, covers 0.2 ha with separate facilities for upkeep of hatchlings, yearlings, and adults. Incubation lasts 63–84 days and clutch sizes vary from 30 to 70 eggs, depending on the size of the female (Stanford et al. 2015; Kar and Singh, unpublished data). We herein report the first two known records of *M. e. phayrei* twins hatched from two eggs incubated at the zoo.

On 30 April 2023, a large female (SCL 51 cm) laid a clutch of 53 eggs. After an incubation period of 74 days at temperatures of 23.9–33.7 °C and relative humidity of 50–99% (misting on alternate days) in a styrofoam box filled with vermiculite topped with a layer of nesting leaf litter, 38 of the eggs hatched. While assessing hatchlings, we noticed the development of twin embryos in two eggs.

In the first egg (Fig.1), the twins were face-to-face and shared the same yolk sac but were not conjoined. The ratio of their body sizes on the seventh day after hatching (SCL 34 mm and 19 mm) was approximately 2:1. In comparison, the average SCL of the 36 sibling tortoises was 56.5 mm. The combined SCL of the two twins was slightly less (3.5 mm) than the average of their siblings. No morphological or developmental abnormalities were evident, suggesting that embryonic development was initiated at the same time and progressed in stages comparable to those of their siblings. At hatching, both twins had very high-domed carapaces, which reverted to normal in the next three days.

After extraction from the shell, we carefully placed the twins into a folded Teak leaf (*Tectona grandis*) (Fig. 1) to avoid infestation and reduce hatchling mobility during continuous monitoring. We placed them in a wooden indoor enclosure equipped with heat lamps. No food was offered until the yolk sac dried up, as chelonians often survive on the yolk for extended periods (Vleck and Hoyt 1991). We cleaned the abdominal area with a dilute (10%) betadine solution and replaced the leafy cover every day. After seven days of intensive care, the hatchlings separated on their own and appeared to be healthy. However, after three more days, the smaller hatchling died.

One hatchling from the second pair of twins was well developed and healthy (Fig. 2) but the other one failed to complete embryonic development, indicating that they were developing at different rates. Nevertheless, we placed the pair in a folded Teak leaf but the weaker hatchling died after the first night. We carefully separated the healthy hatchling (SCL 39 mm), treated the contact area with a dilute antiseptic solution, and placed it on a water-soaked towel until the yolk sac fully dried. Although smaller than their clutch mates, the stronger hatchlings from both sets of twins survived. The stronger hatchling from the second egg died at 11 months, whereas the stronger one from the first egg suffered mortality at 04 months from the birth month.

Twinning has been documented in both marine and freshwater turtles. The former include Olive Ridley Turtles (*Lepidochelys olivacea*) from Kerala, India (Sreeram et al. 2016), Green Sea Turtles (*Chelonia mydas*) from Sri Lanka (Hewavisenthi 1989), and Leatherback Sea Turtles (*Dermochelys coriacea*) from Malaysia (Chan 1985), whereas the latter include Three-toed Box Turtles (*Terrapene triunguis*) (Crooks and Smith 1958), Common Snapping Turtles (*Chelydra serpentina*) (Yntema 1971), and Red-eared Sliders (*Trachemys scripta elegans*) (Tucker and Janzen 1997) from the USA. To the best of our knowledge, no published information has reported twinning in either subspecies of *Manouria emys*.

The twin pairs of *Manouria emys phayrei* documented herein shared characteristic noted in most other twinned turtles, including sharing a single yolk sac, differing in sizes, and mortality of the smaller individual. In addition to genetic



**Figure 2.** The second pair of Asian Giant Tortoise (*Manouria emys phayrei*) twins at Nagaland Zoological Park, Dimapur, India. Photograph by Sushmita Kar.

and environmental variables (e.g., temperature, humidity), twins of oviparous animals have to compete for the limited resources in an egg (Bowden et al. 2011), which could result in hatchlings differing in rates of development and size (Louro and Pereira 2009; Hirasawa et al. 2019).

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