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Plastral deossification zones in the Endangered Spiny Hill Turtle *Heosemys spinosa* (Testudines: Geoemydidae) on Borneo

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Abstract: Studies of the evolution of turtle shell morphology have raised numerous questions. In this study, five adult females and two adult males of the Endangered Spiny Hill Turtle *Heosemys spinosa* from two localities in western Sarawak in East Malaysia (Borneo) were examined for the presence of plastral deossification zones, reported as indicative of kinesis in earlier studies. A total of 52 (34 females, 18 males) radiographs demonstrated distinct poorly-ossified areas across the hypo-hyoplastral and the hypo-xiphoplastral junctures in females of straight carapace length (SCL) 143.4–211.4 mm. The feature is here interpreted as a permanent plastral hinge, which was not observed in either of the males examined. The smallest female with a weak but discernible line at the contact of these bones had a SCL of 125 mm, suggestive of minimum size of maturity in *Heosemys spinosa* in this population.

Keywords: Biology, growth, maturity, osteology, plastron, reproductive biology, sulcus, turtle.

Bahasa: Kajian mengenai evolusi morfologi berkenaan cengkerang kura-kura merupakan topik yang menimbulkan pelbagai persoalan. Lima ekor betina dan dua ekor jantan yang matang dari spesies Terancam yang dikenali sebagai Spiny Hill Turtle (*Heosemys spinosa*), dari dua lokaliti di barat Sarawak (Malaysia Timur, Borneo) telah dikaji untuk kehadiran zon deossifikasi plastral, yang dilaporkan sebagai indikasi kinesis menurut kajian awal. Sebanyak 52 pengimejan radiografi menunjukkan kawasan ossifikasi yang kurang ketara di antara hypo-hyoplastral dan hypo-xiphoplastral dalam kumpulan kura-kura betina yang mempunyai straight carapace length (SCL) 143.4–211.4 mm. Ciri-ciri ini dikategorikan sebagai engsel plastral yang kekal, dan tidak dilaporkan dari kumpulan kura-kura jantan yang dikaji. Betina yang terkecil menunjukkan garis yang kabur tetapi kelihatan di antara dua tulang ini pada size SCL 125 mm, yang seterusnya mencadangkan saiz kematangan minimum untuk populasi ini.

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Author contributions: SNB carried out the research work, providing data (ie: photographs, measurements) and drafted the manuscripts. ID coordinated the data and participate in writing and reviewing the manuscript. All author agreed to the final version.

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INTRODUCTION

Evolution of turtles has produced variations in shell morphology and pelvic elements (Rivera 2008; Williams & Stayton 2019) and shell kinesis, although female plastral kinesis remains speculative in most species. The degree of kinesis depend on specializations in muscle and ligament systems, and active kinesis usually involves modifications of kinetic structures that allow flexibility through voluntary muscle connections (Pritchard 2003). In passive kinesis, modifications of muscles are not necessary and may rely on pressures exerted over certain periods, including for respiration. For instance, pelvic kinesis in *Homopus signatus* (Hofmeyr et al. 2005), posterior plastral lobe kinesis in *Heosemys spinosa* (Mertens 1942, 1971), carapacial kinesis in *Dogania subplana* (Pritchard 1993) and *Kinixys erosa* (Pritchard 2008) have been assumed on the basis of structure, although their mechanism and function is poorly known. Shell kinesis has been reported in smaller individuals of terrestrial or semi-terrestrial species as a possible adaptation to escape predation and aid locomotion (Berlant & Stayton 2017; Cordero et al. 2018). In contrast, posterior plastral lobe kinesis reported in *H. spinosa* has been hypothesized to be part of its reproductive strategy (Waagen 1984; Moll 1985), even though the shell was once considered as akinetic, and the possibility of plastral muscle specialization has been proposed (Bramble 1974). The hinge in *H. spinosa* reportedly develops in mature females (Moll 1985), resembling the hinge position displayed in the genus *Cuora*, albeit the kinesis in *H. spinosa* is limited to the posterior part of the plastron (Pritchard 1993).

Shell kinesis harbours numerous advantages, including predation survival, locomotion and facilitation of the passage of large eggs in small turtles. Accordingly, variation in the turtle shell has been speculated to be affected by multiple pressures, including phylogenetic, environmental and reproduction, in which plastral kinesis is one result of those pressures (Angielczyk et al. 2011). It is important to note that plastral modifications is usually expressed strongly in adults of both sexes in kinetic species (Ernst & Barbour 1989). Consequently, a number of studies (reviewed in Cordero & Quinteros 2015) discusses the adaptations of shell kinesis to habitat preferences, while sexually dimorphic kinesis must be related to reproduction strategy benefits, although a recent study suggests that the evolutionary structure of shell kinesis may stem from more complex relationships between ecological, phylogeny, and developmental processes in turtles (Cordero et al. 2018).

In the present study, we examine adults of the Spiny Hill Turtle from two free-ranging populations in Sarawak, East Malaysia (northwestern Borneo), in order to understand plastral kinesis (represented by plastral sulcus). Specifically, we will try to ascertain if the feature is restricted to females, the specific plastral bones associated with sulcus, the minimal size of animal that correlates with the development of the feature, and finally, if there are seasonality in variation of the feature.

MATERIALS AND METHODS

Data on plastral morphology were collected within a larger study on the spatial and thermal ecology of the Spiny Hill Turtle from two localities in western Sarawak, northern Borneo. The first was from Kubah National Park (headquarters at 1.6115°N, 110.1964°E, WGS 84), a protected area of 2,230 ha, located within the Matang Massif, the second from forests attached to a privately-owned farmland (1.3073°N, 110.5037°E, WGS 84), around the township of Serian (Image 1). Vegetation types represented include mixed dipterocarp forest, Kerangas (Bornean heath forest) and submontane forests (Hazebroek & Morshidi 2000). Data were obtained between 11 April 2017 and 30 January 2019 from five females that were fitted with temperature sensitive transmitters (Holohil™ Ri2B and Holohil™ PD-2T) for a study of spatial and thermal ecology, during which the present study was conducted. Animals were brought to a veterinary clinic for radiography and released at the point of encounter within a week.

Radiography procedures were conducted by a qualified veterinarian. A Sedecal Apr-Vet (Model E7239X) radiographic unit was used to produce three views per individual (dorsal, ventral and lateral positions). Each exposure was 78 kV (25 mA to 320 mA) for 0.08 sec, following which individuals were weighed using a digital scale (Camry/ ACS-3—JC31). General anaesthesia was not used in the procedure, and depending on the mobility of the individual turtle, manual restraints with tape was used. An Xscan Radiology Application (Version 2.10) was used to edit the image obtained, prior to examination.

OBSERVATIONS AND DISCUSSION

A total of 34 radiographic images were taken of five females, which displayed a distinct sulcus, presumably comprising connective tissue, across the midbody,

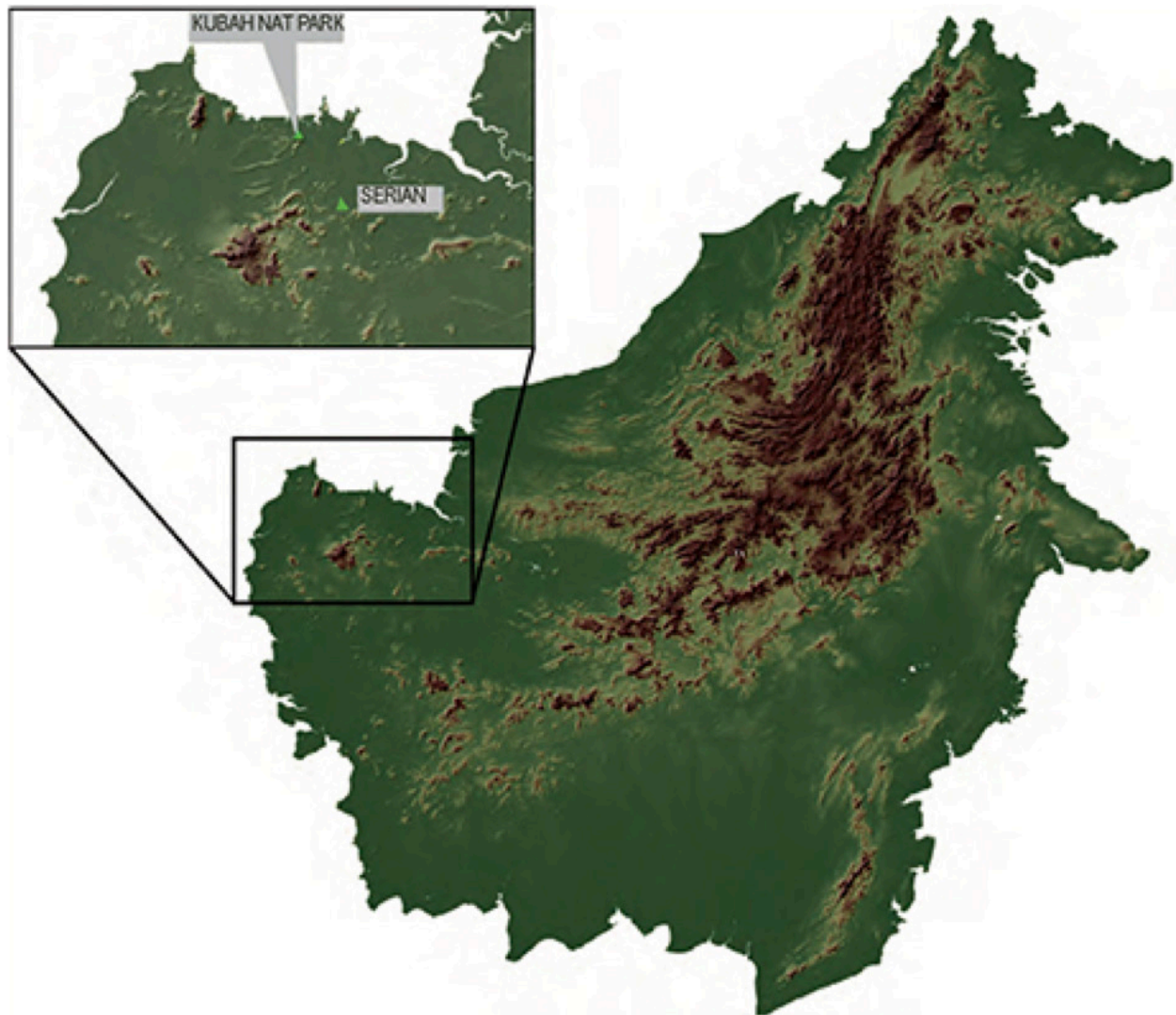


Image 1. Map of north-western Borneo; expanded area to top right show locations of study sites at Kubah National Park and the township of Serian, Sarawak State, East Malaysia.

specifically transversely between the hyoplastral-hyoplastral bones, and the hypoplastral & xiphoplastral bones, under the abdominal and femoral scute regions. None of the 18 radiographs of the two males showed evidence of a sulcus (refer to Image 5), which has been referred in the literature as indicative of a plastral hinge (Mertens 1942, 1971; Bramble 1974; Waagen 1984). The feature is known to exist only in adult females of the species (Moll 1985), presumably to facilitate oviposition and perhaps to allow the passage of large eggs (Yasukawa et al. 2001; Joyce et al. 2012). Other sexually dimorphic features have been listed in Baizurah & Das (2021). Images 2–4 indicate that kinesis of plastral elements may be shown by *H. spinosa* as seen in two females (SNB 638 and SNB 641) across time. The smallest female (mean SCL of 125.1 mm) was beginning to display

presence of such a gap (Image 3) indicating possible size at maturity of females in *H. spinosa*. Examination of the radiographs of these individuals do not indicate a greater development of the hinge at any particular month, as might be expected if the hinge becomes functional only periodically, for the passage of the eggs.

Previous studies have discussed that pelvic aperture dimensions may be a limiting factor in reproductive output, and how it relates to sexual dimorphism in some turtles (Clark et al. 2001; Matysiak et al. 2017; Cordero et al. 2018). Apart from pelvic size variation and reduced relative plastral length in males in increasing reproduction output, plastral kinesis can serve a similar function in increasing reproduction output. Plastral kinesis is thought to reduce pelvic strain in females during egg-laying (Legler 1960; Yasukawa et al. 2001). The first

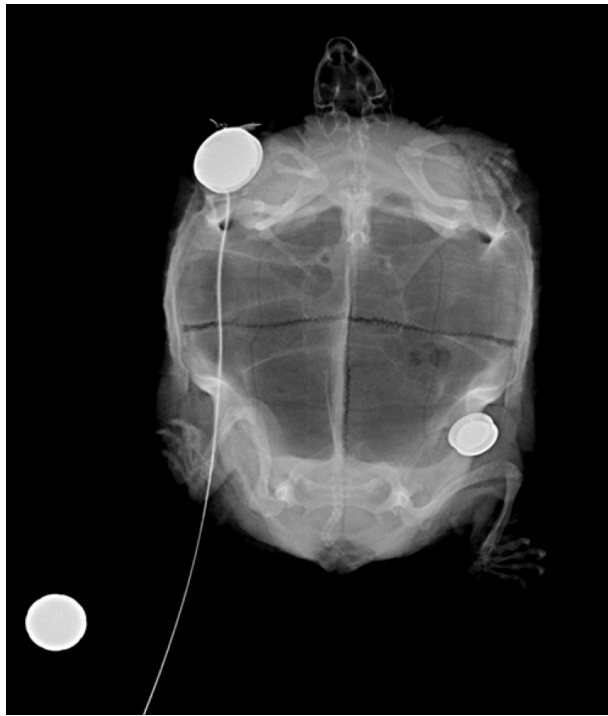


Image 2. Radiographs of plastron showing sulcus at juncture of hyo-hyoplastra of an adult (SCL 191 mm) female *Heosemys spinosa* (SNB 643). Image taken in November 2018. Also visible in this and the subsequent images are the externally-attached radio-transmitter, and an iButton, implanted for a larger study of movement and thermoregulation in the species.

Table 1. Details of *Heosemys spinosa* radiographically examined.

Identification	Sex	Total radiographs	Locality
SNB 637	Male	16	Kubah NP
SNB 638	Female	14	Kubah NP
SNB 639	Male	2	Kubah NP
SNB 641	Female	12	Kubah NP
SNB 642	Female	2	Kubah NP
SNB 643	Female	4	Kubah NP
SNB 640	Female	2	Serian

record of a plastral hinge in *H. spinosa* was by Mertens (1942, 1971), followed by the detailed examination of two females by Waagen (1984), who speculated that the structure is rather weak in adult females. Hence the likelihood that kinesis serves a probably protective mechanism is unlikely. It is important to note that these descriptions are not supported by histological data hence lacking the information needed to functionally validate female-specific plastral kinesis in *Heosemys*. Waagen (1984) described the hinge as consisting of fibrous tissues internally that did not appear to

change with body size, although the possibility of temporal change was mentioned. As described in that study, the present one found that the structure does not remain distinct year round, the deossification of bones presumed related to oviposition, not affected by seasonal changes, as evidenced in our radiographs (see Images 1–3). However, we have no indication that any of our females were preparing to reproduce, and no mating behaviour was observed. Previous literature on reproduction of *H. spinosa*, albeit in captivity, stated that copulation is typically triggered by rain showers, and egg deposition usually occurs in March–July (Herman 1993; Goetz 2007). In Sarawak (northwestern Borneo), high humidity and temperatures are encountered throughout the year, and periods associated with high rainfall events occur between November and March, with the passage of the north-east monsoons, and a weaker one between May and September, coinciding with the south-west monsoons (Sa’adi et al. 2019).

Numerous functional traits usually emerge late in turtle ontogeny, including development of fibrous tissues, which are known to progress slowly via repatterning of tissue which is acquired over the growth period (Cordero et al. 2018). The gradual process may explain the changes in hyo-hyoplastral, and hypo-xiphoplastral regions we noted across time. For instance, hatchling plastron shape of kinetic-shelled species undergoes differentiation post embryonic stages, especially in area where the hinge presumably occurs, as they reached maturity, in contrast to akinetic species which undergoes plastron differentiation at extreme and posterior ends (Cordero et al. 2019). Lastly, extrinsic factors such as abundance of resources and rainfall are known to affect reproductive cycles in some species (Akani et al. 2005; Loehr et al. 2011; Graham et al. 2015). Our radiographic observations suggest that hyo-hyoplastral and hypo-xiphoplastral kinesis in *H. spinosa* is possibly influenced by reproductive needs, developing during ontogeny, and may not be associated with local climate.

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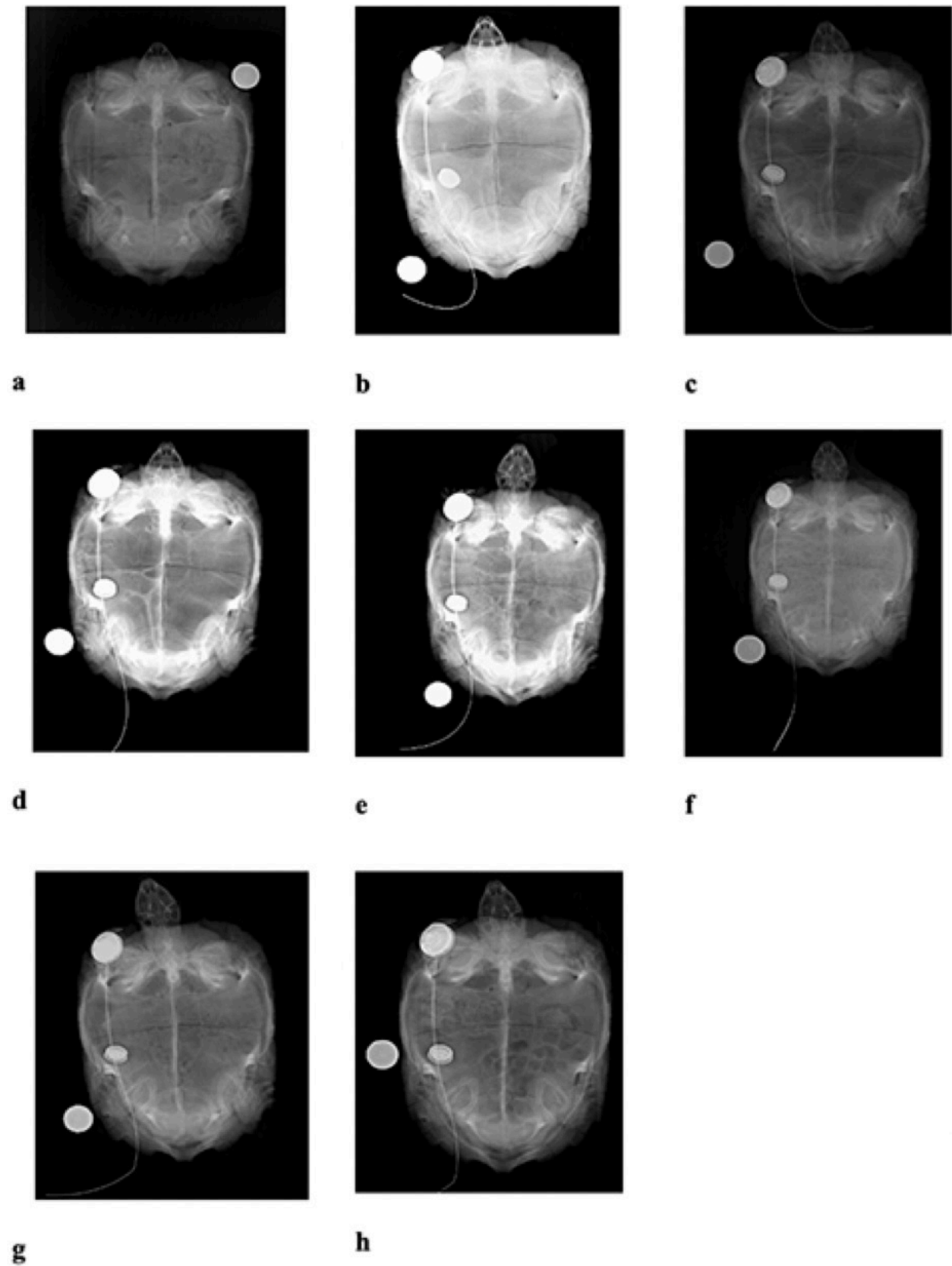


Image 3. Radiographs of plastron showing sulcus at junctures of hyo-hyoplastra and hypoplastra-xiphiplastra of a female *Heosemys spinosa* (SNB 638). Straight carapace length (SCL in mm) as follows: a) 191.33; b) 195.46; c) 198.12; d) 198.12; e) 201.3; f) 208.31; g) 210.06; h) 210.53. Diameter of scale markers = 22 mm. a) Aug-2017; b) Oct-2017; c) Dec-2017; d) Feb-2018; e) April 2018; f) Nov-2018.

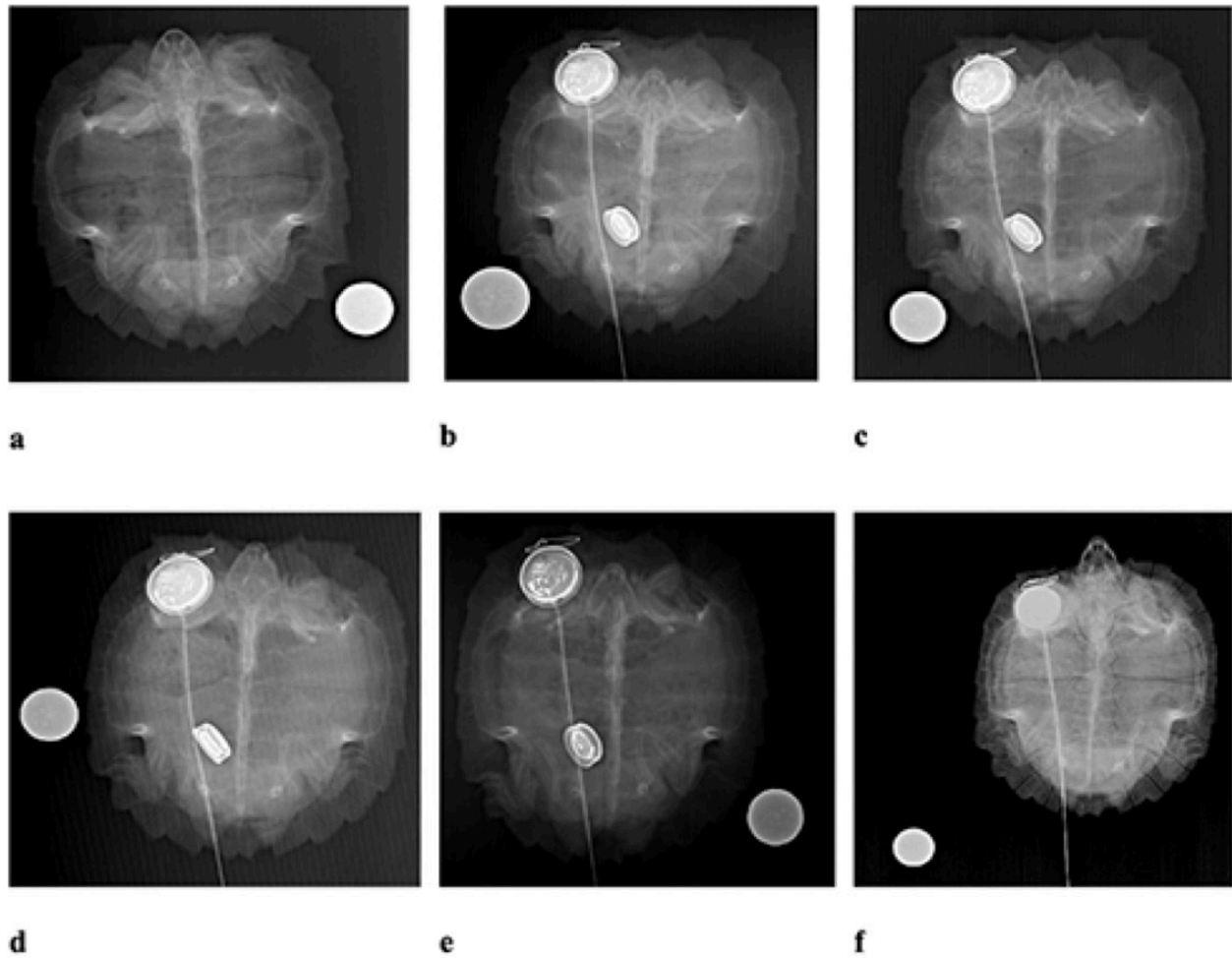


Image 4. Radiographs of plastron showing sulcus at junctures of hyo-hypoplastra and hypoplastra-xiphoplastra of a female *Heosemys spinosa* (SNB 641). Straight carapace length (SCL in mm) as follows: a) 125.1; b) 126.2; c) 126.96; d) 128.52; e) 131.07; f) 143.29. Diameter of scale markers: 22 mm.

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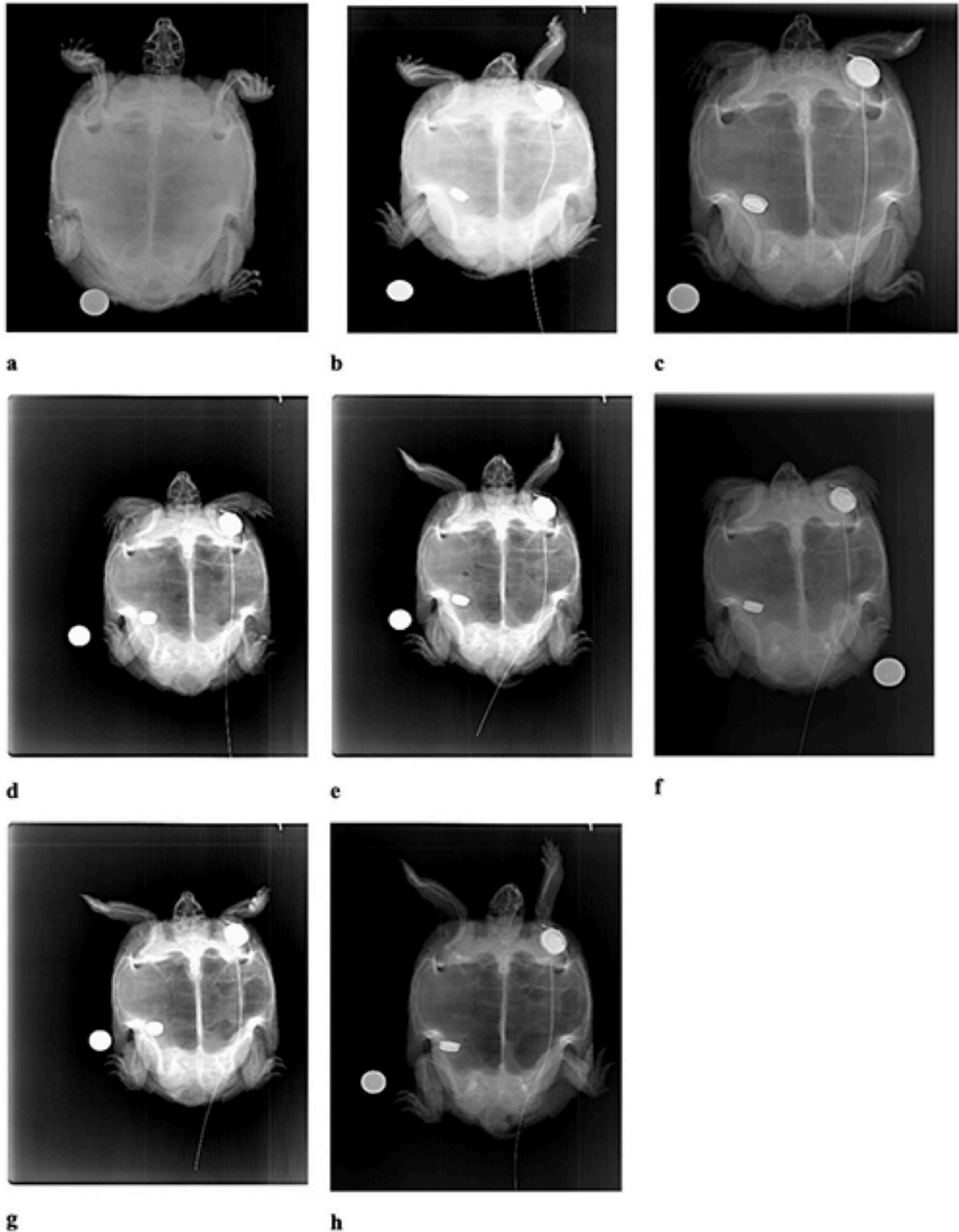


Image 5. Radiographs of plastron of a male *Heosemys spinosa* (SNB 637). Straight carapace length (SCL) 637 mm. Straight carapace length (SCL in mm) as follows: a) 198.2; b) 200.8; c) 205.1; d) 218.5; e) 223.19; f) 225.91; g) 231.27; h) 237.72. Diameter of scale markers: 22 mm.

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