forest still exist west of the lake. To the east, modification of college grounds and other human activities have converted the upland forest to private and commercial land use. These anthropogenic changes have been shown to significantly increase the temperature of streams draining into Lake Matoaka by almost 2°C (Murphy et al., unpubl. data). Less shading by forest may also increase soil temperatures, which in turn would affect sex determination of incubating turtle eggs (Janzen, 1994). Soil temperatures where female turtles nest may be lower in the undeveloped watershed surrounding the western arm of the lake, relative to the more developed eastern arm. Incubation temperature differences of 1-2°C have been shown to yield variation in the proportion of male musk turtle hatchlings from 23 to 94% (Vogt et al., 1982; Ewert and Nelson, 1991). Since most musk turtles generally show site fidelity, differences in hatchling sex ratios may contribute to location-specific variation in sex ratio of the adults.

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## LITERATURE CITED

- CAGLE, F.R. 1939. A system for marking turtles for future identification. Copeia 1939:170-172.
- CAGLE, F.R. 1942. Turtle populations in Southern Illinois. Copeia 1942:155-162.
- CONGDON, J.D., GREENE, J.L., AND GIBBONS, J.W. 1986. Biomass of freshwater turtles: a geographic comparison. American Midland Naturalist 115:165-173.
- Cox, G.W. 2002. General Ecology Laboratory Manual. New York, NY: McGraw-Hill Higher Education, 312 pp.
- DODD, C.K., JR. 1989. Population structure and biomass of Sternotherus odoratus (Testudines: Kinosternidae) in a northern Alabama lake. Brimleyana 15:47-56.
- EBERHARDT, L.L. 1969. Population estimates from recapture frequencies. Journal of Wildlife Management 33:28-39.
- EDMONDS, J.H. AND BROOKS, R.J. 1996. Demography, sex ratio, and sexual size dimorphism in a northern population of common musk turtles (*Sternotherus odoratus*). Canadian Journal of Zoology 74:918-925.
- ERNST, C.H. 1986. Ecology of the turtle, *Sternotherus odoratus*, in Southeastern Pennsylvania. Journal of Herpetology 20:341-352.
- ERNST, C.H., LOVICH, J.E., AND BARBOUR, R.W. 1994. Turtles of the United States and Canada. Washington, DC: Smithsonian Institution Press, 578 pp.
- EWERT, M.A. AND NELSON, C.E. 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. Copeia 1991(1):50-69.
- GIBBONS, J.W. 1970. Reproductive characteristics of a Florida population of musk turtles (Sternotherus odoratus). Herpetologica 26:268-270.
- JANZEN, F.J. 1994. Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. Ecology 75(6):1593-1599.
- JORDAN, F. AND ARRINGTON, D.A. 2001. Weak trophic interactions between large predatory fishes and herpetofauna in the channelized Kissimmee River, Florida, USA. Wetlands 21:155-159.

MAHMOUD, I.Y. 1969. Comparative ecology of the kinosternid turtles

of Oklahoma. Southwestern Naturalist 14:31-66.

- MITCHELL, J.C. 1988. Population ecology and life histories of the freshwater turtles *Chrysemys picta* and *Sternotherus odoratus* in an urban lake. Herpetological Monographs 2:40-61.
- REAM, C. AND REAM, R. 1966. The influence of sampling methods on the estimation of population structure in painted turtles. American Midland Naturalist 75: 325-338.
- Risley, P.L. 1930. Anatomical differences in the sexes of the musk turtle, *Sternotherus odoratus* (Latreille). Papers of the Michigan Academy of Science, Arts, and Letters 11: 445-464.
- RISLEY, P.L. 1933. Observations on the natural history of the common musk turtle, *Sternotherus odoratus*. Papers of the Michigan Academy of Science, Arts, and Letters 17:685-711.
- SACKS, D. 1984. The history of the William and Mary campus. Honors Thesis, College of William and Mary.
- TINKLE, D.W. 1961. Geographic variation in reproduction, size, sex ratio and maturity of *Sternotherus odoratus*. Ecology 42:68-76.
- VOGT, R.C., BULL, J.J., MCCOY, C.J., AND HOUSEAL, T.W. 1982. Incubation temperature influences sex determination in kinosternid turtles. Copeia 1982:480-482.
- WADE, S.E. AND GIFFORD, C.E. 1965. A preliminary study of the turtle population of a northern Indiana lake. Proceedings of the Indiana Academy of Science 74:371-374.
- WILLIAMS, J.E. 1952. Homing behavior of the painted turtle and muskturtle in a lake. Copeia 1952(2):76-82.

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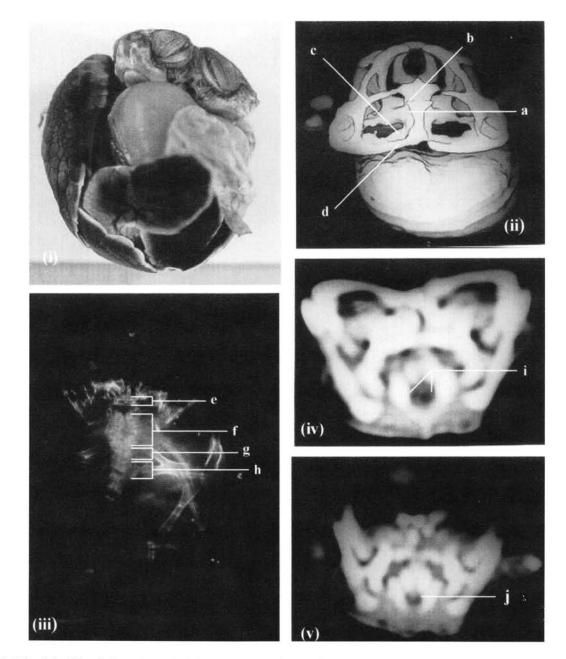
## Axial Bifurcation in a Bicephalic Chelonia mydas Embryo

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Developmental anomalies in reptiles are reported only occasionally and have received little attention in general (Cunningham, 1937; Davies, 1974; Branch, 1979; Bellairs and Kamal, 1981; Miller, 1985). In marine turtles, the incidence of developmental abnormalities appears to be low, judging from the scant literature on the subject. Of the abnormalities known to occur in turtles, the common ones are albinism, pigmentation or pattern variations, malformation in scute patterns, size reduction or loss of body parts, malocclusion of jaws, twinning (Harrisson, 1963; Bellairs, 1983; Miller, 1985; Chan, 1985; Frye, 1991), and a recent case of conjoined twins (Haft, 1994). In this note, we describe, what is to our



**Figure 1. (i)** Conjoined bicephalic embryo of *Chelonia mydas* with fully developed flippers and residual yolk, (ii) CAT scan of embryonic heads showing point of separation: a, jugal bones; b, postorbital bones; c, maxilla; d, mandible, (iii) Radiograph of dorso-ventral view of embryo: e, laterally extended atlas, f, C2–C6; g, C7; h, first dorsal thoracic vertebrae, (iv) CAT scan of cervical region: i, two separate neural spaces with septum, (v) CAT scan of thoracic region showing one neural space (j) without any septum in a normal vertebra.

knowledge, a first case of incomplete bicephaly in the green turtle, *Chelonia mydas*.

*Methods.* — A dead deformed *C. mydas* embryo with incomplete head bifurcation was recovered from an unhatched egg collected at the sea turtle hatchery on Talang Talang Besar Island, the largest of three turtle nesting islands situated off the southwest coast of Sarawak (109°46'E, 1°44'N), Malaysia. A beach hatchery on this island has been used since the early 1950s to manage stocks of green turtles. In this hatchery operation, entire clutches of eggs were collected for incubation in the beach hatchery. In recent years, a proportion of the nests have been left to incubate *in situ* (Diong et al., 1999a, 1999b). The deformed embryo was staged (Miller, 1985) and xrayed at 25 kV, 28 mAs (Siemens Mammomat X-ray machine) to study the skeletal structures associated with the abnormality. As radiography alone was not able to analyze fully the skeletal anomaly, the embryo was subjected to a computerized axial tomography (CAT) scan to determine skeletal malformation arising from its incomplete head duplication. The specimen was deposited in the Sarawak Museum, Kuching, Malaysia (MU/480/3.49a).

*Results.* — The near-term embryo was aged at stage 28; its body was darkly pigmented but lighter than in hatchlings, the skin at the base of the limbs was not pigmented. The malformed embryo showed incomplete cranial bicephaly

(Fig. 1-i). The right and left heads were equally developed and exact counterparts of each other. The two heads were symmetrically aligned with the axis of the body. Each head had two eyes, one mouth, and a fully formed beak. Both heads shared a thickened neck which joined the separate heads to one common body. The body had one set of front and hind flippers, one tail, one cloaca, and one residual yolk mass.

Computerized axial tomography (CAT) scans showed that the bicephaly was incomplete. The right and left embryonic heads (midplane) were angled 90° to each other, separated proximally at a common jugal bone and along a bony ridge on the distal border of the proximal orbits of the two heads comprising the postorbital-jugal bones (Fig. 1-ii). Partial separation was limited to the skulls; the mandibles were entirely separate, without any fusion point. The distal unseparated skull was connected to one common axial skeleton (Fig. 1-iii). Radiography revealed that the cervical vertebrae (C) 1 and 2 were conjoined mesially to form one common atlas which was unusually broad and supported the two separated heads. Cervical vertebrae 2 to 6 were incompletely bifurcated (Figs. 1-iii, 1-iv). The spinal column from C2 to C5 was expanded laterally; neural tissues from C2 to C7 appeared as two distinct bundles separated by a soft tissue septum (Fig. 1-iv). The centra in these vertebrae were not joined; they had two lateral ventral walls but no medial wall. The neural tube was incompletely divided. Radiographically, the last cervical vertebra, C7, was normal, as were the other vertebrae that form the thoracic and caudal regions of the axial skeleton (Figs. 1-iii, 1-v).

*Discussion.*—The bicephalic embryo described here is a case of conjoined twins in *Chelonia mydas* involving partial head separation. It differs from one other known case of conjoined twins in *C. mydas* (see Haft, 1994) in which separation occurred further distally, resulting in a hatchling with two fully functional, independent heads, each with a neck of its own, one common body, and single pairs of front and hind flippers.

Incomplete head bifurcations initiate in early development and typically start at the tip of the head fold. The axial bifurcation that forms later during somite formation may have resulted from lateral dichotomization of the cervical vertebrae. This could have happened from the partial separation of blastomeres at some stage of early embryonic development. Developmental anomalies may also arise due to mutation or the genetic constitution of the zygote (Ewert, 1979). The most likely cause of the incomplete bicephaly is a miscoding or misdirection in the genetic code that controls the division of cells in the head. The frequency of malformation in marine turtles has been reported to be low, ranging from 0.17 to 0.6% in samples ranging from about 5000 to 90,000 eggs (McGehee, 1979; Blanck and Sawyer, 1981; Miller, 1982; Miller, 1985). The incidence of malformed embryos in the Sarawak green turtle population is unknown. Nest contents in the hatchery that are routinely dug out for disposal after hatchling emergence should be analyzed for incidence of malformations. A comparison of incidence of malformations

from hatchery and *in situ* nests may shed some light as to whether hatchery practice and hatchery incubational environment or other genetic factors may be a contributory factor to abnormal development in this population.

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## LITERATURE CITED

- BELLAIRS, A.D'A. 1983. Partial cyclopia and monorhinia in turtles. In: Rhodin, A.G.J. and Miyata, K. (Eds.). Advances in Herpetology and Evolutionary Biology. Essays in Honor of Ernest E. Williams. Cambridge, MA: Museum of Comparative Zoology, pp. 150-158.
- BELLAIRS, A.D'A. AND KAMAL, A.M. 1981. The chondrocranium and the development of the skull in recent reptiles. In: Gans, C. and Parsons, T.S. (Eds.). Biology of the Reptilia. Vol. 11. London: Academic Press, pp. 1-263.
- BLANCK, C.E. AND SAWYER, R.H. 1981. Hatchery practices in relation to early embryology of the loggerhead sea turtle, *Caretta caretta* (Linne). Journal of Experimental Marine Biology and Ecology 49:163-177.
- BRANCH, W.R. 1979. Dicephalism in the African worm snake Leptotyphlops bicolor. Herpetological Review 10(1):6-7.
- CHAN, E.H. 1985. Twin embryos in unhatched egg of *Dermochelys coriacea*. Marine Turtle Newsletter 32:2-3.
- CUNNINGHAM, B. 1937. Axial bifurcation in serpentes. Durham, NC: Duke University Press, 117 pp.
- DAVIES, M. 1974. A double-headed grass snake, *Natrix natrix helveticus*, found at Ladock, Truro, Cornwall. British Journal of Herpetology 5(3):452-453.
- DIONG, C.H., LIM, S.S.L., AND LEH, C.M.U. 1999a. Isolated reincubation of five-day old white-spotted eggs of *Chelonia mydas* in a beach hatchery: is hatch rate improved? Sarawak Museum Journal 54:301-308.
- DIONG, C.H., LEE, C.S., YAP, S.F., AND LEH, C.M.U. 1999b. Nesting pattern, clutch size variation and estimates of female population size in the Sarawak green turtle *Chelonia mydas*. Sarawak Museum Journal 54:309-318.
- EWERT, M.A. 1979. The embryo and its egg:development and natural history. In: Harless, M. and Morlock, H. (Eds.). Turtles: Perspectives and Research. New York: John Wiley and Sons, pp. 333-413.
- FRYE, F.L. 1991. Developmental anomalies. In: Frye, F.L. Biomedical and Surgical Aspects of Captive Reptile Husbandry. Second Enlarged Edition. Malabar, FL: Krieger Publ. Co., Vol. II, pp. 393-419.
- HARRISSON, T. 1963. Notes on marine turtles: 14 Albino green turtles and sacred ones. Sarawak Museum Journal 11(21/22):304-306.
- HAFT, J. 1994. Bemerkungen zu den Suppenschildkröten bei Xcacel, Halbinsel Yucatan, Mexiko. Salamandra 30(4):254-259.
- MCGEHEE, M.A. 1979. Factors affecting the hatchling success of loggerhead sea turtles. M.S. Thesis, University of Central Florida.
- MILLER, J.D. 1982. Development of marine turtles. Ph.D. Thesis, University of New England, Australia.
- MILLER, J.D. 1985. Embryology of marine turtles. In: Gans, C., Billett, F., and Maderson, P.F.A. (Eds.). Biology of the Reptilia. Vol. 14. New York: John Wiley and Son, Inc., pp. 269-328.

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