First Report of Twinning in the Hawksbill Sea Turtle (*Eretmochelys imbricata*) from Khram Island, Chonburi Province, Thailand.

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ABSTRACT
Twinning in reptiles and especially in the sea turtle, occurs rarely. During an investigation (May – August, 2011) of the nesting monitoring program on Khram Island, Chonburi, where an important nesting site in Thailand is located, we found that the number of female turtles has decreased. Unexpectedly, twinning in hawksbill turtle embryos has also been reported. This is the first discovery of such a phenomenon in the Gulf of Thailand. An un-hatched egg, with two fully developed embryos has been found. The embryos shared one yolk sac and differed substantially in size. The larger one has a carapace deformity with a 31.5 mm curved carapace width and a 22.5 mm length, while the smaller embryo has a normal carapace with a 16.5 mm width and a 18.5 mm length. However, the causes of twinning in sea turtles are still not well understood. Low temperature has been suggested as a possible factor. Other environmental or genetic factors could also be involved. For further studies, the pattern of the twins' and parental DNA should be investigated.

KEYWORDS: Twinning, hawksbill sea turtle (*Eretmochelys imbricata*), deformation, abnormal, embryo

INTRODUCTION
There are many important sea turtle nesting sites in the Gulf of Thailand. One of the biggest sites is on Khram Island, where the nesting beaches are protected by the Royal Thai Navy (Charuchinda & Monanunsap, 2000). Only two species have been recorded nesting on the island: the green turtle (*Chelonia mydas*) and the hawksbill turtle (*Eretmochelys imbricata*). Due to the habitat protection program of sea turtles in Thailand since 1956, activities overseeing sea turtle conservation on Khram Island have been undertaken (Penyapol, 1957; Phasuk, 1992; Monanunsap & Charuchinda, 1994; Charuchinda & Monanunsap, 1998).

More than 100 females of each turtle species have been reported as nesting here each year (Charuchinda & Monanunsap, 2000). Problematically, the number of eggs hatching has decreased. Twinning in reptiles is a rare but widely known phenomenon that includes both the development of separate individuals of equal or unequal size, as well as different degrees of the malformation known as axial bifurcation. Completely separated, freshwater turle twins have been observed surviving hatching in captivity and under laboratory conditions (Crooks & Smith, 1958; Obst, 1976; Heimann, 1993 and Messinger & Patton, 1995) and Hewavisenthi (1989) recorded the larger twin of a pair found hatching in the nest of a green turtle during post-hoc examination. Conjoined twin embryos in sea turtles usually die (Miller, 1985) in the early stages of development before pipping (Kaska & Downie, 1999).

MATERIALS AND METHODS
The Eastern Marine and Coastal Resources Research Center and The Royal Thai Navy staying on Khram Island investigated the island's nesting beach during May – August, 2011. (Fig. 1) The female turtles were tagged internally using passive, integrated, transponder tags and externally, using metal tags on their fore left flipper. The total turtle eggs were observed and the hatching rate was calculated. The characteristics of un-hatched eggs were note.

RESULTS and DISCUSSION
The finding of twinning was reported during a nesting monitoring program undertaken on Khram Island, Chonburi between May and August, 2011. In this study, data have been recorded from 140 clutches of eggs laid by the green turtle (*Chelonia mydas*) and the hawksbill turtle (*Eretmochelys imbricata*). During July 2011, twins of hawksbill turtles were found in one out of every 9,712, unhatched eggs (twinning rate on Khram Island was 0.01%). The low incidence of twinning that was found in unhatched eggs (0.01 %) was not statistically different

to those reports taken from other nesting colonies of *Caretta caretta* (0.036 % from Blanck & Sawyer, 1981), for the sea turtle *Chelonia mydas* (0.152 % from Fowler, 1979) and the loggerhead sea turtle (from Susanna. P et al., 2011). The twins differed from each other in size. The larger one had a carapace deformity with the normal number of lateral (costal) scutes on both side.

Its curved carapace measurements were 31.5 mm in width and 22.5 mm in length. The smaller embryo had 4 left and 5 right lateral scutes and its carapace measured 16.5 mm in width and 18.5 mm in length. They were connected by a shared yolk sac (Fig.2A-C)

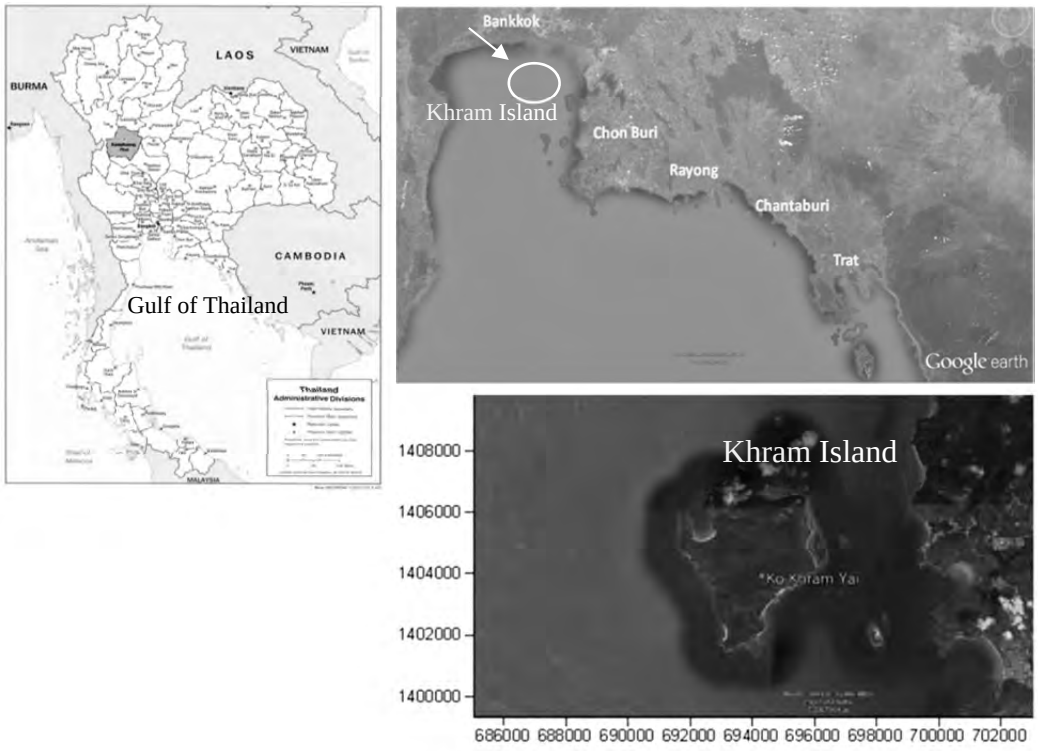

Fig.1: The map of Khram Island, Chonburi Province, Thailand, showing the location of the nest where the twin hawksbill turtle embryos were found.

Figs.2: A-C: The twin hawksbill sea turtles, connected by the yolk sac differed from each other in size (see text).

Twinning is one form of abnormality found in sea turtle embryos and hatchlings. There have been reports of twinning found in loggerhead turtles (*Caretta caretta*) from South Carolina (Caldwell, 1959), Japan (Fujiwara, 1964), Florida (McGehee, 1979), Australia (Miller, 1982; Limpus, 1985), North Carolina (Ferris, 1986 - reviewed by Dodd, 1988), Cyprus and Turkey (Kaska et al., 2000) and southern Mozambique (Louro, 2009), leatherback turtles (*Dermochelys coriacea*) from Sri Lanka (Deraniyagala, 1930 and 1932) and Malaysia (Chan, 1985) and in the green turtle (*C.mydas*) from Sri Lanka (Hewavisenthi, 1989).

The characteristics of twinning from Khram Island were similar to those described by the embryos in that they too shared a common yolk sac. They differed in size, had abnormal number of lateral scutes and did not

survive. Only a few studies are available on embryonic development of sea turtles, especially focusing on cases of twins. Causes and incidents of twinning in sea turtles are not yet well understood. Kaska et al. (2000) found that the frequency of abnormalities in sea turtle embryos in the Eastern Mediterranean were found in approximately 0.1% of all studied samples.

Naturally, twinning is a result of the development of two blastodiscs from a single yolked ovum (Deeming and Ferguson, 1991). In cases of sea turtles, the causes of this process are yet to be determined. Some studies had suggested low temperatures as one of the key factors, with other environmental and genetic conditions as further indicators (Louro, 2009).

Nevertheless, more research and reporting is still required to enable better understanding of the occurrences and mechanisms specific to this phenomenon. In further studies, the genetic pattern of the twins and their parents should be considered.

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REFERENCE

Blanck, C.F. & Sawyer, R.H. (1981) Hatchery practices in relation to early embryology of the loggerhead sea turtle, *Caretta caretta* (Linné), *J. Exp. Mar. Biol. Ecol. no.49*, pp 163–177.

Chan, EH. (1985) Twin Embryos in Unhatched Egg of *Dermochelys Coriacea*. *Marine Turtle Newsletter*no.32, pp 2-3.

Charuchinda, M & Monanunsap, S. (1998) Monitoring survey on oea turtle nesting in the inner gulf of Thai1and, 1994-1996.*Thai Marine Fisheries Research Bulletin,no 6*, pp 17 - 25.

Crooks, F.D. & Smith, P.W. (1958) An instance of twinning in the box turtle, *Herpetologica no.14*, pp 170–171.

Deeming DC & Ferguson MWJ. (1991) *Egg incubation: its effects on embryonic development in birds and reptiles*, (Cambridge University Press, New York), pp 94-96.

Deraniyagala, PEP (1930) The Testudinata of Ceylon, *Ceylon J. Sci. B.,no 16*,pp 43-88.

Deraniyagala, PEP. (1932) Notes on the development of the leathery turtle, *Dermochelys coriacea*. *Ceylon J. Sci. B.,no 17*, pp 73-102.

Dodd, CK. (1988) Synopsis of the biological data on the loggerhead sea turtle (*Caretta caretta*) (Linnaeus 1758) .*U.S. Fish Wildlife Service Biological Report,, no 88(14)*, p 110.

Fowler, L.E. (1979) Hatching success and nest predation in the green sea turtle, *Chelonia mydas*, at Tortuguero, Costa Rica. *Ecology, no 60*, pp 946–955.

Heimann, E. (1993) Drei Zwillingspaare bei Testudo marginata, *Salamandra; no 29*, pp 167–172.

Hewavisenthi, S. (1989) Twin Green Turtle Hatchlings, in *Sri Lanka Marine Turtle Newsletter , no 46*, p 9.

Kaska, Y. & Downie, R. (1999) Embryological development of sea turtles (*Chelonia mydas*, Caretta caretta) in the *Mediterranean. Zool. Middle East no 19*, pp 55–69.

Kaska, Y., Downie, R. &Furness, RW. (2000)Abnormal development in sea turtle embryos, in Abreu-Grobois, FA., Briseño-Dueñas, R., Márquez, R & Sarti, L (comp), *Proceedings of the Eighteenth International Sea Turtle Symposium, U.S. Dep. Commer. NOAA Tech. Memo*, NMFS-SEFSC-436, pp 271-272.

Louro, MMC & Pereira, MAM. (2009)First report of twinning in the loggerhead sea turtle (*Caretta caretta*) from Ponta doOuro, southern Mozambique, *Indian Ocean Turtle Newsletter ,no 9*, pp 1-2.

Messinger, M.A & Patton, G.M. (1995) Five years of nesting of captive Terrapene carolina triunguis. *Herpetol. Rev, no 26*, pp 193–195.

Miller, J.D. (1985) Embryology of marine turtles, in Gans, C., Billett, F & Maderson P.F.A. (eds.), *Biology of the Reptilia.*

14A. Development, (John Wiley & Sons, New York), pp 269–328.

Monanunsap, S & Charuchinda, M. (1994) Laying egg of Sea turtle around Khram Island, Chonburi Province during 1988-1993. *Technical Paper Sea turtle Conservation Station Department of Fisheries*,p 35. (Thai)

Obst, F.J. (1995) *Schmuckschildkröten: Die Gattung Chrysemys. Westarp*, (Wissenschaften, Magdeburg)

Penyapol, A. (1957) *A preliminary study of the sea turtle in the gulf of Thailand* , (Royal Thai Navy, Bangkok), p 12.

Phasuk, B. (1992) Biology of sea turtle and reproductive biology of green turtle in Thailand, *Thai.Fish.Gaz, no 45(1),* pp 603-650 (in Thai)

Piovano, S., Kaska, Y., Prazzi, E., Nannarelli, S & Giacoma, C. (2001) Low incidence of twinning in the loggerhead sea turtle, *Folia Zool. – no 60 (2),* pp 159–166.