

## SEM Study of the Apical Ectodermal Ridge of the Green Turtles (*Chelonia mydas*)

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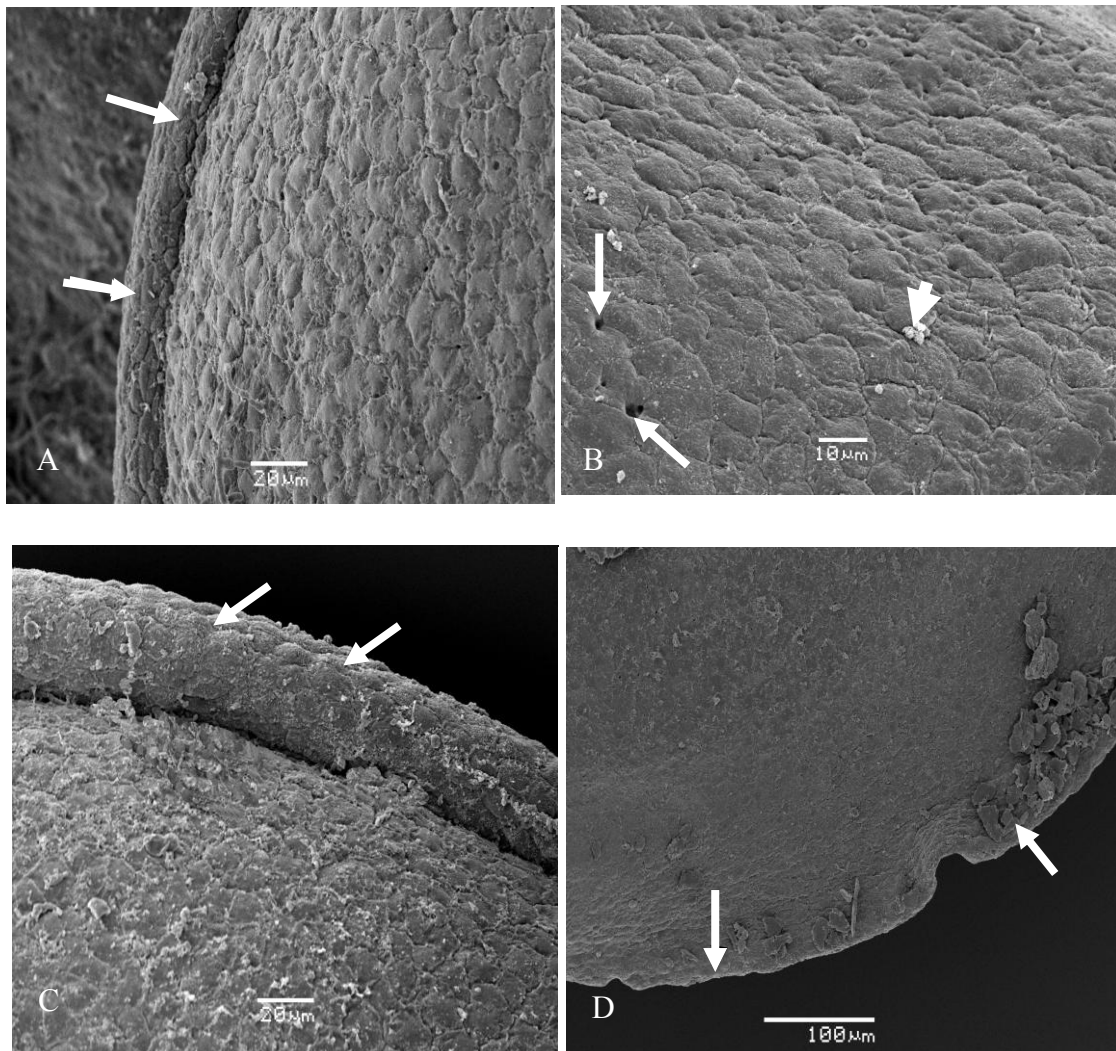
Green turtles (*Chelonia mydas*) are among world widely distributed species in the tropical and temperate waters. The coastlines of Oman and its Islands are considered to be the largest nesting areas in the northern sector of the Indian Ocean.

The apical ectodermal ridge (AER) is a transitional structure which is important to maintain a proliferative undifferentiated region of the mesenchymal cells to make linear growth (proxial-distal) and for the expression of the region, which is called zone of polarizing activity (ZPA) that generates an anterior-posterior axis of the limbs.

Green turtle's eggs were collected randomly immediately after oviposition from 13 nests in Ras Al-Hadd Reserve in Oman (22° 32'N, 59° 45'E to 22° 14'N, 59° 48'E). The eggs were transferred to plastic buckets containing damp sand to the Department of Biology at Sultan Qaboos University (SQU) and were immediately placed in the incubators at constant temperature of 30 °C± 0.05 . Each egg was incubated singly in a 600ml plastic container according to the method of Mrosovsky (1988), with minor modifications. Each container had a plastic cover 10cm in diameter. The upper part of each container was punched with six holes (2.5mm each in diameter). Each egg was placed in the center of the container on top of spongy foam with two layers of vermiculite on the bottom and top of eggs. Distilled water (65 ml) was added to the vermiculite on day 0 of incubation and then another 65 ml on day 19 of the incubation. The eggs were opened on the specific days relative to staging. Limb buds of days (10, 12, 14 and 17) of incubation were dissected out and fixed with Karnovsky fixative and processed for SEM. The limb buds were examined under JEOL JEM-5600LV scanning electron microscope.

On day 10 of incubation, the apical ectodermal ridge (AER) is present in both fore and hind limb buds and measures about 17µm (Fig 1A). The cells covering the apical ectodermal ridge are packed together (Fig 1A). The limb buds apical ectodermal ridges of day 12 are thicker than in the previous stage and measures about 25 µm (Fig.1B) while on day 14, the apical ectodermal ridge of the limb buds increases in thickness to measures about 40µm (Fig. 1C). The apical ectodermal ridge starts to decrease in width on day 17 which indicates the regression of this structure. (Fig.1D)

Reference: Mrosovsky, N. (1988): Pivotal temperatures for loggerhead turtles (*Caretta caretta*) from northern and southern nesting beaches. *Canadian Journal of Zoology*. **66**: 661-669



**Fig. 1(A-D).** Scanning electron micrograph **(A)** on day 10 of incubation the apical ectodermal ridge of the forelimb is present (arrows); **(B)** on day 12, the ectodermal flat cells covering the skin of the limb buds exhibiting mucus drops (arrow head), with the presence of pores in this external skin surface (arrows); **(C)** on day 14, an increase in thickness of the apical ectodermal ridge is showing (arrows) and **(D)** on day 17, a regression of the apical ectodermal ridge of the limb bud has taken place (arrows).