

Introduction of Developmental Biology at Utkal University, (Odisha, India)

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ABSTRACT The paper deals with the background and the establishment of a Developmental Biology Laboratory in Utkal University in Odisha state. It describes the process from a humble beginning with limited facilities into a leading research centre, initially for amphibians and later for the endangered olive ridley (*Lepidochelys olivacea*) turtle. Starting from the biology, reproduction and development in many anurans, the laboratory took up research on regeneration, especially on super-regeneration in tadpoles under the influence of morphogens such as vitamin A (retinoids). Treatment with vitamin A after amputation of the tail inhibited tail regeneration but unexpectedly induced homeotic transformation of tails into limbs in many anurans, starting with the marbled balloon frog *Uperodon systoma*. This was the first observation of homeotic transformation in any vertebrate. The laboratory continues research on histological and molecular aspects of this phenomenon. In addition, taking advantage of the largest rookery of olive ridley sea turtles in Gahirmatha, in the same state the laboratory has contributed significantly to the biology, breeding patterns, development and especially the temperature-dependent sex determination phenomenon (TSD). This research was extended to biochemical and ultrastructural aspects during development for the first time for any sea turtle. The laboratory has contributed significantly to the conservation of olive ridleys as well as the saltwater crocodile (*Crocodylus porosus*). Recognition and awards for the laboratory have been received from both national and international bodies.

KEY WORDS: *Anuran, Crocodylus porosus, homeotic transformation, Lepidochelys olivacea, sex determination*

I went to do my Ph.D. in Zoology at the University of Michigan with a Barbour Fellowship from University of Michigan and Fulbright Travel Grant in 1963. Although I had a M. Sc. in Zoology from Lucknow University, I had to complete a 24 hour credit course requirement to be eligible for the Ph.D. qualifying examination. Studying biology in the U.S. in the early 1960's was an exciting experience—it was in the wave of Watson and Crick era following the cracking of the Double Helix and working out the details of protein synthesis.

After qualifying, I registered under Dr. G. W. Nace to work on the topic "Transfer of maternal serum proteins and their role in development in the American leopard frog *Rana pipiens*" (Mohanty-Hejmadi, 1970) for my Ph. D. work. I had to learn the trick of the trade: raising and maintaining amphibians, which meant starting from collecting frogs, carrying out artificial fertilisation in the laboratory and raising embryos till metamorphosis. In the "Amphibian Facility" of Dr. Nace (Fig. 1), I was also exposed to techniques for raising different types of amphibians, which was to be of great help while setting up my own laboratory at Utkal University.

After completing my Ph. D. and teaching in the USA for a while,

I decided to return to India. Fortunately, I was selected for a post of Reader (Associate Professor in Zoology) in Utkal University, Bhubaneswar, in 1975. Consultations with departmental faculty in Michigan were helpful. Dr. Carl Gans who was very familiar with the herpetofauna of India, advised me of the importance, before beginning any research, of properly identifying my frogs, because the systematics of Indian amphibians had not been worked out properly. He suggested that I should seek the help of J. C. Daniel (<http://www.sanctuaryasia.com/interviews/jcdaniel.php>) of the Bombay Natural History Society for this. Prof. Nace kindly handed over many chemicals that were essential for starting my work. They included stains, hormones and above all the much coveted MS 222, the anaesthetic needed for cold-blooded tadpoles.

Abbreviations used in this paper: CSIR, council for scientific and industrial research; FAO, food and agricultural organization; IUCN, international union for the conservation of nature; MS222, tricaine methanesulfonate; NET, national eligibility test; TSD, temperature-dependent sex determination; UGC, university grants commission; WTO, world trade organization.

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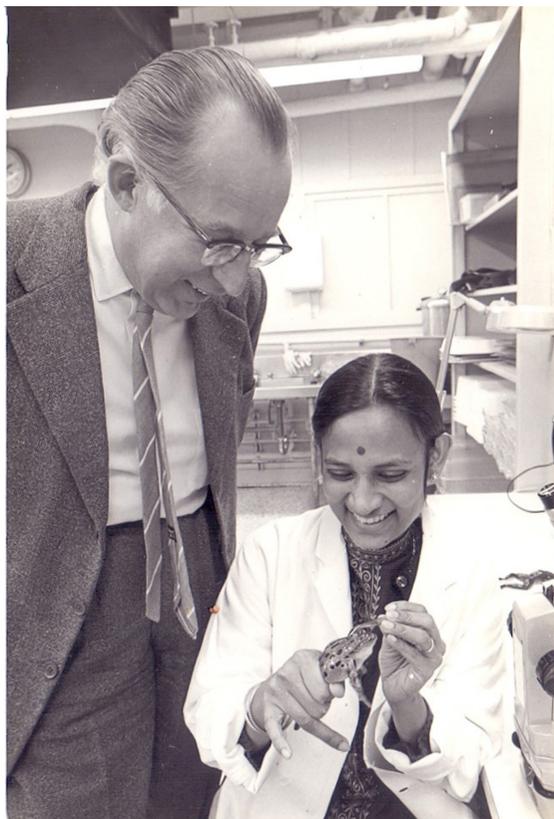


Fig. 1. G.W. Nace and the author at the Amphibian Facility of the University of Michigan, MI, USA (1968).

It is gratifying that a few mundane techniques that I brought with me continue to be used by my students to this day. Some of them I had picked up in my lab, especially Mallory's triple stain, and others I was taught, for example the Alizarine and Alcian Blue stain techniques for skeleton and cartilage, to which I was introduced by Dr. Richard Wassersug during a brief stay at the Field Museum of Natural History in Chicago. My students later transferred techniques related to electrophoresis to other labs in India.

Research in Utkal University

Building an amphibian laboratory

A laboratory had to come up from scratch, literally and figuratively; we did not even have taps for water in the laboratory and had to depend on potable stored water. It was my luck that Sushil Kumar Dutta, who was completing his Master's at the time, volunteered to work with me; he was not only interested in frogs but also developmental biology. He took up a thesis project on the theme "Biology and the effect of pesticides and fertilizers on the developmental stages of Indian Bull frog, *Rana tigerina* (Anura: Ranidae)" (Dutta, 1980). He was extremely skilled in field work and had a knack for catching frogs, and surprised me by bringing new varieties which we had not seen before. Fortunately the Utkal University campus had considerable vegetation with swampy areas suitable for amphibians and reptiles. A colleague of mine, an endocrinologist, was setting up a laboratory to study lizards and snakes, which made it convenient for workers from both groups to go out for collections at night. This led to encounters with police

patrols who thought they were up to no good; the police could not believe that they were actually collecting frogs and snakes. I had to provide them with official certificates to the effect that they were genuine research scholars of our department on official collecting expeditions.

Things started getting better as I started getting research grants. A University Grants Commission (UGC) grant in 1978 was followed by one from the Council for Scientific and Industrial Research (CSIR) in 1980. Both were for research on amphibians. Although the amounts were small, they allowed me to purchase basic equipment and provided running expenses. Above all, the fellowships that came with them enabled me to appoint research scholars who turned out to be extremely hard working and contributed to publications from year to year. As Dr. Gans had suggested, we began with faunal studies, especially the life histories of several frogs including *Rana tigerina*, the jumping frog *Rhacophorus maculatus*, and the microhylids *Microhyla ornata* and *Uperodon systoma* (a few of them have been renamed in the meanwhile).

Homeotic transformation along with regeneration

Having come across a dissecting microscope with attachments for photography in Prof. I. A. Niazi's laboratory in Rajasthan, I decided to acquire one with the CSIR grant, thinking that it might be of use one day. That microscope was to prove its worth much later, when we wrote up our landmark finding on vitamin A-mediated homeotic transformation (Mohanty-Hejmadi *et al.*, 1992; Fig. 2). My interest had been sparked by Prof. Niazi's work on the effects of vitamin A during regeneration in frogs (Niazi and Saxena, 1968 and 1979). By this time Sushil Kumar Dutta had returned from Kansas after picking up a second Ph.D. His thesis was completed

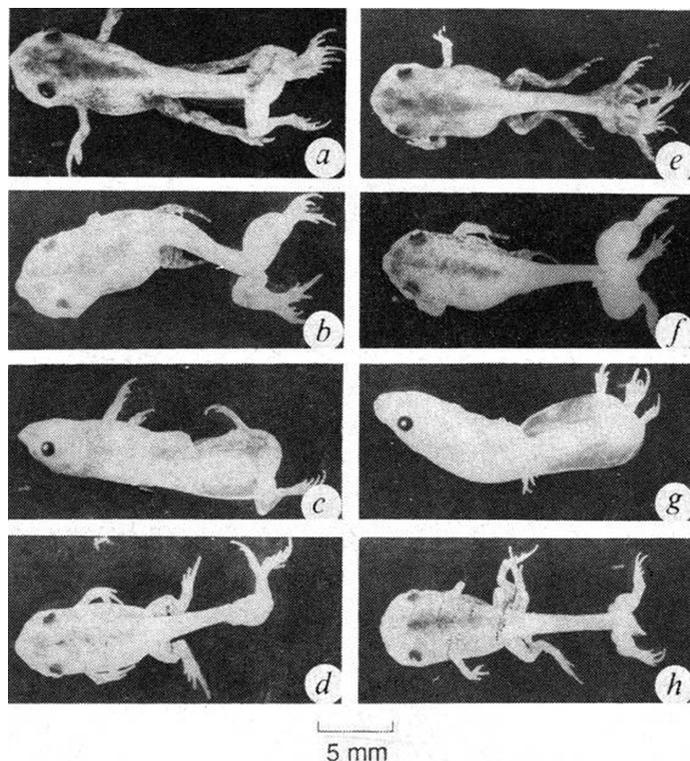


Fig. 2. Homeotic transformation of tails to limbs in the tadpoles of *Uperodon systoma* (Mohanty-Hejmadi *et al.*, 1992).



Fig. 3. In the Developmental Biology laboratory, Utkal University, Odisha, India (January, 2012). The team after 20 years of publication of homeotic transformation, from left to right: Pravati Kumari Mahapatra, Priyambada Mohanty-Hejmadi and Sushil Kumar Dutta. Prof. Mahapatra continues her regeneration work in the same laboratory which was established in 1975 by the author.

under the supervision of Prof. William E. Duellman and was titled “Amphibians of India and Sri Lanka: Annotated checklist and bibliography”. He joined my lab, this time as a post-doc. Pravati K Mahapatra, the first research scholar from our department to qualify for a National scholarship (UGC-NET), also took up regeneration as her area of study.

We noticed that in vitamin A-treated tadpoles of the marbled balloon frog *U. systoma*, sometimes limbs emerged in place of the amputated tail. We had no idea that this was unusual. Our access to international publications being poor, the significance of the finding had escaped us. To our great luck, Utkal University hosted an all-India symposium on developmental biology in 1991. One of the attendees was Prof. I. A. Niazi, pioneer in studying the effect of vitamin A on regeneration in *Bufo andersoni* and other species. After our presentation, I said to Prof. Niazi “You must have seen this kind of limb emergence instead of tail regeneration in your frogs”. Prof. Niazi remained silent for a few moments. Then he replied “Dr. Hejmadi, I have never seen this and I do not think anyone else has either. You must get a paper ready and send it to Nature”. Even after returning to Jaipur in Rajasthan, he kept track of our progress in the matter. We lacked proper library facilities, and I had to make some efforts to find out the “Instructions for authors” before a manuscript for Nature could be put together. Pravati Kumari Mahapatra provided the excellent sequential photographs which she had taken for her thesis.

The photographs, which showed limbs developing in place of tails, proved critical for the acceptance of what was taking place as a homeotic transformation; one of the reviewers said as much. Another reviewer questioned our claim that the limbs that emerged from the amputated tails were hind limbs. We were able to settle the point by showing that the limbs had 5 digits unlike forelimbs with their 4 digits.

International response to the publication was overwhelming, no doubt in part because the journal had highlighted it in Nature’s “This

Week” column. (Possibly tongue-in-cheek, the journal pointed out that the discovery held out the prospect of producing edible frog legs on a mass scale.) Publication of the paper was no less than a drama for us. Even before we knew its fate, some students who had gone to their hostel for lunch learnt from the national news broadcast on TV that some work from our department has been published. This was followed by a copy of the paper sent by an old student from Delhi. We did not have Nature in our laboratory but managed to locate it in another institution in the city. We were delighted when the offprints reached us. Requests for offprints flowed, and it makes me uncomfortable to confess that at that time we could not afford to send them; my belated apologies to all who had asked. We were delighted that Editor Anil Agrawal highlighted our results in the magazine “Down to Earth” in the first issue in June 1992.

Work on homeotic transformation continues to this day and is described in another article from our laboratory in this volume.

Serendipitous entry into sea turtle research

Our lab was working concurrently on amphibians and sea turtles. To make it easier for the reader, I present the part on the turtles now and continue with the work on amphibians later (Mohanty-Hejmadi and Mahapatra, 2020). One day in the early 1980s I had a surprise visitor in the person of Dr. Marie T. Dimond, a nun from the faculty of Biology in Trinity College, Washington. Dr. Dimond was working on the development of the common fresh water snapping turtle (*Chelydra serpentina*), especially on temperature-dependent sex determination (TSD). She was a Visiting Professor at the University of Bhopal. At that time, it was reported that the largest population of olive ridley turtles nest in Gahirmatha beach in northern Odisha. She wondered whether TSD was also found in sea turtles, and whether the phenomenon of TSD could be put to use in conservation efforts. That had brought her to Bhubaneswar, because she needed to apply for a permit to collect eggs to be carried to Bhopal for further study. She soon discovered that Bhubaneswar was home to Utkal University and a full-fledged Zoology department, and more, that there was a full-fledged laboratory of developmental biology (Fig. 4). Orissa has a reputation for being among the less-developed states in India, and she had thought that no facilities to carry out research would be available there; hence the plan to carry turtle eggs to distant Bhopal (in central India) for incubation. She opened up the window for us to the exciting world of the olive ridleys; her visit started a long association.

The plan to collaborate faced an immediate hurdle. She was denied a permit to collect eggs. When I followed up her application, I realised that two factors were contributing to the objections. A government Missile Centre was present in the area (on Wheeler Island), which meant that clearance had to be obtained from the Defence Department. Secondly, Dr. Dimond being a foreigner needed yet another clearance from the Ministry of External Affairs. When these issues came up during my discussions with the authorities, it transpired that there would be no difficulty were I to apply for the permit. It occurred to me that I could bring olive ridley eggs to my laboratory to study their development and develop techniques to raise them as was being done in USA for conservation of Kemp’s Ridleys and Green turtles (“head starting”, as it

was called). Yearlings could be released when they were larger, thereby improving the chances of their survival in the wild. We could learn the techniques from Dr. Dimond to raise the hatchlings. In addition, my research team could collaborate in her studies on temperature-dependent sex determination (TSD).

The Department of Forests helped out, with the Chief Wildlife Warden U. N. Sarangi bringing the first batch of eggs from Gahirmatha for me in 1982. We were not planning to take up serious research on olive ridleys, as we were fully busy with developmental biology of several species of amphibians. However, that changed as we began helping out Dr. Dimond. She visited our laboratory four times up to 1986, taught us the basics of incubation, handling of the delicate turtle embryos and raising the hatchlings. Thanks to the media highlighting our work on the endangered olive ridleys, I was issued a blanket permit to collect eggs from year to year with all facilities extended for collection and observations by my students. Soon our laboratory became well known in the field of olive ridley research. The work led to several publications including a monograph (Mohanty-Hejmadi, 1992) and two Ph. D. theses (Behera, 1989 and Sahoo, 1997). Along the way, techniques for rearing of the hatchlings were standardised (Mohanty-Hejmadi, 1992, 1999).

Temperature-dependent sex determination (TSD) in the olive ridley sea turtle

The thermal tolerance for embryonic development was found to be between 26°C to 34°C. The period of incubation under natural conditions and at different regimes under laboratory was determined. Just 1.54% of unhatched eggs exhibited malformations (Behera, 1989). The 38 developmental stages at cold temperature (27.5°C) were standardised. As expected, the rate of development at warm temperature (33.5°C) was higher than that of cold temperature (27.5°C). However, the rates became equal during the latter part of development. Sex determination was strongly temperature-dependent: incubation at warm (33.5°C) temperature and at cold (27.5°C) temperature resulted in 98% and 0.75% of the eggs developing into females, respectively. By incubation at intermediate temperatures, the threshold was found to be as narrow as 0.5°C (it lay between 29.5 to 30°C). We were able to report the critical temperatures for male and female determination for the first time in this species (Mohanty-Hejmadi and Dimond, 1986; Mohanty-Hejmadi *et al.* 1985; and Behera, 1989). Under natural conditions at Gahirmatha, 13% females were obtained from first nesting (January) when the temperature is cooler and 98.3% females from second mass nesting (March) when the temperature was much higher (Behera, 1989), showing an interesting seasonal bias in hatchling sex ratios.

Sahoo (1997) extended the work to biochemical and ultrastructural aspects in collaboration with the Regional Research Laboratory, Bhubaneswar, which had facilities for such work. The compositional changes in the eggshell of *Lepidochelys olivacea* during development is discussed by Sahoo *et al.* 1998. In addition, changes in the corresponding ultrastructure (Sahoo *et al.* 1996a,b) during embryonic development were reported for the first time for any sea turtle (Fig.5). I was delighted to see that similar work was



Fig. 4. Priyambada Mohanty-Hejmadi examining olive ridley hatchlings with Marie T. Dimond (Developmental Biology laboratory, Utkal University, Odisha, India, 1986).

taken up for other species of sea turtles after the publication of our papers.

Conservation work on sea turtles and crocodiles

A nesting area almost 100km long along the east coast of India that covers Gahirmatha, is mentioned in the travelogue "A New Account of the East Indies" by Alexander Hamilton, who visited Orissa in 1708 (Mohanty- Hejmadi, 2001). Gahirmatha beach remains the largest nesting rookery for olive ridley turtles (*Lepidochelys olivacea*) in the world. When we started our studies in the early 1980's, more than half a million olive ridleys used to nest annually in two batches, the first and larger one during January-February and a second, smaller one during March-April. The shifting as well as the shrinking of nesting towards the northern end of Gahirmatha beach was an important feature during our study period. A 35 km nesting site in 1970's became restricted to 10 km during the early 80s and to only 2 km in the late 80s. A cyclonic storm in May, 1989 separated a 2 km stretch of beach called Babubali. The entire nesting shifted to this isolated island called Babubali. Shrinking of nesting sites is a major problem for Gahirmatha turtles (Mohanty-Hejmadi, 2003).

"Missile Man" Dr. A. P. J. Abdul Kalam, later to become President of India, was Chief Speaker at the Northern Indian Ocean Sea Turtle Workshop held in Bhubaneswar in 1997. Thanks to his interest and grants from the Department of Defence, hatcheries monitored by the Department of Forests, Government of Odisha, were set up in all the three major nesting beaches (Gahirmatha, Rushikulya and Devi) with appropriate temperature regimes to ensure that both male and female hatchlings enter the sea from the beaches every year. Gratifyingly, the report from my laboratory led to the formation of a High-Powered Committee of the Government of Odisha for protection of olive ridleys, which meets prior to the turtle season (November to May) every year. It is one of a kind, with all stakeholders from the coast guard to the fisheries department being involved. In addition, as requested by me, a Turtle Research Centre is being established at Rushikulya, one

of the more accessible nesting sites.

There was an unusual detour from sea turtle research to crocodile conservation. Our area of research Gahirmatha was in Bhitarkanika sanctuary where a very successful salt water crocodile (*Crocodylus porosus*) conservation centre had been set up by the Food and Agriculture Organisation (FAO)-deputed expert Dr. H. R. Bustard in the late 1970's. Eggs were being collected from nature, hatchlings raised for a few years and released to the wild year after year. Unlike in sea turtles, sex can be identified at hatching due to the presence of secondary sexual characters. The District Forest Officer who knew about our TSD work informed me that there was a problem: most of the hatchlings they raised, and thereafter released, were females. Understandably, he was concerned that the skewed sex ratio would come in the way of long-term conservation efforts. Could we help them in getting males by making use of TSD? It turned out to be possible, though unlike in the olive ridley, males were produced at higher temperature in the salt water crocodile. The effort to help worked out successfully (Mohanty Hejmadi *et al.* 1999).

Research on olive ridleys has enabled me to participate in many national and international forums involved in sea turtle protection. I was part of a 19-member expert committee which drafted a global strategy for sea turtles on behalf of International Union for the Conservation of Nature (IUCN), in Mexico in 1994. In a rather unusual assignment I represented Govt. of India in the Shrimp-Turtle dispute in world trade organization (WTO) in May and October 1998 (Mohanty-Hejmadi, 1998).

Recognition for the laboratory

We started publishing papers from 1977 onwards on fauna, development and other aspects on amphibians. This has resulted in a number of M. Phil and Ph. D. theses. The laboratory continues

pioneering research on amphibians which are discussed in an accompanying article (Mohanty-Hejmadi and Mahapatra, 2020) in this volume. My contributions were recognized by the award of a Padma Shri (3rd highest civilian award in the field of Science and Engineering in 1998), by the Government of India. It was a matter of pride for all of us that one of the first ones to get his Ph. D. from the laboratory, Sushil Kumar Dutta has emerged as an expert on amphibians. His expertise was acknowledged by the replacement of the generic designation of the Asian common toad from *Bufo* to *Duttaphrynus* (Frost *et al.*, 2006). This new genus so far includes 29 species from India, Sri Lanka, Pakistan, Nepal, Bhutan, Myanmar, Bangladesh, China and Japan. Research on amphibians continues in the laboratory in tune with the recent developments in the field.

Epilogue

This story would be incomplete without mention of my dual life, about which many of my friends remain curious. Although science happens to be my profession, dance and music are my passion. At times both have run parallel, to the extent that I have two sections to my publications - science and culture. This has not happened by design, but by circumstances beyond my control. I matriculated from high school in 1952 with music as an optional subject but was compelled to choose science for my college studies as there were no facilities to continue with music. I did like science, though, and chose it as a career. At that time very few of us were trained in classical dance; dancing in public was almost a taboo in many parts of India. But due to the encouragement of our parents, who were ahead of their time, I and some others continued with it. I gave performances off and on. It so happened that the First Inter-University Youth Festival, a brainchild of the then Minister of Education Maulana Abul Kalam Azad, was organised in New Delhi in 1954. It was meant to encourage students of different

Universities to interact through art and culture. I was a student of Ravenshaw College in Utkal University and was sent to compete in the Classical Dance category. That was probably the first time that Odissi dance was performed and seen in public outside the state of Odisha. This laid the foundation for recognition of Odissi and it flatters me that I am considered to be a pioneer. Later, the serious pursuit of science made me think of leaving dance; but dance would not let me go. As I have documented in a book, it was rewarding to keep in touch with Odissi through lectures and demonstrations in India and abroad (Mohanty-Hejmadi and Hejmadi Patnaik, 2007). Performances had to be restricted to my time and convenience; my last programme, telecasted nationally, was in 1992. In retrospect, instead of being a distraction, it was the training in dance

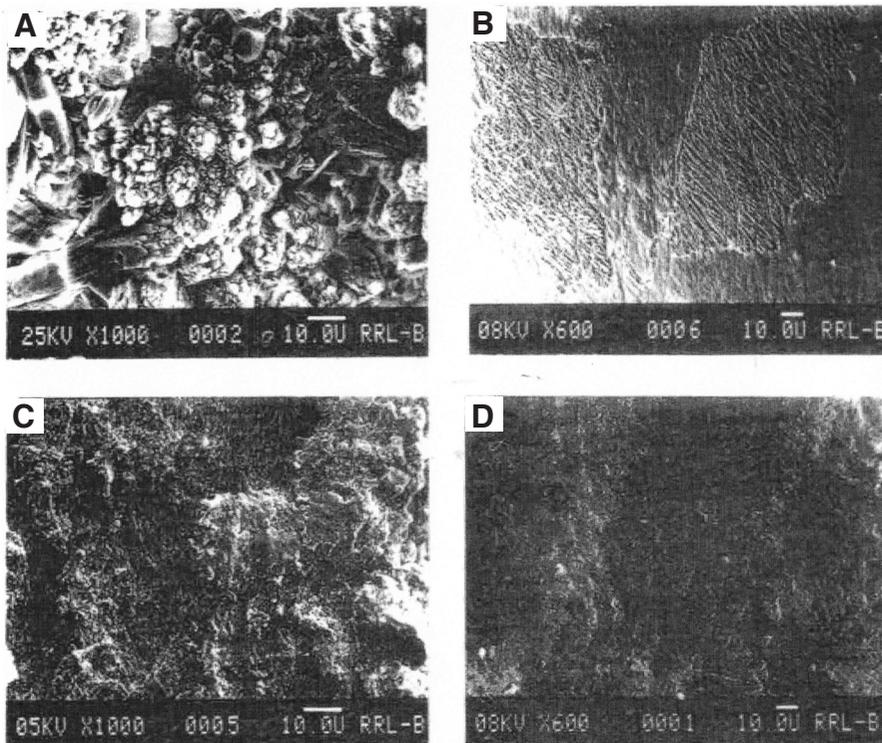


Fig. 5. Scanning electron micrograph of normal eggshells of olive ridley turtles. (A) Upper surface of fresh eggshell showing loosely arranged crystal-lites and spicules. **(B)** Lower surface of fresh eggshell showing the fibrillar network and a thin shell membrane covering it. **(C)** Upper surface of hatched eggshell. **(D)** Lower surface of hatched eggshell (Sahoo *et al.*, 1996a).

and music which helped me cope with several stresses and enriched my life in many ways. I was conferred with the highest civilian award in the field of dance in the country, from the national academy of music and theatre (Central Sangeet Natak Akademi) in 1986. It was not possible for me to carry on doing science seriously after I retired in 1999. That was not true with dance and culture, which continue to keep me engaged in rewarding ways.

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