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SCANNING ELECTRON MICROSCOPY OF THE EGGSHELL OF LIZARD, *CALOTES VERSICOLOR*

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Abstract
The eggshell of the lizard *Calotes versicolor* was studied by scanning electron microscope. The eggshell of the lizard is composed of outer calcareous layer and inner fibrous layer. Outer calcareous layer is not compact. The calcareous blocks are superficially placed. The inner fibrous layer is more thick than the calcareous layer. It appears like meshwork with fibers arranged in random fashion.

Introduction

Oviparity presumably represent the ancestral mode of reproduction in the class-Reptilia. The sub-class Lepidosauria has two orders Rhyncocephalia which includes *Sphenodons* and Squamata which further divides into Lacertia (lizards) and Ophidia (snakes) sub- order. The reptiles lay their eggs at different places as per required environmental conditions necessary for their incubation. During incubation, the eggshell plays an important role in exchange of gases, moisture.

The suborder Lacertilia and Ophidia, include lizard and snakes, possess both viviparous and oviparous populations. Eggshells of oviparous lepidosauria are either flexible, 'Parchment-like' or rigid, calcareous. And have both crystalline and fibrous components (Packard *et al.*, 1982 a,b; Packard and Hirsch 1986; Blackburn, 1993, 1995; Mathies and Andrews, 2000). No other membrane seems to be present on the inner side of the shell of lepidosaurian eggs. Pores seem not to occur in eggshell of lepidosaurian (Packard, *et al.*, 1977).

The structure of eggshell is studied extensively in poultry science. The structure of eggshell is of prime importance for the developing embryo. Although India is rich in reptilian fauna representing the members of all existing sub-classes very little is known about their eggshells except few reports on turtle eggshell (Sahoo, *et al.*, 1996). Therefore in the present investigation an attempt has been made to study the eggshell morphology of reptilian representative one belonging to lepidosauria (*Calotes versicolor)* using scanning electron microscope. Our observation would serve as a base line for investigating the effects of shell structure and strength on hatchling trauma, influence of maternal diet, gases exchange metabolic exchange and the evolution of viviparity

Material and Methods

In the present study of eggshells the garden lizards have been selected. Their eggs were collected from the nesting habitats during their reproductive cycle. The female Indian garden lizards, *Calotes versicolor* lay clutches of 7 to 16 eggs in wet soil in the month of August to October. These eggs were collected from local garden in Nagpur.

Preparation of Sample :

The collected eggs were brought to the laboratory, at Department of Zoology. The samples were prepared scanning electron microscopy in the following manner.

The inner content of eggs was removed by breaking eggs and the eggshells were washed thoroughly with distilled water to remove any forgein material on the outer surface as well as yolk present on the inner surface of the eggshells. The eggshells were dried for overnight in an oven at 35^{0}C to remove the moisture.

After complete drying of eggshells the pieces of eggshells were processed for scanning electron microscopy. Pieces were affixed with adhesive on the metal stub used for scanning electron microscope (SEM) in the following manner.

1.The outer surface :

The fragments of eggshells were mounted on stub having outer surface facing up words upper most.

2.The radial or cross section :

The shell fragments broken into small pieces and mounted with broken edges longitudinally facing upper most.

3.The shell membrane :

From some fragments egg-membrane peeled and mounted the eggshell facing shell membrane the uppermost.

4.The inner most surface of shell adjacent to shell membrane :

From some fragments the shell membrane was removed and such fragments of shell were mounted with the inner surface of shell uppermost.

5.The inner surface of the egg membrane :

The fragments of eggshell mounted with inner surface upper most.

All the mounted samples were labelled. Since the eggshell is a biological material which is non-conducting, it was coated with gold in Poleron Sputter Coater (England made) for 70 seconds to make it conducting. The thickness of coat observed as around 70A°. The stub with coated material was scanned using Cambridge scanning electron microscope. The photographs were taken by automatic camera attached to the SEM unit on 100ASA Sakura Photofilm at suitable magnifications. The scanning was carried out at RSIC of Nagpur University Nagpur. The scanned material was observed critically and the structures are described in the following chapter.

Observations

The Indian garden lizard, *Calotes versicolor* is found throughout the India. The female lays clutches of 7 to 16 eggs in the months of August to October. The eggs are slightly elliptical in shape (Fig. 1). The eggshell is parchment-like or flexible. The eggs swell during the incubation. The outermost layer is calcareous (Packard *et al.*, 1977).

The Radial Section :

The radial fracture of eggshell has two distinct outer and inner layers. The outer layer is compact crystalline layer and the inner one is a fibrous shell membrane (fig. 7-8).

The radial section of the eggshell of *C. versicolor* shows crystalline layer is poorly developed consisting of coarse granular matrix with no typed structure. It is about 14 µm thick. The organic material is also not present on the crystalline layer (Fig. 2-7-8). The fibrous shell membrane is thick and well developed as compared to crystalline layer. It is about 95 µm thick.

The cuticle is also not present as that of some bird eggs (Board, 1982; Parson, 1982). The shell units are not close to each other to form a crystalline layer (Fig. 2).

The Outer Surface Of Crystalline Layer :

The eggshell surface of *C. versicolor* has calcareous crystalline blocks of random size, shape and orientation and they are free from each others (Fig. 3-4). In *C. versicolor* the crystalline matrix is calcium carbonate in the form of calcite (Simkiss, 1966; Tyler, 1969; Board and Love, 1980). These crystals of calcite show a rosette structure with a pore in the center at higher magnifications (Figs. 4, 5). It has different layers of calcite deposition (Fig. 5).

The outer calcareous layer is not compact. At low magnifications on the outer surface, the pores are not visible. The calcareous blocks are not extended deep into the fibrous membrane moreover they are superficial.

The Inner Surface Of Crystalline Layer:

In *C. versicolor* as the crystalline layer is very thin composed of blocks of crystals, while peeling of shell membrane the integrity of crystalline layer is not maintained. Hence it is very difficult to scan the inner surface of the crystalline layer.

The Shell Membrane :

The shell membrane of *C. versicolor* is porous and fibriller in nature, sheet like (Figs.6-7). The shell membrane of eggshell of *C. versicolor* is fibrous but thicker than that of crystalline calcareous layer. The fibrils of crystalline layer facing side are smooth and elongated forming a meshwork in random fashion. Shell membrane side facing albumen showing fibrillar arrangement with thin film of unknown substance (fig. 9).

Discussion

The eggshells of *Calotes versicolor* have entirely different structural pattern. The presence of flexible or parchment-like porous eggshell in *C. versicolor* may facilitate the absorption of water from the soil to maintain the hydric condition during embryogenesis as reported in the turtle, *Chelydra serpentina* (Morris *et al.*, 1983).

The eggs of oviparous lepidosauria usually have a flexible, parchment-like shell containing relatively small amount of calcium carbonate as observed in *C. versicolor* (Dendy, 1899 a, b; Bellairs, 1959; Dawbin, 1962; Sharell, 1966; Bellairs, 1970; Benoit, 1990). In *Hemiductylus turcicus* they have described the oviparous mode of reproduction and a hard calcareous eggshells, whereas *Saltuarius wyberba,* though exhibit oviparity produces a soft, parchment-like eggshell. The difference in eggshell structure with in the gekkonid lizards may be for maintaining the favorable water balance required for embryogenesis.

In lepidosauria, the eggshell of *Urosaurus ornatus* did not have a cuticle, whereas a cuticle has not been reported on the eggshell of any squamate reptile (Mathies and Andrews, 2000). The cuticle as such is not present on the crystalline layer of eggshell of *C. versicolor*

The crystalline layer in the eggshell of *C. versicolor* is composed of calcium carbonate and it is very thin as compared to other eggshell studied earlier. The calcium carbonate in the form of crystalline material is deposited between the fibres in outer layers of shell membrane (Giersberg, 1922; Harris, 1964). The structure and arrangement of crystalline material varied considerably among the lepidosaurian species and except for *S. undulatus consobrinus* and *S. virgatus,* it completely cover the underlying shell membrane (Mathies and Andrews, 2000). In *C. versicolor* the calcium carbonate deposition is superficial. It is in the form of crystalline blocks. It is not in between the fibres of outer layer, as that of *Sphenodon punctatus,* where the crystalline matrix is calcium carbonate in the form of calcite. The eggshell of *C. versicolor* is flexible or parchment-like and the egg swells during the incubation. The calcareous crystalline layer of calcium carbonate is poorly deposited and loosely organized on the eggshell, due to this outer surface of the eggs of *C. versicolor* has a rough and weathered appearance. The irregular sized blocks of crystalline material are superficially placed and are not extended into the shell membrane as found in tuatara *S. punctatus* (Packard *et al.*, 1982 b; Packard *et al.*, 1988; Benoit, 1990). Some of the crystalline blocks of calcium carbonate have rosette structure with a pore at the centre. Since these blocks are loosely organized, during the incubation water may enter through the membrane and eggs imbibe. The gases exchange may also takes place through these gaps of blocks of crystalline layer. The spaces and fissures between the shell units of crystalline layer were reported in some species of Phrynosomatid lizards performing the same functions (Packard and De Marco, 1991; Mathies and Andrews, 2000).The reduced calcification in the eggs of *C. versicolor* is not the morphological change occurred during the transition of oviparity to viviparity in lacertilia as found in green snakes, *Opheodrys vernalis* (Blanchard, 1933). The eggs of oviparious lizard, *Lacerta agilis*, have a lightly calcified eggshell, Such thinning of the calcareous layers of the eggshell facilitate the respiratory gas exchange (Blackburn, 1993, 1995). In *C. versicolor* poor calcification of eggshell as compared to those of other squamate may be the adaptation, as the females lay their eggs in wet soil.

Shell Membrane :

The parchment-like eggshell of lepidosaurian eggs seems to be formed from a single shell membrane, composed of approximately five layers of minute fibers (Giersberg, 1922; Jacobi, 1936; Packard *et al.*, 1977). The eggshell of *C. versicolor* has also a single shell membrane, which is thicker than that of crystalline layer. The radial as well as any other view of the eggshell does not show the presence of five layers of minute fibrils with clear demarcation which may be the characteristic feature of this species.

The inner side of the shell membrane of *C. versicolor* facing to the albumen is similar to those of chelonia (Packard *et al.* 1984 a, b), avian eggs and the eggs of other reptile (Bellaris and Boyde, 1969; Tung and Richards, 1972; Packard and Packard, 1979; Packard *et al.*, 1982 a, b; Guillette *et al.*, 1989) except having a thin film of unknown substance and fibrils.

Summary

The eggs of garden lizard, *Calotes versicolor* are slightly elliptical in shape with flexible eggshell. The eggshell is described as parchment-like or flexible.

Eggshells have two distinct layers outer crystalline layer and inner fibrous shell membrane.

Outer Crystalline Layer :

The outer surface of the eggshell of *C. versicolor* is composed of individual blocks of calcareous material which are irregular in shape, size and orientation. They are free from each other forming a crystalline layer. These blocks shows rosette structure with a pore in the center having different layers of calcite deposition. The outer calcareous layer is not compact. The calcareous blocks are superficially placed. The crystalline layer is calcium carbonate in the form of calcite.

Shell Membrane :

In. *C. versicolor* the shell membrane is more thick as compared to that of the calcareous layer. It appears like a meshwork with fibres arranged in random fashion . The inner surface of the shell membrane nearer to the albumen is little different, having thin film of unknown substance and fibers.

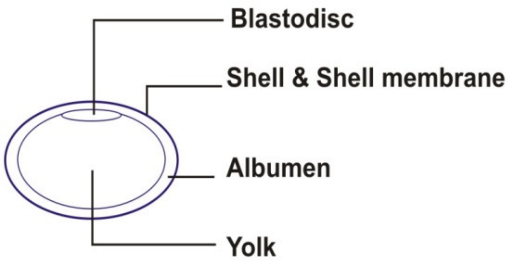

Figure 1: Egg of Lepidosauria

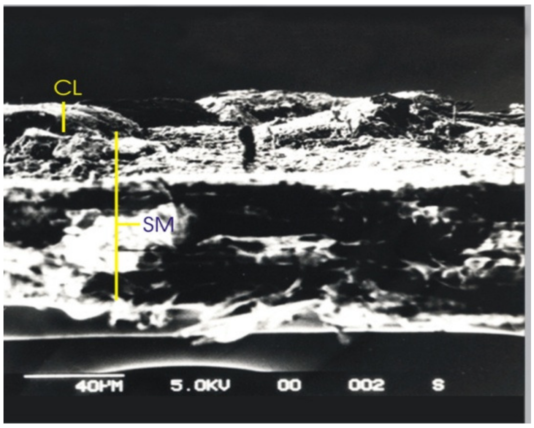

Figure 2: Radial View showing organization of egg shell of *C. versicolor.* Note a thin crystalline layer (CL) without any differentiation into zones and thick fibres shell membrane (SM). Laying below (CL). Scale bar =40 µm

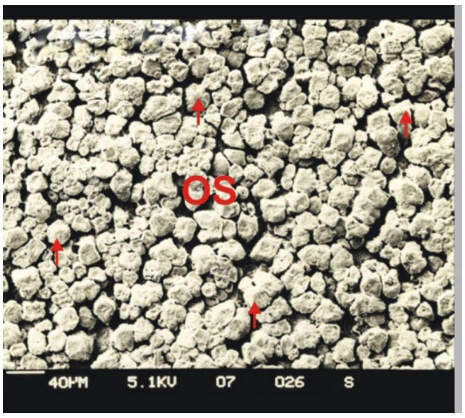

Figure 3 : Outer Surface (OS) of eggshell showing calcareous crystalline blocks (Arrows). Scale bar=40 µm

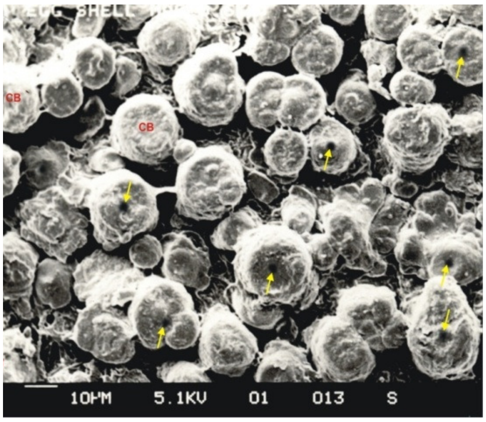

Figure 4 : Outer Surface (OS) of eggshell showing distribution of calcareous crystalline blocks (CB) with centrally situated pores(Arrows). Scale bar=40 µm

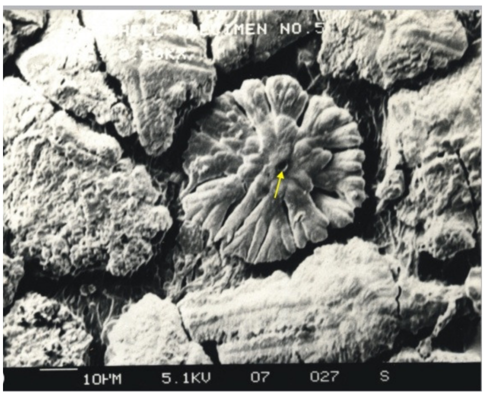

Figure 5 : Magnitized view of Figure no. 4 showing rosette structure with pore (Arrows) in the centre. Note calcite deposition on the roses. Scale bar=10µm

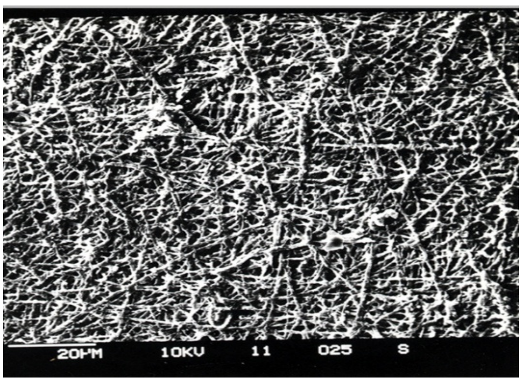

Figure 6: Shell membrane facing crystalline layer. Showing fibrillar mesh work. Scale bar = 40 µm

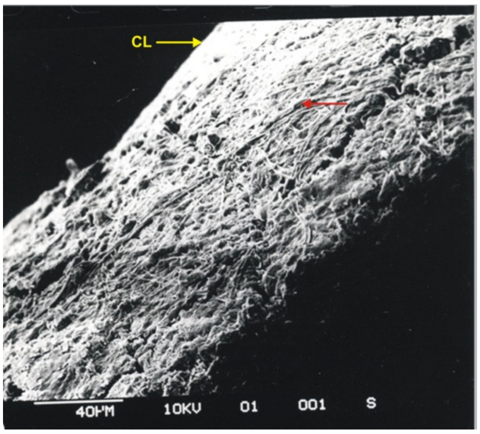

Figure 7 : Radial view of shell membrane showing thick fibrous structure.

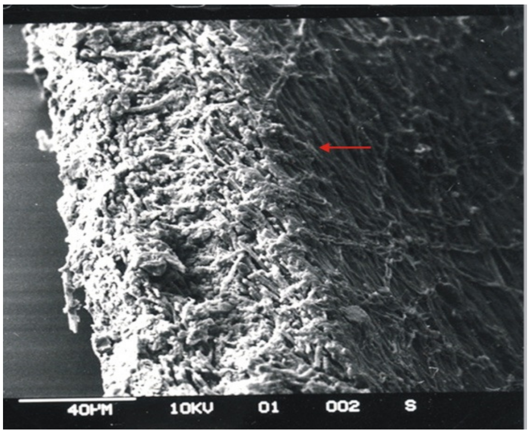

Figure 8: Radial view of shell membrane showing fibres coated with thin film of unknown substance (Arrow).

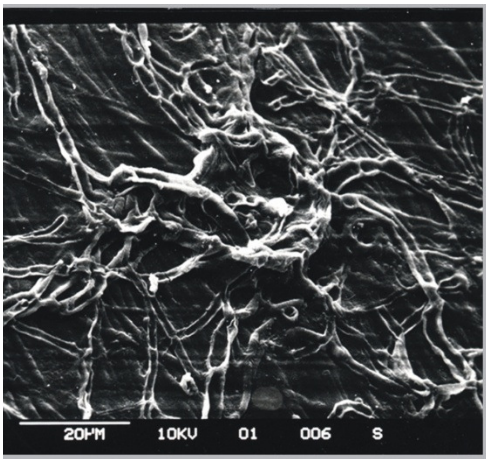

Figure 9: Magnified view of shell membrane side facing albumen showing fibrillar arrangement with thin film of unknown substance.

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