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Scanning Electron Microscopy of the Post-Embryonic Stages of the Climbing Perch, *Anabas testudineus*

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Abstract Different developmental stages, fertilized eggs through hatchlings, of the climbing perch, *Anabas testudineus*, have been studied by scanning electron microscopy. The surface specialization of eggs and hatchlings reveals that while the egg surface is reticulate in appearance, the hatchlings are covered with microridges. Vitelline arteries are seen at the pharyngeal and abdominal regions. They supply nutrients directly from the yolk sac to the developing embryo. Three pairs of such arteries are distinctly seen in the pharyngeal region. Mucous glands are discernible at places over the entire body surface of the embryo before the formation of scales. The skin seems to be helpful in gaseous exchange till the gills and accessory respiratory organs develop and become functional.

The spawning behaviour of *Anabas testudineus* has been observed by Moitra et al. (1979). Some workers have studied its embryonic and post-embryonic developmental stages (Moitra et al., 1978; Singh and Mishra, 1980; Hughes et al., 1986). The gills and air-breathing organs of fingerlings (1–2.5 g) and adult (30 g) *Anabas testudineus* have recently been studied by scanning electron microscopy (Munshi and Hughes, 1986). The hatchlings come out of egg membrane about 10 h after fertilization. During the yolk sac stages the gaseous exchange takes place through the well vascularised skin. But no information is available on the relationship between the yolk sac and the developing embryo.

The present paper reports a SEM study of the surface architecture of the fertilized eggs and the developing embryos including their relationship with the yolk sac.

Materials and methods

Properly conditioned adult males and femaels of *Anabas testudineus* were induced to breed in the laboratory by intramuscular injection of heteroplastic pituitary gland extract (15–20 mg/100 g body wt.) during the monsoon months (June–August). Collapsible plastic pools (1 m diameter) and glass aquaria (60×30×30 cm) were used as breeding containers. During the experiment the temperature of ambient water was 29±1°C.

Fertilized eggs and different developmental stages were fixed in chilled 2.5 percent glutaraldehyde prepared in 0.1 M phosphate buffer (pH 7.4) and kept at 4°C. The eggs and hatchlings were then washed thoroughly in phosphate buffer and dehydrated in graded alcohol and in a mixture of absolute alcohol and acetone in different concentrations and finally in anhydrous acetone at room temperature. Eggs were air-dried and hatchlings of different age groups were critically point dried using carbon dioxide. The samples were fixed on standard aluminium stubs by plasticised micro-cellulose solutions, coated with gold and then studied under Phillips PSEM-500 scanning electron microscope at RSIC, Bose Institute, Calcutta.

Results

6–9 h stage. The fertilized eggs (6 h) of *Anabas* are somewhat spherical in shape measuring 0.466 mm in diameter (Figs. 1, 2). At this stage the embryo measures 0.472 mm in length and 0.067 mm in breadth. At nine hours the eggs bearing the developing embryos become oval shaped with increase in the length of the embryo. The head part of the embryo becomes broader than the tail region (Figs. 3, 4) (Table 1). The yolk surface is reticulate in appearance having numerous depres-

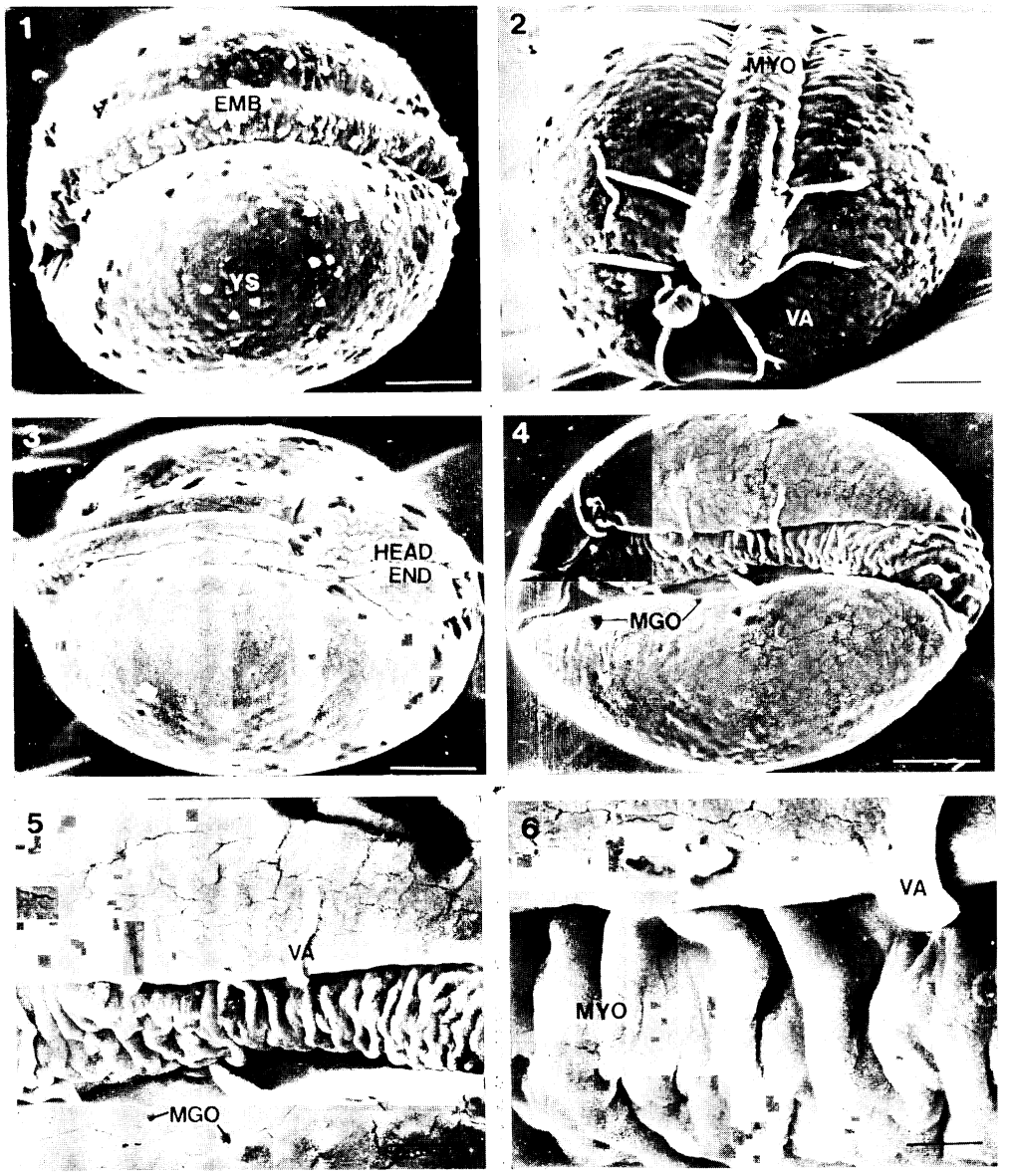

Fig. 1. Developing embryo within the egg (6 h). EMB, embryo; YS, yolk sac. Scale bar indicates 100 μm.

Fig. 2. Head end of the developing embryo with vitelline arteries entering into the pharyngeal region (6 h). MYO, myotome; VA, vitelline artery. Scale bar indicates 100 μm.

Fig. 3. Lateral view of the embryo and yolk mass (9 h). Scale bar indicates 100 μm.

Fig. 4. Aboral view of the embryo and yolk mass (9 h). MGO, mucous gland opening; VA, vitelline artery. Scale bar indicates 100 μm.

Fig. 5. Mid-aboral view of the embryo showing the vitelline arteries emerging from the yolk sac and entering into the myotomes (9 h). Abbreviations as in Fig. 4. Scale bar indicates 50 μm.

Fig. 6. Magnified view of the myotomes and the vitelline artery (9 h). Abbreviations as in Fig. 2. Scale bar indicates 10 μm.

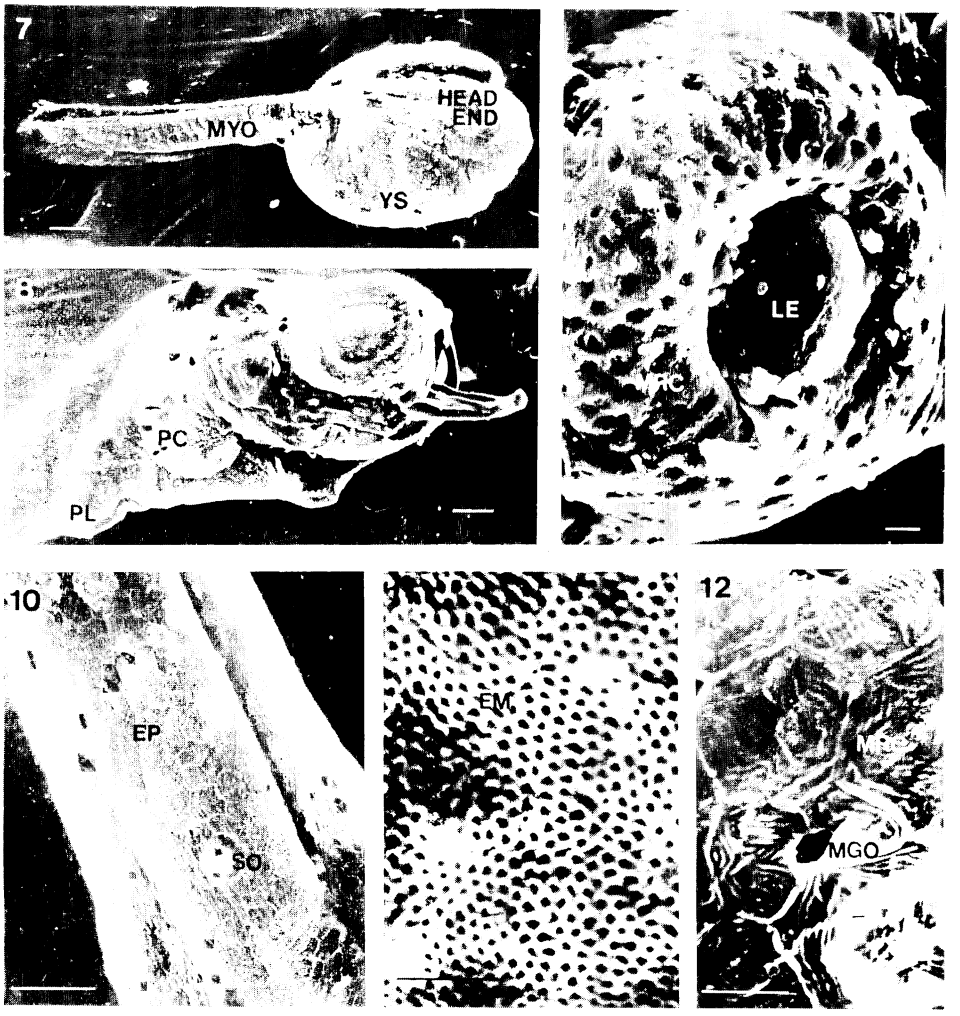

Fig. 7. Lateral view of the hatchling showing the developing myotomes in the tail region (12 h). Abbreviations as in Figs. 1 and 2. Scale bar indicates 1 mm.

Fig. 8. Head end of the hatchling with opened jaws and fin primordia (16 h). PC, pectoral fin; PL, pelvic fin. Scale bar indicates 1 mm.

Fig. 9. Depressed epithelial cells with microridges, encircling the orbit (42 h). LE, lens; MRC, microridged cell. Scale bar indicates 10 μm.

Fig. 10. Sense organs of the lateral line canal towards the tail region of the hatchling (96 h). EP, epithelium; SO, sense organ. Scale bar indicates 0.5 mm.

Fig. 11. Reticulate surface of the egg membrane (6 h). EM, egg membrane. Scale bar indicates 5 μm.

Fig. 12. Microridged epithelial cells and mucous gland opening over the general body surface of the hatchling (12 h). Abbreviations as in Figs. 4 and 9. Scale bar indicates 10 μm.

sions on its surface (Fig. 11). In the next three hours the rate of growth is further accelerated. The embryo becomes more elongated (0.743 mm) and it is at this stage that the hatching takes place at about 10 h after fertilization.

At the head end of the embryo, the vitelline arteries are seen arising from the yolk sac and entering into the pharyngeal region (Fig. 2). Similar blood vessels also arise in the trunk region in between the myotomes as seen from the ventral side (Figs. 4–6).

12–16 h stage. The newly hatched larva is

1.889 mm long and the yolk sac measures 0.250 mm in diameter (Fig. 7). It has not yet developed its mouth and operculum. A continuous dorsal and ventral fin is seen. The chromatophores make their appearance on the head region. They are somewhat oval or stellate in shape and remain scattered over the surface of the head and the body.

The hatchling measures about 2.411 mm at 16 h. At this stage the entire body surface remains covered with microridged epithelial cells. The eyes and pectoral fins are very conspicuous in structure (Figs. 8, 12). The operculum and jaws have developed. Amongst the microridged epithelial cells, the lateral line canal pores, measuring 0.0043 mm to 0.0045 mm in diameter, are seen. The distance between the pores increases with the gradual development of the embryo.

At 96 h, the whole body of the hatchling remains covered with microridged epithelial cells. The pores of lateral line sense organs are also discernible in the trunk and tail regions of the hatchling (Fig. 10). The yolk sac is completely absorbed.

The diameter of the eye is nearly 0.059 mm at 22 h (Fig. 9) which gradually increases to 0.200 mm at about 60 h (Table 1).

Chromatophores are present on the body surface of the developing embryo. They have also been observed over the yolk sac. The mucous gland openings are also discernible between the microridged epithelial cells of the body surface. At about 60 h the chromatophores change the colour from grey to black.

Discussion

Assessment of the degree of development of the respiratory surfaces in the post-embryonic stages of *Anabas testudineus* has recently been made (Hughes, et al. 1986). It has been seen that during the yolk sac stages the gaseous exchange takes place through the well vascularised skin. The present SEM study gives information on the supply and distribution of vitelline arteries. There are three distinct pairs of vitelline arteries in the head region of the 6 h old embryo. They are not only involved in gaseous exchange but also supply nutrition from the yolk sac region. The nutrient vessels have also been discovered on the ventral aspect of the embryo between the myotomes.

The reticulated surface of the developing embryo may hold on the aerated water. The pitted surface of the eye may be better adapted for gaseous exchange.

Recently, Fischelson (1984) has made an extensive study of the microridges on the surface epithelial cell membrane of fish scales and has classified them into three major types with their subtypes. The microridges on the epithelium of *Anabas* hatchlings are of coil maze pattern. Two distinct subtypes have also been identified as helix or spirema and acentric type. But the ridge pattern of the epithelial cells at the lateral line canal are different. Fischelson (1966) has also observed similar modifications in *Tilapia* juveniles,

Table 1. Morphometric measurements of the different developmental stages of *Anabas testudineus*.

Age	Developmental Stage	Diameter of egg (mm)	Length of embryo (mm)	Breadth of embryo (mm)	Length of hatchling (mm)	Diameter of yolk sac (mm)	Diameter of eye (mm)
06 hrs	Egg	0.466	0.472	0.067	—	—	—
09 hrs	Egg	0.431	0.507	0.130	—	—	—
12 hrs	Hatchling	—	—	—	1.889	0.043	—
16 hrs	Hatchling	—	—	—	2.411	0.035	—
20 hrs	Hatchling	—	—	—	2.778	0.020	0.059
24 hrs	Hatchling	—	—	—	2.841	0.015	0.062
30 hrs	Hatchling	—	—	—	2.944	0.005	0.070
36 hrs	Hatchling	—	—	—	3.111	—	0.098
40 hrs	Hatchling	—	—	—	3.278	—	0.115
42 hrs	Hatchling	—	—	—	3.333	—	0.130
46 hrs	Hatchling	—	—	—	3.555	—	0.150
50 hrs	Hatchling	—	—	—	3.652	—	0.160
56 hrs	Hatchling	—	—	—	3.667	—	0.180
60 hrs	Hatchling	—	—	—	3.722	—	0.200

where, with the gradual sinking of the neuromasts into the lateral line canal, the involved cell surface becomes completely modified.

The microridges on the epithelial cell surface have been assigned with various functions by different workers, viz., increase in the functional surface area (Hughes and Wright, 1970; Olson and Fromm, 1973), spreading of mucus away from the mucous secreting cells (Sperry and Wassersug, 1976); anchorage of mucus (Hughes and Wright, 1970; Hughes, 1979) and to withstand the mechanical stress (Harding, 1973; Lanzing and Higginbotham, 1974; Ezeasor and Stokoe, 1980). The present study indicates two possible functions of microridges. Since the microridges are seen on the epithelial cells of the body at 16 h stage when the respiratory organs have not developed, the sculped surface probably increases the functional surface area for the gaseous exchange. The microridges of the epithelial cells on the body of the delicate embryo may also serve to withstand the mechanical stress.

The mouth with prominent jaws develop almost simultaneously with the opercular slits and operculum at the 24 h stage. The gill differentiation also takes place at this stage. The development of labyrinthine organs begin after 51 h but the hatchlings do not take air-gulps till 13/14 days (Hughes et al., 1986). The entire body becomes covered over by scales in about 10–12 days. The appearance of scales prevents cutaneous respiration and therefore correlates with the onset of aerial respiration.

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キノボリウオの後胚期の走査電子顕微鏡的研究

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キノボリウオ *Anabas testudineus* の受精卵から孵化仔魚にわたる各発生段階を，走査電顕によって観察した．卵表面は網状であるが，後期胚では微小堤で覆われるようになる．卵黄動脈は，咽頭域と腹域に存在し，卵

黄から発育中の胚へ養分を供給する．鱗形成前の胚体全表面にわたって，粘液腺が開口している．鰓並びに補助呼吸器が発達し，機能を営むまでは，皮膚がガス交換に役立っていると思われる．