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M Akilan
P.G. & Research Department of Zoology, Voorhees College, Vellore, Tamil Nadu, India

M Md Ismail
Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India

P Venkatesan
Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India

Correspondence
M Akilan
P.G. & Research Department of Zoology, Voorhees College, Vellore, Tamil Nadu, India

Scanning electron micrographic studies on the morphology of the eggs of Nepids (Hemiptera: Nepidae)

M Akilan, M Md Ismail and P Venkatesan

Abstract
Water bugs are well known to exhibit diversity in ovipositional behavior and sites. Among nepids *Laccotrephes* oviposit their eggs under the sand grains in bunches while *Ranatra* oviposit inside the soft parts of floating vegetation of the fresh water habitats. Scanning electron microscopic study was carried out on egg chorion of nepids shows that bead shaped projections are distributed in clusters over the chorionic surface of both the insects. Tips of the respiratory horns showed pores for respiration and posterior part of the egg contain micropylar region. An oval parchment like area was observed in the eggs of *Laccotrephes* termed as complex chorionic hydropyle and the significance of the investigation is discussed.

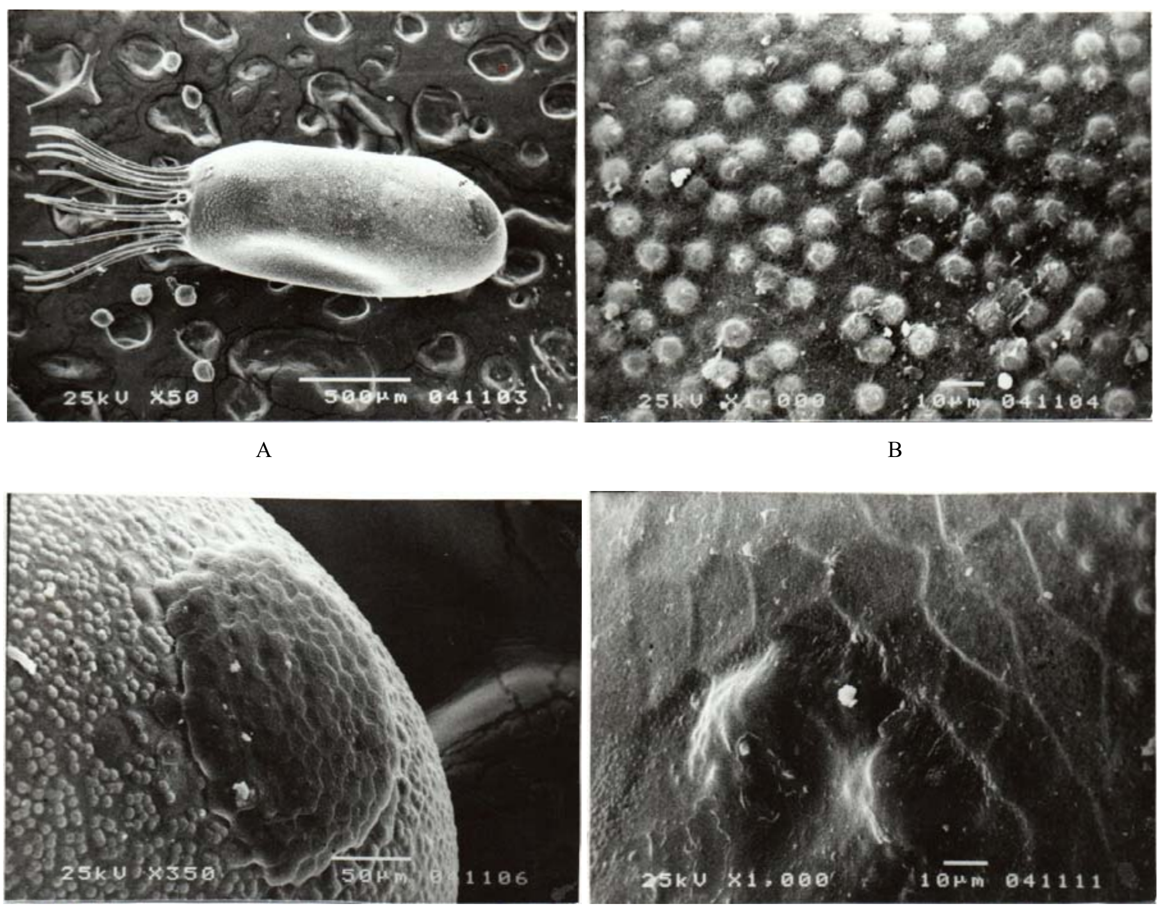
Keywords: SEM, Egg Chorion, Hydropyle, Respiratory horns, Nepids

Introduction
Aquatic insects are known to prefer specific ovipositional sites in fresh water bodies [15, 4, 2] that vary among various groups of water bugs. Nepids *Ranatra* and *Laccotrephes* are unique in depositing their eggs by inserting them within the submerged vegetation [3] and on the sub-soil surface within the water bodies respectively. Both these water bugs share a common feature in their egg structure. The egg shell is drawn anteriorly into many respiratory horns [5]. Water scorpion (*Laccotrephes*) eggs bear two or more respiratory horns at their anterior pole and are oviposited either in mud (Nepini) or vegetation (Ranatrini) [11]. Egg structure of these Hydrocorisae has not been studied except for the work of earlier authors who have described the characteristics of the eggs including the colour and measurements [7, 12, 13].
In the view of the above considerations, a detailed investigation of the egg structure of the nepid water bugs with electron microphotographs was undertaken in order to understand whether the fresh water environment and its characteristics have a direct impact on the architecture of such eggs.

Materials and methods
Adult nepids brought from the fresh water bodies were maintained in an aquarium containing twigs of *Hydrilla,* sand and water. They were fed with larvae of mosquito in a photoperiod of 12:12. Female *Ranatra* oviposited the egg by inserting in the twigs of submerged vegetation, whereas the *Laccotrephes* oviposited on the sand base of the aquatic habitates. Fresh eggs of *Ranatra filiformis* were isolated from the twigs of *Hydrilla* plants; similarly fresh eggs of *Laccotrephes griseus* were removed from the sand base. For Scanning Electron Microscopic studies, eggs fixed in 70% alcohol were kept in glutaraldehyde overnight and washed several times with phosphate buffer at pH 7. They were then transferred to osmium tetra oxide. After 2 to 5 minutes they were again washed in phosphate buffer before dehydration in the series of ethyl alcohol up to 100% and finally treated with propylene oxide for 2 to 5 minutes. The egg samples were then dried at 15 ºC. From such eggs, unshrunken ones were isolated and sputter coated in Jeoul Sputter Coating Unit for 3 minutes and were observed under Jeoul Scanning Electron Microscope for the ultrastructure of the eggs chorion. Entire egg, chorionic structure, tip of respiratory horns, hydropylar and micropylar regions were scanned and photographed.

Results
The egg of *Laccotrephes griseus* is oval in shape and blunt at both ends. While the posterior end has the complex chorionic hydrophyle,

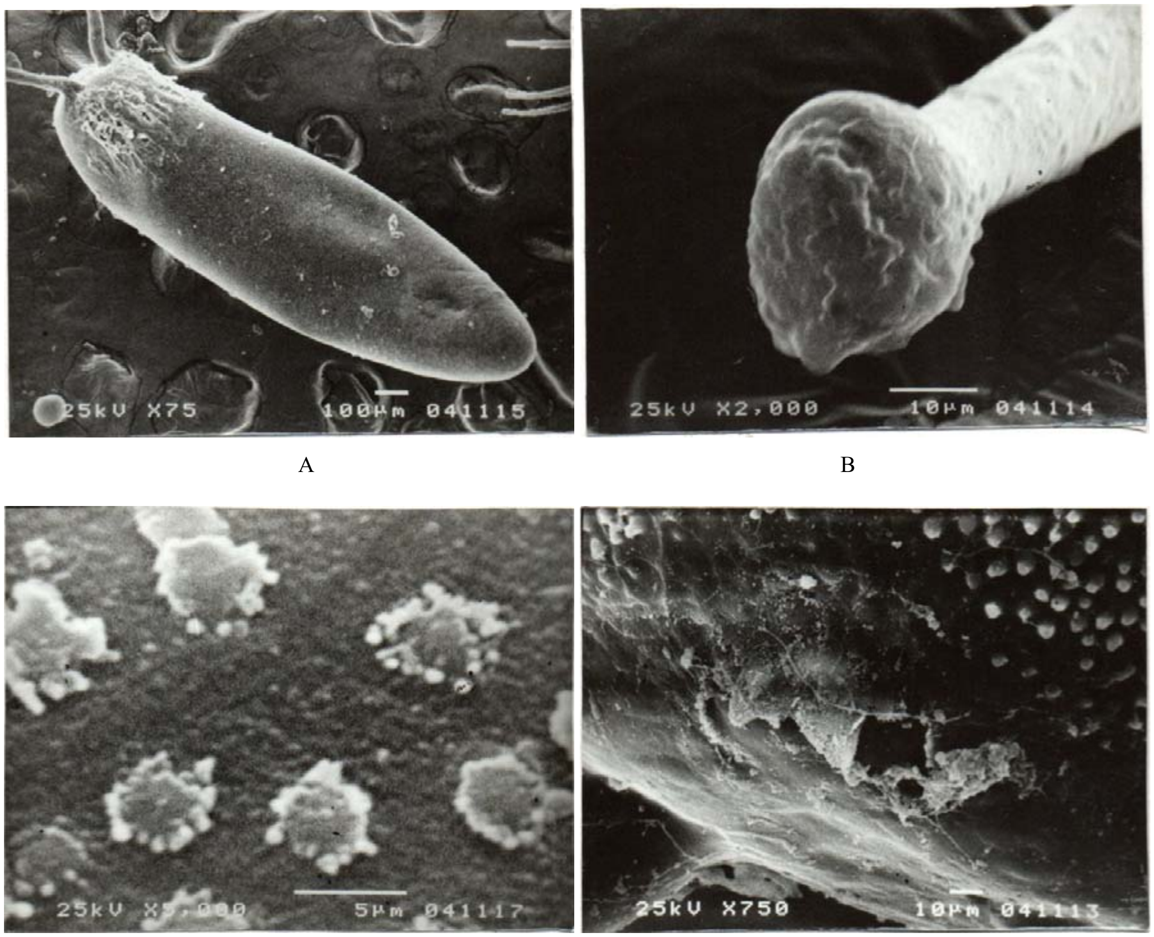
the egg is drawn anteriorly in to the respiratory horns numbering 9-13 in the eggs [Plate1 A] of the same species. The tip of the horn is club shaped possessing numerous hexagonal shaped pits. The general egg chorion shows a number of bulb or bead like structure [Plate1 B] except in the complex chorionic hydropylar region. The respiratory hydrophylar region is elevated than the general chorion structure within the slightly irregular margin. Higher magnification of the complex chorionic hydrophyle [Plate 1C] shows stratification of hexagonal like structures and each such structure indicates a rough surface with small blunt projections. The anterior region of the egg chorion possesses the micropyle which is a depressed area with a least cellular differentiation. It is surrounded by a number of honey comb like structure on the egg chorion. The length of the egg measures 1382 µm, width of the egg 647 µm and the length of the respiratory horn 735 µm. Under magnification, the tip of respiratory horn was found to be round in shape and on the respiratory horn are distributed a number of pores measuring about 0.76 µm which play an important role in the exchange of air. The inter distance between the pores was measured as 2.3 µm. The beads present over the chorion measures 3.71 µm and the distance between two groups of beads measures 11.42 µm. The posterior pole possesses complex chorionic hydropyle and its radius measures 212.5 µm, which is unique in the eggs of *Laccotrephes griseus*. Further higher magnification shows a honey comb like structure with a number of spherical nodules [Plate 1D]. The length and width of these cells are 18 µm and 10 µm respectively. Two microphyles situated at the anterior side are also surrounded by hexagonal honey comb structures with spherical nodules. The width of the microphyle measures 2.16 µm. The egg of *Ranatra filiforms* is more oblong in shape with a tapering posterior end. Anteriorly, the egg is drawn into a pair of respiratory horns. The anterior tip of the horn is tubular ending in a swollen bulb like structures with numerous undulations [Plate2B]. The general surface of the egg chorion has tightly packed bead like structures. The egg surface between the base of the respiratory horns shows the absence of the beaded structures and the presence of numerous fibroid structures with depressions. The length and width of the egg measure 1600 µm respectively. The space between the two respiratory horns at the anterior pole measures 100 µm. Under higher magnification, the tip of the respiratory horn is found to be spherical with a radius of 29.2 µm and the surface area of respiratory horn is 2.3 µm. The chorionic architecture shows a uniform distribution of bead like structures [Plate 2C] which may also help in the diffusion of water and other nutrients. The width of the beads presents over the chorion measures 5 µm and the space between two beads was 8.3 µm. The micropylar opening [Plate 2D] is situated at the anterior end between the respiratory horns and it is rectangle in shape measuring 22 µm and 16 µm respectively surrounded by the plain surface of the chorion.



A B C D

A. Egg entire view (X 50)
B. Architecture of the chorion (X 1000)
C. Complex chorionic hydropyle (X 350)
D. Micropylar region (X 1000)

Plate 1: Scanning Electron Photographs of the Eggs of *Laccotrephes griseus*



A. Egg entire view (X 75)
B. Respiratory horn (X 2000)
C. Architecture of the chorion (X 5000)
D. Micropylar region (X 750)

Plate 2: Scanning Electron Photographs of the Eggs of *Ranatra filiformis*

Discussion

The distribution of nepid bugs in a wide variety of substrata such as stones, muddy bottom and vegetation of the freshwater environment has led to a selective pressure on their biodiversity. On reproduction observations were noted to be peculiar in nepids. In *Laccotrephes* eggs are laid along the edge of the pond and glued in mass to fine sand particles [17] and insertion of the eggs into the soft parts of aquatic vegetation in *Ranatra* [12]. The ovipositional behavior exhibited by *Ranatra filiformis* is another interesting feature. Females search for the vegetation and pierce the stem of aquatic plants with their ovipositors. The tip of the ovipositor plays a role in the deposition of the eggs in to the softer parts of aquatic vegetation. The respiratory horns are helpful in the gaseous exchange during development. The respiratory horns are of greater significance in nepid bugs. Nepid eggs are essentially terrestrial in their respiration, since the horns normally project above the surface of the water [6]. The central region of each horn consists of a fine meshwork and this polarity opens with the atmosphere through a peripheral plastron mostly confined to the tip of the horn [5]. The egg of *Laccotrephs griseus* possesses two microphyles whereas the egg of *Ranatra filiformis* has a single microphyle at the anterior end. The egg chorion is a complex structure produced by the follicle cells as the eggs develop in the ovary. The outer surface of the chorion is often sculptured with a pattern which is basically hexagonal and reflex the form of the follicle cells. In other cases the surface may be ribbed or ridged and pitting also occurs, resulting in uneven laying down of chorion by the follicle cells [7] that prevents water loss.

In the present investigation, the egg chorion of *Laccotrephs griseus* is opaque throughout its surface except for a small crescent shaped transparent chorionic hydrophyle at the posterior end of the egg. The micropylar area at the anterior end.of the posterior polar region, an oval parchment like area is found which is slightly raised above the general surface of the egg represent a complex chorionic hydropyle as reported by Cobben. The chorionic architecture revealed the disposition of group of bead like structures which may probably increase the surface area for the diffusion of water during development. The eggs of *Ranatra filiformis* reveal an elongated counter with two respiratory horns. The chorionic architecture shows a uniform distribution of bead like structures which may also help in the diffusion of water and other nutrients. It has been observed in the eggs of *Lethocerus indicus* chorionic wall comprises of a number of polygonal cells that accommodate numerous aeropyles with a mosaic effect [9]. Observations of Ismail [8] in the eggs of *Heleocoris indicus* revealed the presence of a number of hexagonal cells with nodules distributed uniformly throughout the chorionic surface, which increases the area of absorption of nutrients and also the imbition of metabolites. In *Hydrometra butleri*, the egg chorion shows a number of longitudinal ridges that may avoid loss of water molecules and at the same time the depression enhances the surface area [10]. In view of these considerations a comparative electron microscopic study on the egg chorion of water bugs may further reveal the significance of chorionic architecture and its phylogenetic relevance.

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