Ultrastructural observation of the vitelline cells of diplozoon sp (from Arunachal Pradesh) (Monogenea, Polyopisthocotylea)

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Abstract- An Electron microscopic studies of the vitellaria of three polyopisthocotylean fish-gill flukes,Diplozoon paradoxum, Diclidophora merlangi andD. Denticulata are composed of cells in different stage of development.(Halton et al 1974) Immature cells are embryonic and undifferentiated. Differentiation into maturing vitelline cells involves the development of extensive GER, Golgi complexes and the production of dense droplets of shell-protein. With the onset of maturity, protein synthesis stops and, as the GER disintegrates, the cell develops food-reserves in the form of yolk bodies, glycogen and lipid and released in the ciliated vitellian duct. Vitelline development is continuous and all of the cellular stages involved can be found in each follicle.

Index Terms- Diplozoon sp- ultrastructure- vitelline cells – Monogenea- Polyopisthocotylea

I. Introduction

It was regarded that the reproductive organs , especially the ovary and vitelline glands from female schistosomes were most sensitive to the changes in invitro conditions (Hua , Zhou ,1988). Ultra structures and their dynamic changes of the cultured cells from *Metamicrocotyla macracantha* were also studied in detail (Baptista-Farias, Maria de Fatima D, & Kohn, Anna. 1998). The present study details ultrastructural observations of vitelline cells of monogenea , Polyopisthocotylea parasite and then evaluate the culture conditions used in the experiments.

II. Materials and Methods

The parasites were collected from the gills of *Schizothorax richardsonii* from the Tenga River through the Himalayan ranges of Arunachal Pradesh within the Kameng river system following examination in a saline medium under stereoscopic microscope. For light microscopy, worms were fixed in 5 % formalin under slight cover-slip pressure stained in alcoholic chlorhydric carmine (Langeron 1949), dehydrated through an alcohol series, cleared in beechwood creosote and mounted in Canada balsam . For transmission electron microscopy worms were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium phosphate buffer (PH 7.3) for 12 hrs at 4°C. After wash in buffer samples were post fixed in 1% OsO4 for one hour at 4° C. The samples were dehydrated in an ascending grade of acetone , infiltrated and embedded in araldite CY 212 (TAAB UK). Thick sections (1µm) were cut with an ultramicrotome, mounted onto glass slides , stained with aqueous toluidine blue and observed under light microscope for gross observation of the area and quality of tissue fixation. For electron microscope examination, thin sections of grey-silver colour interference (70-80 nm) were cut and mounted onto 300 mess copper-grids. Sections were stained with alcoholic uranyl acetate and alkaline lead citrate, washed gently with distilled water and observed under a Morgagni 268D transmission electron microscope (Fei Company , The Netherlands) at an operating voltage 80 KV. Images were digitally acquired by using a CCD camera (Megaview III, Fei company) attached to the camera.

III. Results

The details ultrastructural observations of vitelline cells of *Metamicrocotyla macracantha* (Monogenea, Microcotylidae) by Baptista –Farias et al(1998) has unique similarity in the result of the ultrastructural observation with the Ultrastructural observation of the vitelline cells of diplozoon *sp* (Monogenea, Polyopisthocotylea).

Observed by transmission electron microscopy, the vitelline follicles are juxtaposed separated by profiles of parenchyma and small amounts of fibrous material and muscle fibres . These cells can not be confused with the parenchymatous cells for : (1) the cells were collected from follicles and these cells significantly differ in their morphology from the parenchymatous cells of parasitic helminths (Morris and Threadgold 1968, Reissig and Colucci 1968 and Reissig 1970).

Immature vitelline cells are irregularly- shaped and each contains a large nucleus which occupies almost all the volume m a single nucleolus and dense area of heterochromatin . The cytoplasm is filled with numerous unattached ribosomes and few mitochondria .(Fig.1)

Developing vitelline cells are larger , have nuclei similar to immature ones , cytoplasm with golgi apparatus and granular endoplasmic reticulum. At this stage , droplets of egg shell protein appear and cytoplasm shows large amounts shell material due to continuous protein shynthesis.(Fig2)

Mature cells increase in size and in the quantity of membrane- bound protein globules that form clusters. In the mature cells, the amount of heterochromatin decreases in the nucleus and size of nucleolus increases in size. It is characterized by a growing number of egg-shell droplets while the cells are active state of secretion .(Fig 3&4)

At the end of development , the vitelline cell cytoplasm is filled with parallel arrays of GER and homogenous droplets of

shell-protein that gradually increase in size alongside deposits of lipid and glycogen which with disintegrated portion of GER form into large yolk bodies . All of these inclusions can be observed as isolated components or in groups ,throughout the cytoplasm, in variable number, or present and largely free in ciliated vitelline ducts ready to be expelled.

IV. Discussion

Histochemical and electron microscopic studies of the vitellaria of three polyopisthocotylean fish gill flukes ,*Diplozoon paradoxum*, *Diclidophora merlangi* and*D. denticulata*, and one monopisthocotylean fish-skin fluke,*Calicotyle kröyeri* have shown that, in each case, vitelline cell development is basically similar.Halton et al. (1974). The vitelline cell development of *M. macracantha* is similar to that of these polyopisthocotyleans. Baptista –Farias et al(1998). An electron microscope study of the vitelline follicles of *Fasciola hepatica* also shows the similar result (Irwin and Threadgold 1970), *Halipegus eccentricus* by Holy and Wittrock (1986)

Differentiation into maturing vitelline cells involves the development of extensive GER, Golgi complexes and the production of dense droplets of shell-protein. With the onset of maturity, protein synthesis stops and, as the GER disintegrates, the cell develops food-reserves in the form of yolk bodies, glycogen and lipid. It is then released into the vitelline ductlet . These processes occur in all the follicles at the same time, and all stages of development can be found in any one follicle.(Halton et al 1974)

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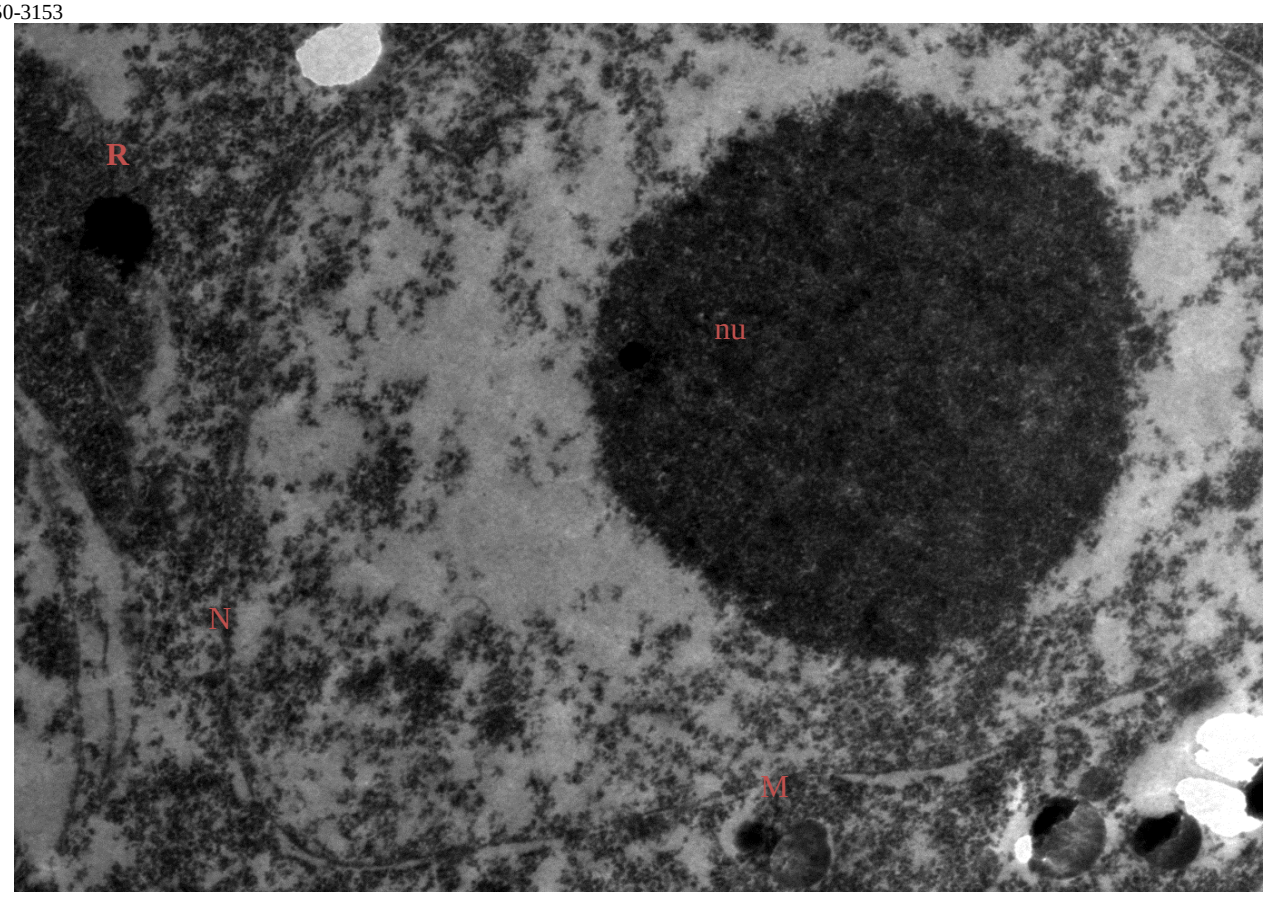
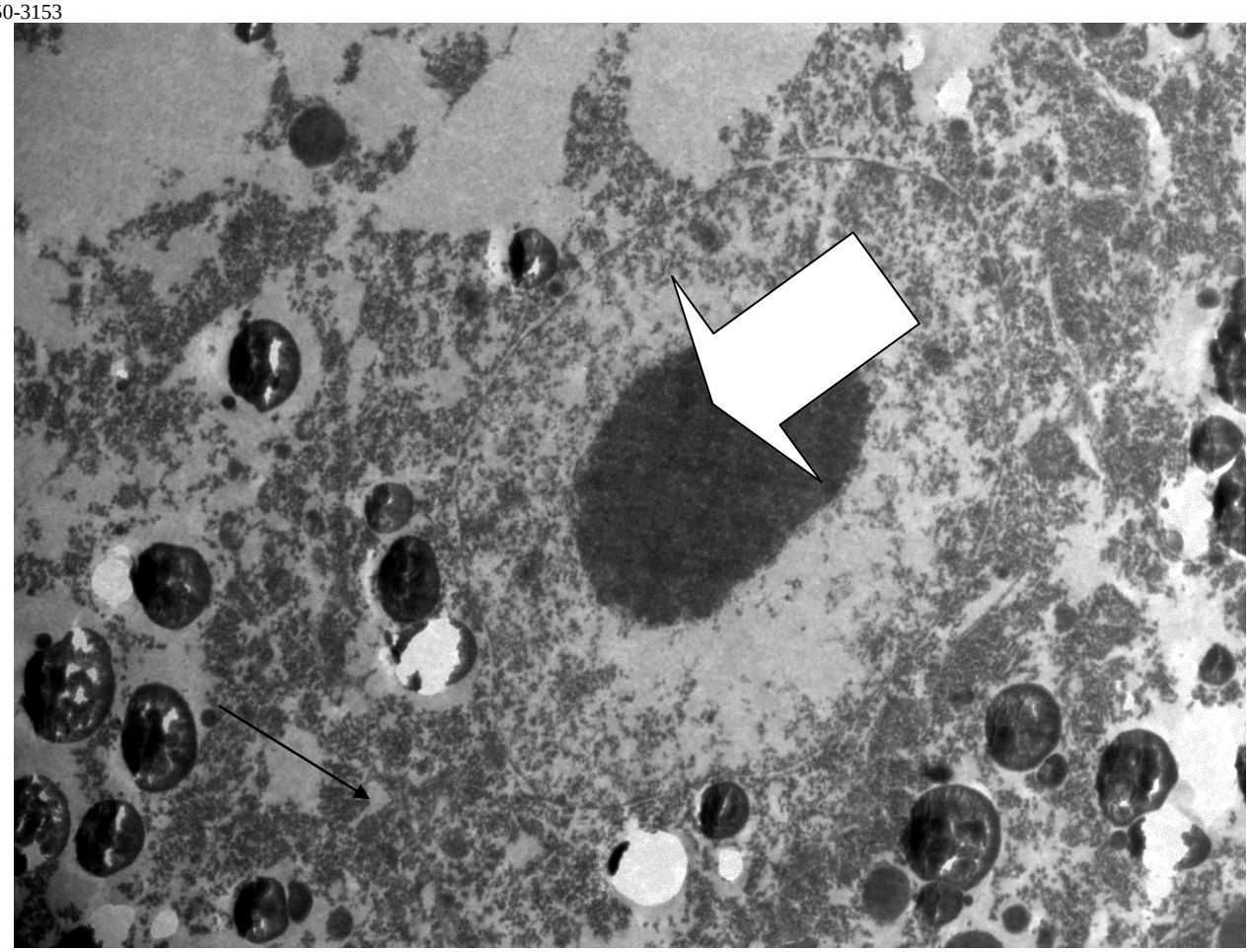
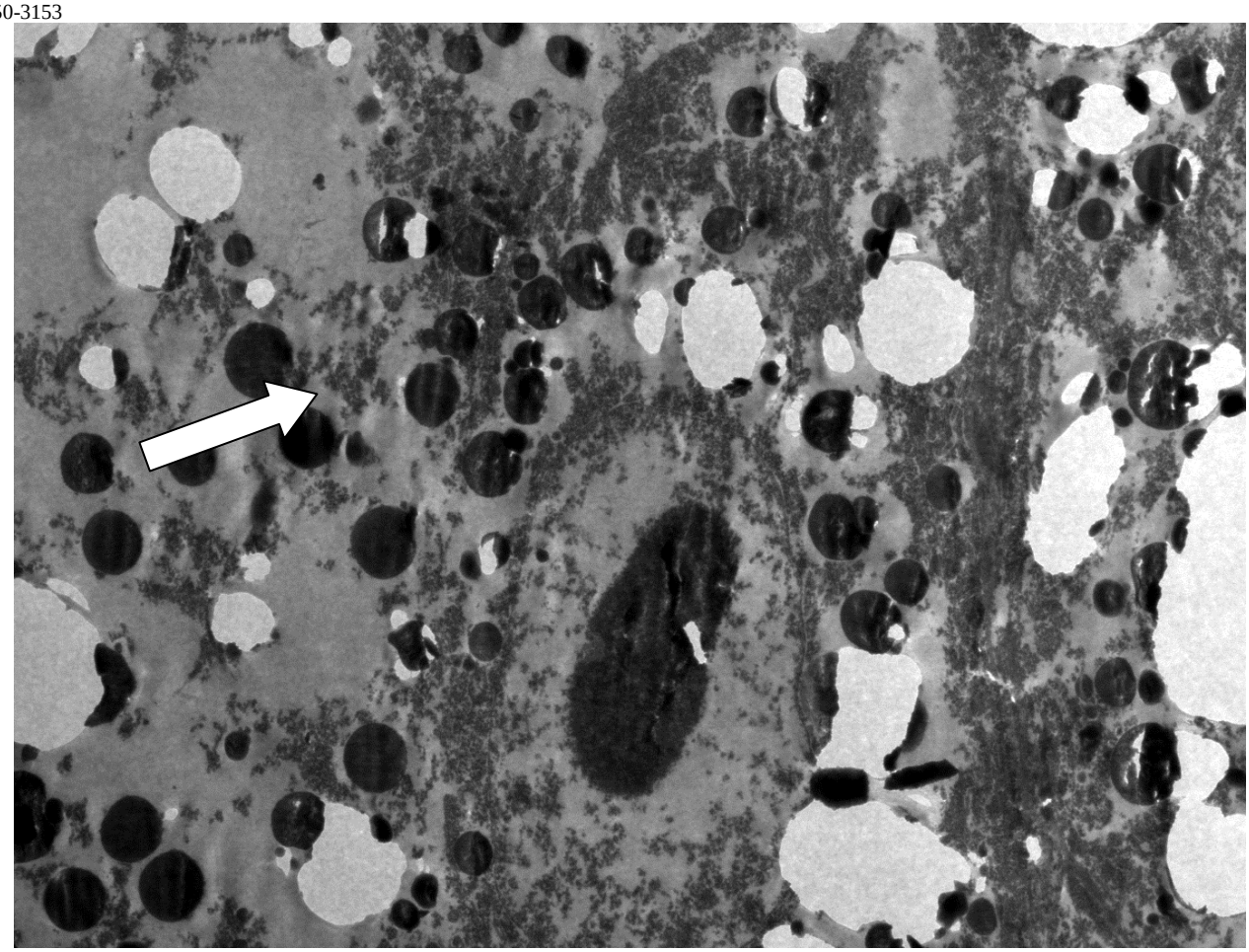


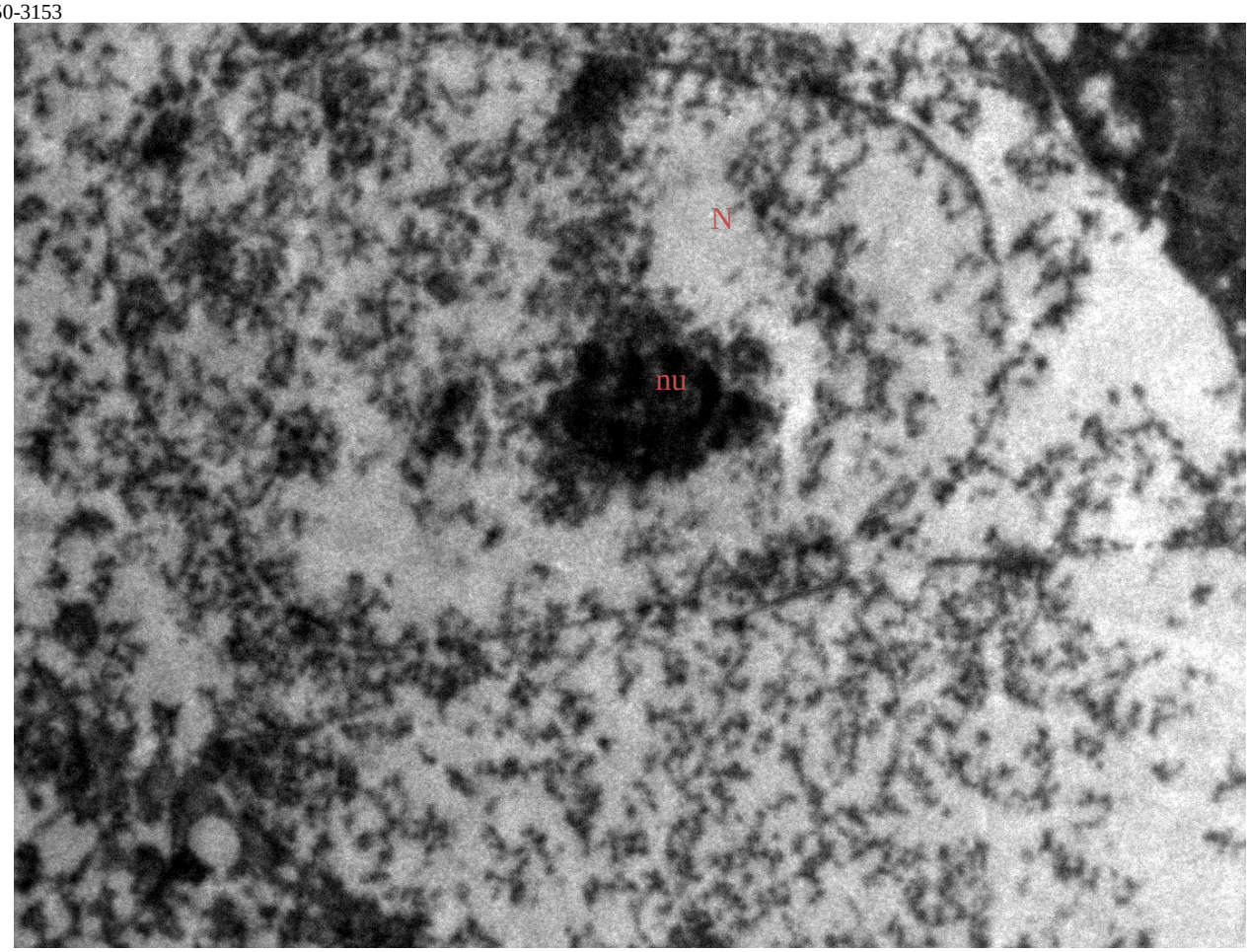
Diplozoon sp **Fig.1 : immature cells of the vitelline follicles, with a large nucleus (N), nucleolus (nu), mitochondria (M), and free ribosomes (R)**



Diplozoon sp **Fig .2 : developing vitelline cell with nucleus, large nucleolus (big arrow), granular endoplasmic reticulum (little arrow) and droplets of egg- shell protein . X 9,000.**



Diplozoon sp **Fig.3 : mature vitelline cell showing the increase of the shell-protein droplets (arrow) and decrease of the nuclear heterochromatin . X 8,000**



Diplozoon sp **Fig. 4: mature vitelline showing the decrease of nuclear heterochromatin X. 8,000.**