Studies of the Seasonal Variations in the Protochordate, *Branchiostoma lanceolatum*: A Cell Biology Investigation by the Use of Light and Electron Microscopes.

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Abstract

Cephalochordates – commonly known as "Lancelets" – are the key to understanding the vertebrate origins, hence comes the importance of this study. *Branchiostoma lanceolatum* (Amphioxus) specimens were collected monthly – with the exception of few months – from the Red Sea shores near Fayed region. These specimens were submitted to morphological, histological, and ultrastructural investigations. Researches on *B. lanceolatum* had never been carried out before in this locality.

The present study reports that spawning of this animal occurs all over the year, and its highest values were encountered in summer. Therefore, the present study suggests that the breeding period extends all over the year instead of only few months as was suggested before. The present study also states that the spermatogenic epithelium follows the basic pattern of all vertebrates: spermatogonia – with two forms "A" and "B" – divide to give spermatocytes; which in turn divide to give spermatids that undergo morphological transformations to produce spermatozoa at the end. The ovary of such magnificent animal has three developmental stages: the previtellogenic (immature) stage, the vitellogenic stage, and the mature stage.

The ovary is lined externally with a layer of germinal and non-germinal cells. The germinal epithelium comprises oogonia and oocytes. Three stages of the oocyte can be seen. Stage one: which is the smaller-growth-stage oocyte; stage two: this is the medium-growth-stage oocyte; and stage three: which is the large-growth-stage oocyte. Each of these is responsible for a different metabolic process. Non-germinal follicular cells can be found in the form of flattened cells with tapering ends, adherent to the outer surface of the oocytes as they grow. The histological and ultrastructural studies were conducted in the summer season when the gonads are full and ripe.

1. INTRODUCTION

The origin of chordates and the consequent genesis of vertebrates are major events in the natural history. The Amphioxus (lancelet) is now recognized as the closest extant relative to the stem-chordate and is the only living invertebrate that retains a vertebrate-like development and body plan through its lifespan, despite more than 500 million years of independent evolution from the stem-chordate. **(Garcia-Fernàndez and Benito-Gutiérrez, 2009).**

Phylum Chordata comprises three extant clades: Cephalochordata, Tunicata and Vertebrata **(Holland N. D. and Holland L. Z., 2010)**. Sub-phylum Cephalochordata contains around thirty-two species of small fish-like animals known as lancelets. Twenty-four of these species belong to the Genus *Branchiostoma* which is also called "Amphioxus", seven species in the Genus *Asymmetron,* and only one species in the Genus *Epigonichthys.* **(Nishikawa, 2004).** Traditionally, myomere counts, counts of fin chambers, and the position of the atriopore

have been used to distinguish different species within the genus *Branchiostoma* **(Desdevises *et al.*, 2011).**

The cephalochordate "Amphioxus" is considered a promising model animal for studying both the evolutionary and developmental mechanisms of vertebrates due to its unique phylogenetic position, simple body plan and sequenced genome. To date, four of the thirty discovered species have been commonly used for biological studies: *Branchiostoma belcheri*, *B. japonicum*, *B. floridae*, and *B. lanceolatum* **(Li *et al.*, 2012).** Because of great interests among the scientific community, several milestones have been achieved recently in Amphioxus research; these included the completion of the genomic sequencing projects of *B. floridae* and *B. belcheri* **(Putnam *et al.*, 2008)**

Lancelets have been widely known as one of the most important species in the zoological world; these have been studied for over than 200 years in the various faculties and universities around the globe. *Branchiostoma* has been meticulously studied at all levels of the biological organization **(Garcia-Fernandez, 2006).**

In Asia, these fascinating creatures are harvested commercially as food for humans and domesticated animals. Lancelets serve as intriguing comparison point for tracing how vertebrates have evolved and adapted. Although lancelets split from vertebrates more than 520 million years ago, their genomes hold clues about evolution, particularly how vertebrates have employed old genes for new functions. They are regarded as the archetypal vertebrate form **(Fang, 1998)**.

Lancelets are small, rather slender, animals that grow up to about 5 cm long, reaching 7 cm at the longest. They are of pale flesh colour, which changes – when seen by reflected light – to a beautiful display of metallic iridescence. However, male gonads take a white colour, while those of female take a creamy colour. Lancelets have a translucent, somewhat fish-like body, but without any paired fins or other limbs. They possess a smooth, very muscular, much compressed – from side to side body – that tapers gradually to the extremities, which are pointed (**Rice, 1880).** Despite their relatively sessile life style, adult lancelets are capable of swimming through the water by rapid eel-like undulations powered by their extensive trunk muscles **(Stokes and Holland N. D., 1998).**

They are also small fish-like filter-feeders which are common in shallow coastal waters of temperate and tropical seas where they spend most of their time in shallow burrows filter-feeding on small particles especially phytoplanktons. These marvelous creatures live in coarse sand **(Stokes and Holland N.D., 1998).** *Branchiostoma lanceolatum* specifically is widely distributed along Northeast Atlantic coasts, from northern Norway to the Mediterranean Sea and Black Sea (may be absent from the Atlantic Spanish coasts). It entered the Indian Ocean after passage through the Suez Canal; widely distributed in the northern Indian Ocean and tropical Southwestern Indian Ocean along the East African coast **(Poss and Boschung, 1996).**

In common with vertebrates, lancelets have a hollow nerve cord running along the back, pharyngeal slits, and a tail that runs past the anus. Also like vertebrates, the muscles are arranged in blocks called myomeres or myotomes. Unlike vertebrates, the dorsal nerve cord is not protected by bone, but by a simpler notochord – that lies below the nerve cord – made up of a cylinder of cells that are closely packed to form a toughened rod. The lancelet notochord, unlike the vertebrate spine, extends into the head. This gives the Subphylum its name. The nerve cord is

only slightly larger in the head region than in the rest of the body, so that lancelets cannot be said to possess a true brain. Neither do they have any eyes, or other complex sense organs comparable to those of the vertebrates. Also, unlike vertebrates, there are numerous unsegmented gonads. *B. lanceolatum* is sex separate; however, the occurrence of hermaphrodites has been reported among some specimens **(Romer *et al.*, 1977).**

Spermatogenesis appears to be fairly conserved process throughout the vertebrate series. Thus, spermatogonia develop into spermatocytes that undergo meiosis to produce spermatids which enter spermiogenesis, where they undergo a morphological transformation into spermatozoa. There is, however, variation amongst the vertebrates in how germ cell development and maturation is accomplished. The difference can be broadly divided into two distinct patterns; one present in anamniotes (Fish and Amphibia) and the other in amniotes (Reptiles, Birds and Mammals). For anamniotes, spermatogenesis occurs in spermatocysts (cysts) which – for most species – develop within seminiferous lobules. Concerning anamniotes, the oocytes generally have three distinct layers, namely, an outermost follicular layer, a median zona radiata, and an inner oolemma or

oocyte plasma membrane. The follicular layer consists of an outer theca and an inner granulosa layer. The theca has a protective function, whereas the granulosa layer is involved in various functions such as aeration, nourishment of developing oocytes and embryos, and secreting the enzymes that participate in the lysis and/or reorganization. **(Pudney, 1995)**.

The phylogenetic ambiguity among chordates and the limitation of laboratory investigations do not reduce the importance of lancelet studies, but rather further studies are required on this animal. A better understanding of chordate evolutionary history will not be achieved until all chordate groups are well-studied.

To the best of my knowledge, this is the first Egyptian thesis been donated to the protochordate, *Branchiostoma lanceolatum*, and offered to an Egyptian university.

2. AIM OF THE WORK

The present study deals with certain aspects that concern the magnificent chordate *Branchiostoma lanceolatum*; the taxonomic group of such animal is characterised with unique criteria among the different Phyla of the Animal Kingdom. In the present study the specimens of such animal were collected from the Red Sea near Fayed city. The study aims to:

- Investigate the seasonal variations of the body length, the gonadal length, the gonadal status, and width and weight of such animal around the year.
- Assess the spawning period of this animal.
- Determine the histology and the ultrastructure of the testis and ovary in the summer season when the gonads were full and ripe.

It worth mentioning that no earlier study tackled such biological aspects that appertain to this animal.