[HMS#102]

Ecdysis Failure in Asian Horseshoe Crab Larvae of *Tachypleus gigas* under Laboratory Observation

Zaleha Kassim[1]*, Mohd. Razali Md. Razak[2], Noor Yuslida Hazahari[3], Kamaruzzaman Yunus[4]

[1]Institute of Planetary Survival for Sustainable Well-being, International Islamic University Malaysia, Jln Penjara, 25100 Kuantan, Pahang

[2]Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

[3]International Institute for Halal Research and Training, International Islamic University Malaysia, Jln Gombak, 53100 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur

[4]Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

*Corresponding author's e-mail: drzack@iium.edu.my

ABSTRACT

Horseshoe crab has recently been categorized as a halal commodity which exposed them to unsustainable exploitation. As grouped among the arthropods, Asian horseshoe crab, *Tachypleus gigas*, rely on ecdysis to grow. The stage of ecdysis itself is the most crucial step in their life cycle and could cause death if it cannot be completed successfully. This study investigated the different types of ecdysis failure in the larval stage using Scanning Electron Microscope (SEM) technique. Samples of the moulted skeleton that were left behind after the ecdysis process were immediately collected and prepared for SEM. Three types of moulting failures were identified from the observation prosomatic failure, opishosomatic failure, and appendage failure. These failures were classified according to the position of the larvae body part where the exuviae were stuck at. Two out of three failures, prosomatic and opisthosomatic ecdysis failure would cause death instantly as the exuviae stuck at the vital organ, gills, and prevent it to function well in the breathing process. The micrograph obtained from the SEM study could be an important record for an evolutionary and growth study of this living fossil. It will also contribute to the understanding of the conservation effort for the species.

Keywords: Ecdysis failure; Larval stage; Asian horseshoe crab

1 Introduction

The horseshoe crab is also known as a living fossil due to their first era of existence more than 400 million years ago and maintained the features in this modern age. *Tachypleus gigas* is one of the four species which still exist in the world ocean and is also common in Malaysian water along with a smaller size species, *Carcinoscopius rotundicauda* [1]. Even though horseshoe crabs have been included in the International Union for Conservation of Nature (IUCN) list as nearly threatened species, the status of Malaysia and some other Asian countries is still unknown [2]. Thus, researchers are still struggling to compile good data and information to assure the protection of this invaluable living fossil from the increased activity of unsustainable catch [3] and nesting beach destruction [4,5].

DOI: 10.21467/abstracts.155 ISBN: 978-81-961472-9-7 (eBook)

The exoskeleton of arthropods provides protection and structural support but has the disadvantage of needing to be moulted for the animal to grow. Ecdysis is a moulting process of the exoskeleton and involves several stages that could take days or weeks to complete [6]. During the ecdysis, the old skeleton will be removed following enzymatic activity under the skin, leaving behind a new skeleton. It is the most crucial step in their life cycle because death is inevitable if it cannot be completed successfully [7]. Due to several factors, the ecdysis could fail to be completed.

In Malaysia, eating horseshoe crab was previously forbidden under Islamic law. After reviewing the law and discussing it with scientific experts, the Islamic authority changes the forbidden status into allowable [8].

SEM study on the exoskeleton of horseshoe crab was done as early as 1978 on the Atlantic species, *Limulus polyphemus* L [9]. The focus was on the different layers of cuticles which made up the exoskeleton. Observation on the development of the book gill of the larval stage was recently done for the same species *Limulus polyphemus* L [10]. Ecdysis in the Asian horseshoe crab is not well-known and the SEM study for the species is not known. The information about the failure during ecdysis which leads to larvae mortality is also very scarce. Thus, the aim of this study is to investigate the morphological features of the failed ecdysis through examination of the moulted exoskeleton using a scanning electron microscopic observation. The findings from this study will contribute more information for the evolutionary and growth study of this living fossil.

2 Materials and Methods

The field sampling activities were carried out in the full moon lunar cycle during a non-rainy season. The procedure for egg incubation and ecdysis experiment has been described in the recent report for the same project [11]. The exuviae then were fixed in 4% glutaraldehyde in 0.05M cacodylate buffer for 4 – 5 days and further prepared for the scanning electron microscopic study [10]. Any abnormalities and deformities that lead to moulting failure were recorded. A comparison was made to differentiate between deformed and normal exuviae. Deformities will be identified by the presence of abnormal morphology with no sign of damage namely; the broken and torn part that is caused by handling or ecdysis process.

3 Results

There are three types of moulting failures were observed from the SEM images (Figure 1). Prosomatic failure is indicated by the prosoma of the new instar being stuck in between the exuvia and the emergence becoming impossible (Figure 1 A). On the other hand, there is a possibility that the prosoma passed the exuviation suture but stuck at the opisthosoma. This is called opishosomatic failure (Figure 1 B). It is also possible for the prosoma and opisthosoma to successfully leave the exuviae, but not the appendages. The exuvia is still attached to the new instar ventrally where the appendages are located (Figure 1 C). The prosomatic and

ISBN: 978-81-961472-9-7
Series: AIJR Abstracts
DOI: 10.21467/abstracts.155

opisthosomatic ecdysis failure would cause instant death as the exuvia stuck at the vital organ, the book gills and prevent it to function well in the breathing process.

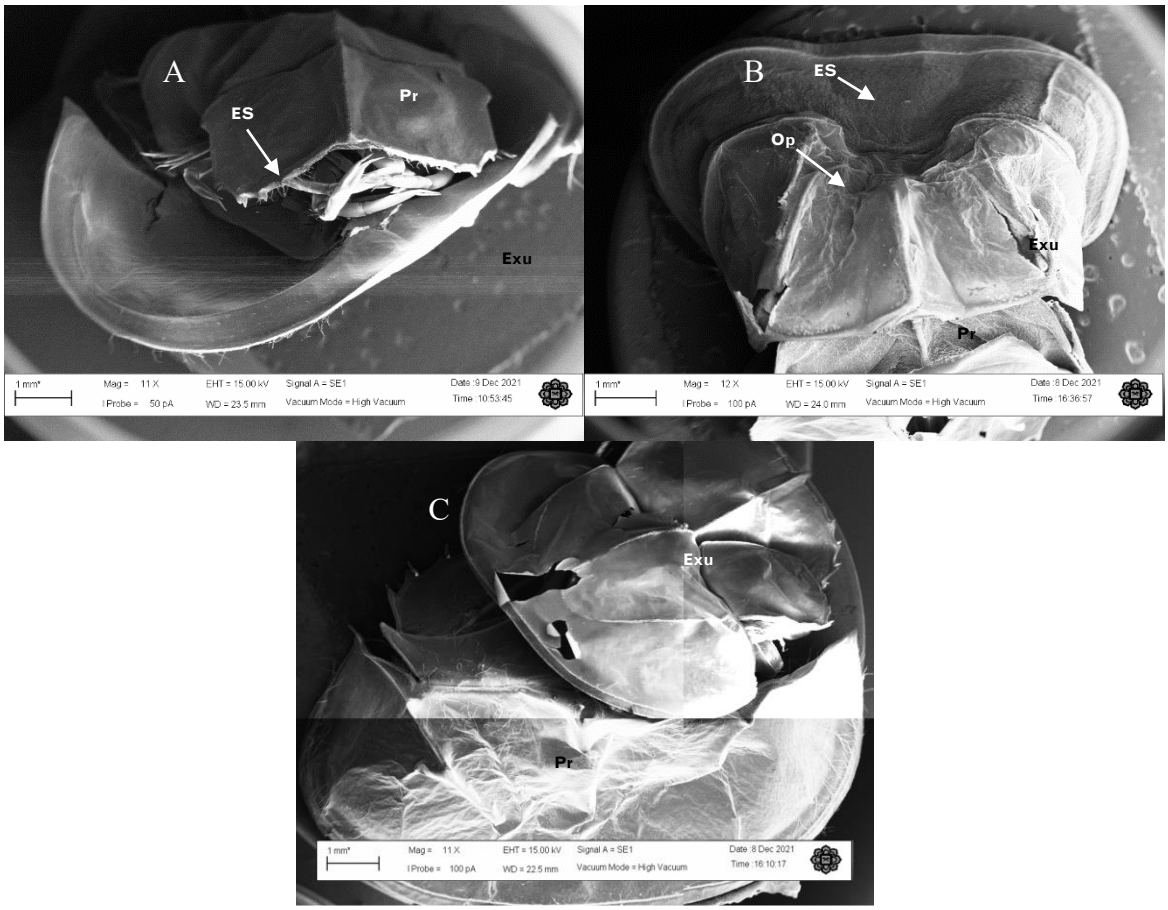

Figure 1: *Three types of moulting failures commonly happen during the ecdysis process. A. Prosomatic ecdysis failure. B. Opisthosomatic ecdysis failure. C. Appendages ecdysis failure. ES, exuviation suture; Op, opisthosoma; Exu, exuvia; Pr, prosoma.*

4 Discussion

The ecdysis failure at the prosoma, opisthosoma, and appendages stages was noted in the earlier report [11]. The ultrastructure of the parts involved in this failure was shown clearly through the SEM. Since the rupture for the new instar to emerge from the old exoskeleton is at the exuviation suture along the prosomal margin, the opening process is possibly controlled physiologically by chemicals produced in the process of ecdysis as shown in other types of crabs [7]. Further study is needed to relate the physiological aspect and mechanism of ecdysis in the larval stage of the horseshoe crab to give a comprehensive perspective and other related factors that influence success in the ecdysis process. This could help to minimize the percentage of failure thus increasing the new population.

Funding

This research was funded by FRGS, MINISTRY OF HIGHER EDUCATION MALAYSIA, grant number FRGS19–162–0771

ISBN: 978-81-961472-9-7

Series: AIJR Abstracts

DOI: 10.21467/abstracts.155

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ISBN: 978-81-961472-9-7

Series: AIJR Abstracts

DOI: 10.21467/abstracts.155