

# The Living Fossil (Horseshoe crab)

**Kamaruzzaman Yunus**

**Akbar John**

**Ahmed Jalal Khan Chowdhury**

**Zaleha Kassim**



**IIUM PRESS**

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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**Editors,**

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**IIUM Press**

Published by:  
IIUM Press  
International Islamic University Malaysia

First Edition, 2011  
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Perpustakaan Negara Malaysia

Cataloguing-in-Publication Data

Kamaruzzaman Yunus  
The Living Fossil (Horseshoe crab)  
Kamaruzzaman Yunus  
Include index  
Bibliography: p.  
ISBN

ISBN: 978-967-418-042-3

Member of Majlis Penerbitan Ilmiah Malaysia – MAPIM  
(Malaysian Scholarly Publishing Council)

Printed by :  
IIUM PRINTING SDN.BHD.  
No. 1, Jalan Industri Batu Caves 1/3  
Taman Perindustrian Batu Caves  
Batu Caves Centre Point  
68100 Batu Caves  
Selangor Darul Ehsan

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### Revision on the molecular phylogeny of horseshoe crabs – Part 1

<sup>1</sup>Akbar John, B., <sup>2</sup>Prasanna Kumar, <sup>1</sup>Kamaruzzaman, B.Y., <sup>1</sup>Jalal, K.C.A.

<sup>1</sup>*Institute of Oceanography and Maritime studies (INOCEM), Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200, Kuantan Pahang, Malaysia*

<sup>2</sup>*Centre of Advanced Studies in Marine Biology (CASMB), Annamalai University, Tamil Nadu, India*

#### Abstract

Present study was aimed to reinvestigate the existing molecular phylogeny of cryptic horseshoe crabs using universal barcode gene (cytochrome oxidase subunit 1). Phylogenetic trees were constructed using Distance matrix method: NJ and UPGMA. Genetic distance (GD) data analysis revealed the distant genetic relatedness of American horseshoe crab (*Limulus polyphemus*) with Asian conspecifics. More interestingly, the monophyletic origin of *Tachypleus gigas* and *Tachypleus tridentatus* was noted in constructed phylogram which other molecular markers failed to address.

**Key words:** molecular phylogeny, genetic conservation, horseshoe crabs, COI gene.

#### Introduction

The little morphological differentiation among horseshoe crab lineages has resulted in substantial controversy concerning the phylogenetic relationship among the extant species of horseshoe crabs, especially among the three species in the Indo-Pacific region. Earlier studies suggest that the three species constitute a phylogenetically irresolvable trichotomy (Xia, 2000). For elucidating their phylogenetic relationships, two proteins, coagulogen and hemocyanin, have been investigated (Shishikura *et al.*, 1982; Srimal *et al.*, 1985). Miyazaki *et al.* (1989) first investigated tropomyosin which is one of the major structural proteins involved in many types of cells, to elucidate prevailing phylogenetic relationships among horseshoe crabs and his result suggested that *L. polyphemus* is phylogenetically differentiated far from the three Asian species.