

Some Interesting News about the Understudied Mangrove Horseshoe Crab in Peninsular Malaysia



By

B. Satyanarayana¹, N. Fairuz-Fozi¹
N. Ashikin Mat Zauki¹, A.M. Kaben¹, A.M. Muslim¹,
B.R. Nelson², L. Triest³, F. Dahdouh-Guebas^{3,4}



¹ *Institute of Oceanography and Environment (INOS), UMT*

² *Institute of Tropical Biodiversity and Sustainable Development (IBTPL), UMT*



ULB

³ *Laboratory of Plant Biology and Nature Management (APNA), VUB*

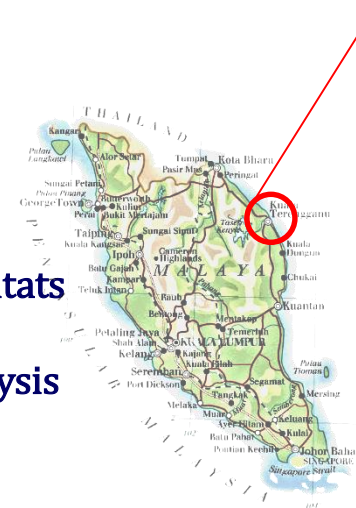
⁴ *Laboratory of Systems Ecology and Resource Management (SERM), ULB*

Profile

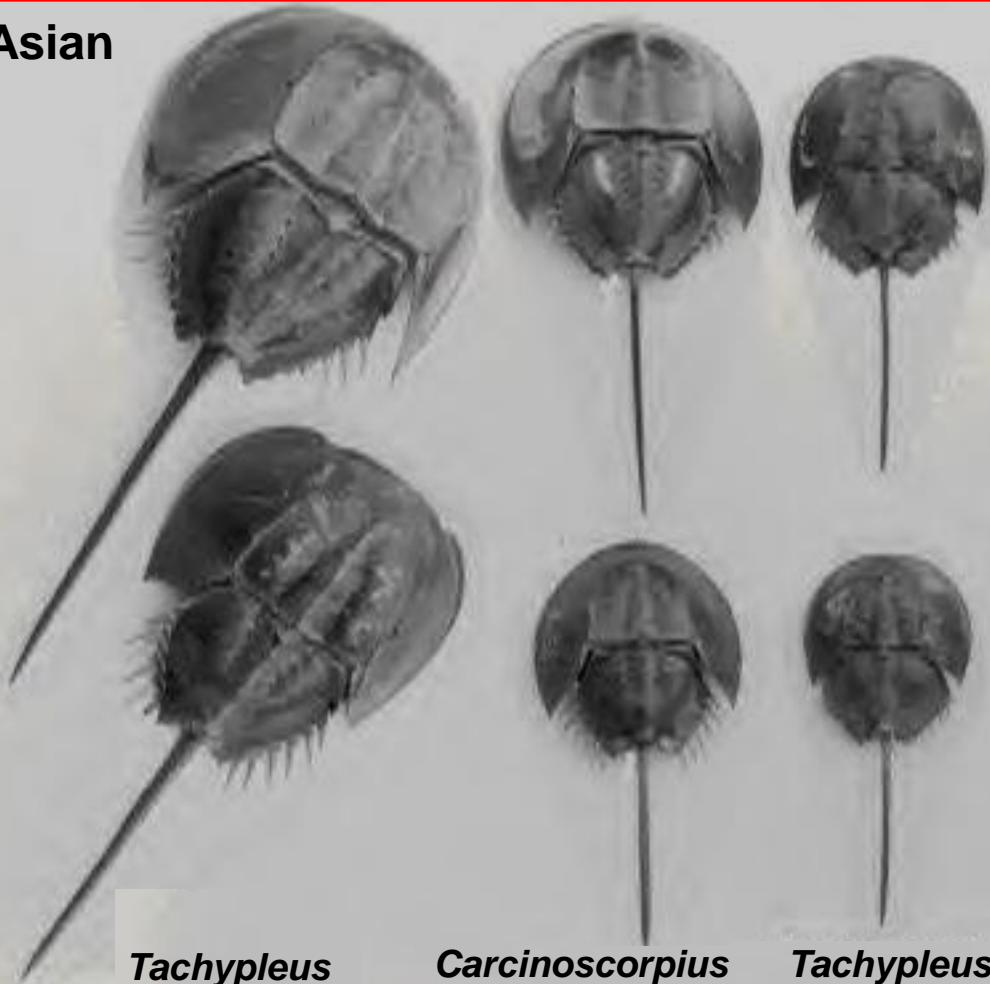
- Name** : Behara Satyanarayana (Satyam)
- Educational Qualifications** : MSc, PhD
- Current Position** : Associate Professor
- Organization** : Universiti Malaysia Terengganu (UMT), Malaysia (2013 - to present)
- Specialization** : Mangrove Ecology with Remote Sensing & GIS

- Research Interests**
- Ecosystem assessment (Flora & Fauna)
 - Spatio-temporal dynamics (RS & GIS)
 - Conservation and management

- Technical Expertise**
- Ground-inventory for wetland/coastal habitats
 - Air photos acquisition through UAVs
 - Remote sensing image processing and analysis
 - Univariate and multivariate data analyses
 - Species-environment relationship



Asian



Tachypleus tridentatus

Carcinoscorpius rotundicauda

Tachypleus gigas

American



Limulus polyphemus

Brockmann & Smith, 2009



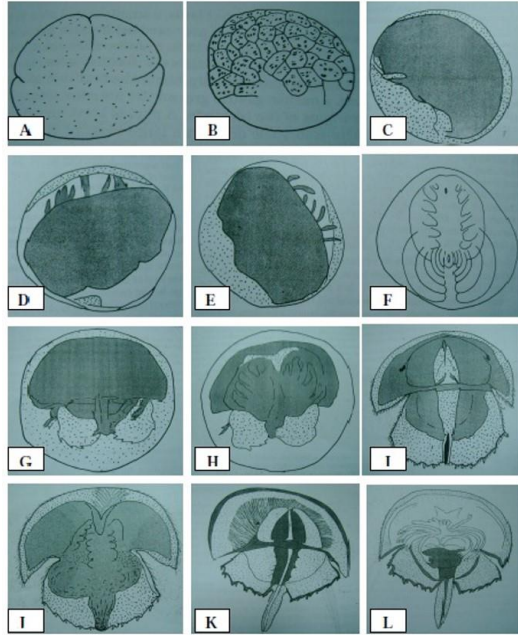
Early colonists in America called the horseshoe crabs as “king crabs”

Loveland, 2001
(Limulus in the Limelight, Kluwer Academic/Plenum Publishers)

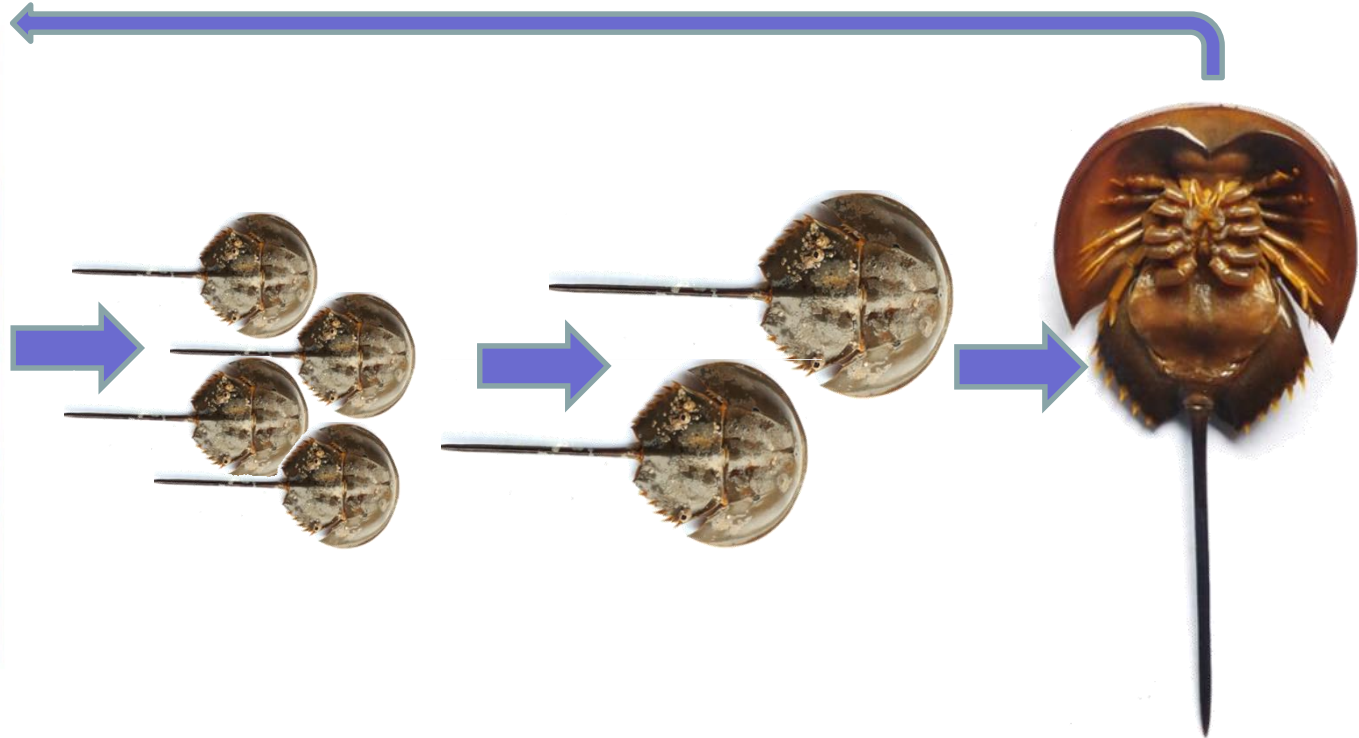
Scientist Dr. Anil Chatterji with a largest *Tachypleus gigas* collected from Malaysian waters
(Science Reporter 2013: 19-22)

Life history

Horseshoe crab is a long lived marine arthropod



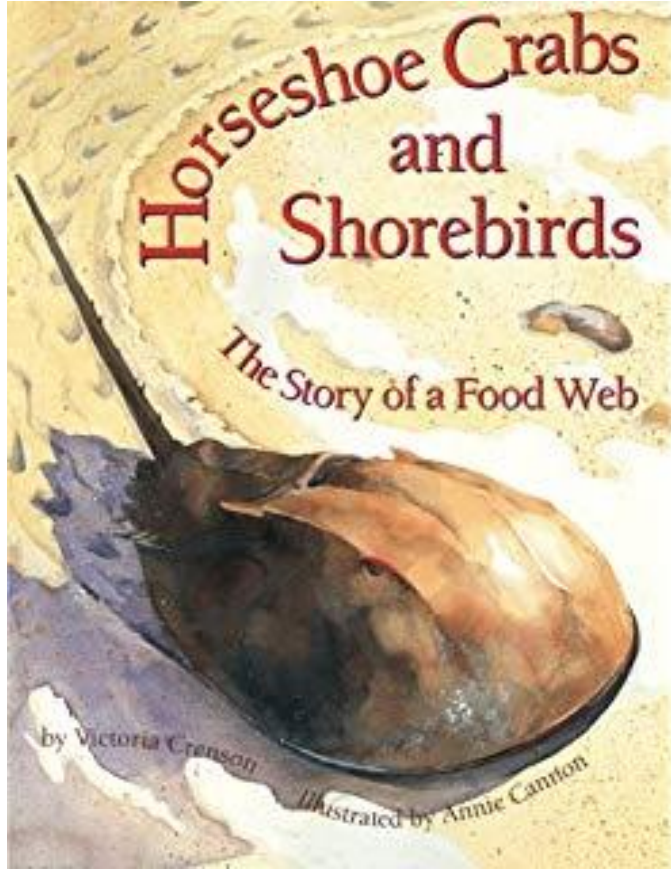
Chatterji, 1994



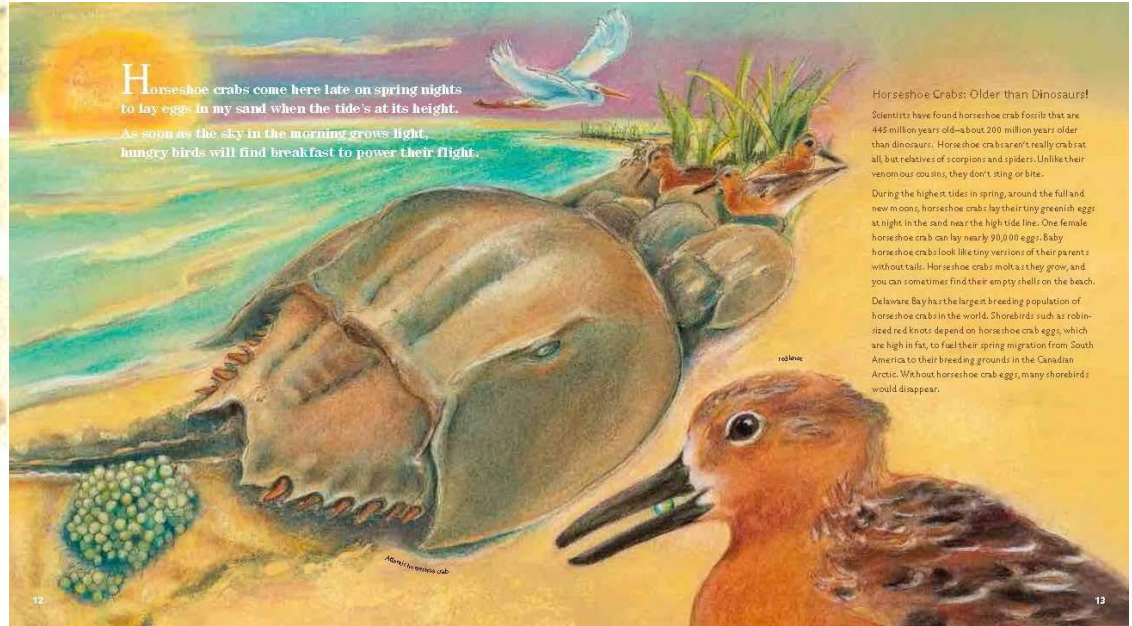
Embryogenesis: up to 45 days
(along the coastlines)

Juvenile to adult : 8-9 years
(in deep waters)

Adult : 8-10 years
(coast / deep sea)



<https://www.amazon.com/Fossil-Claire-Ewart/dp/0802737374>



<https://www.amazon.in/Secret-Tilbury-House-Nature-Book/dp/0884484335>

The coast of New Jersey in the vicinity of Delaware Bay is the site of the largest population of horseshoe crabs in the world

POWER OF THE HORSESHOE CRAB

TAL Kit Set To Change
Endotoxin Detection



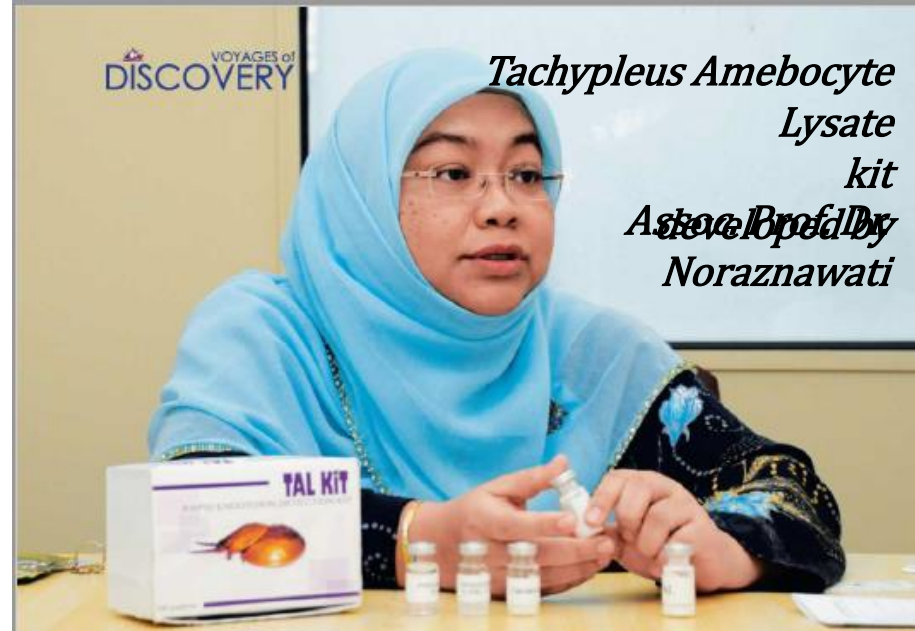
Tachypleus Amebocyte

Lysate

kit

Asst. Prof. Dr.

Noraznawati



The Horseshoe Crab is a 'living fossil' and the most endangered marine species, yet callously ignored. It has genes that could give clues to promoting human longevity, immunity from disease, regeneration of severed organs, and navigational tools in the darkest environment.

But, unfortunately, it has been left to bleed and die the most miserable death by pharmaceutical companies. It is left to the mercy of the beach predators that do not spare even its eggs. It is trapped in nylon nets and thrown dead in the high seas by unwary fisher folks. Horseshoe Crab is found only at Balasore in India's east coast of Odisha and Sunderbans in West Bengal.

The first fossil records of the Horseshoe crab date back to 425 million years, yet this creature still lives on along its chosen coasts. Its tail, which allows it to walk with ease across the



Horseshoe Crab

A Living Fossil, Seriously Endangered, Callously Neglected

IUCN Status

Old Red List website 2018-1

Names - common, scientific, regions etc...




Tachypleus tridentatus

CITATION
World Conservation Monitoring Centre 1996. *Tachypleus tridentatus*. *The IUCN Red List of Threatened Species* 1996: e.T21309A9267047. <http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T21309A9267047.en>. Downloaded on 28 October 2018.



NOT EVALUATED DATA DEFICIENT LEAST CONCERN NEAR THREATENED VULNERABLE ENDANGERED CRITICALLY ENDANGERED EXTINCT IN THE WILD EXTINCT
NE DD LC NT VU EN CR EW EX

Names - common, scientific, regions etc...



Tachypleus gigas


CITATION
World Conservation Monitoring Centre 1996. *Tachypleus gigas*. *The IUCN Red List of Threatened Species* 1996: e.T21308A9266907. <http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T21308A9266907.en>. Downloaded on 28 October 2018.

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DATA DEFICIENT
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Names - common, scientific, regions etc...



Carcinoscorpius rotundicauda

Back to search results

CITATION
World Conservation Monitoring Centre 1996. *Carcinoscorpius rotundicauda*. *The IUCN Red List of Threatened Species* 1996: e.T3856A10123044. <http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T3856A10123044.en>. Downloaded on 28 October 2018.

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Names - common, scientific, regions etc...



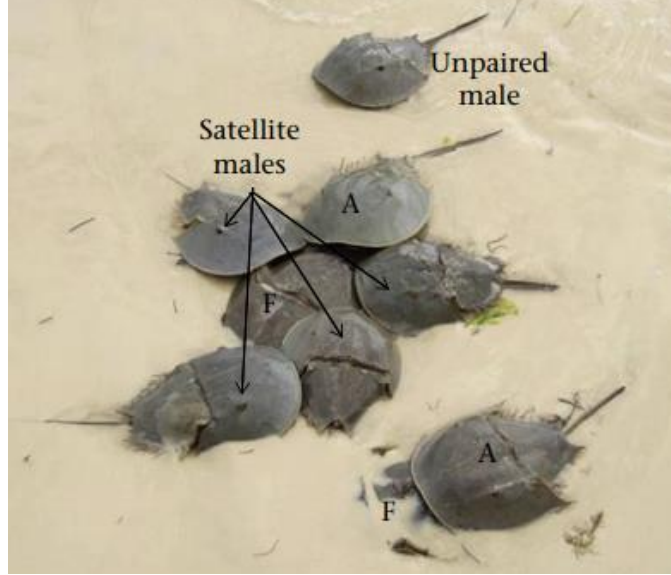
American Horseshoe Crab
Limulus polyphemus

CITATION
Smith, D.R., Beekey, M.A., Brockmann, H.J., King, T.L., Millard, M.J. & Zaldivar-Rae, J.A. 2016. *Limulus polyphemus*. *The IUCN Red List of Threatened Species* 2016: e.T11987A80159830. <http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T11987A80159830.en>. Downloaded on 28 October 2018.

NOT EVALUATED DATA DEFICIENT LEAST CONCERN NEAR THREATENED **< VULNERABLE >** ENDANGERED CRITICALLY ENDANGERED EXTINCT IN THE WILD EXTINCT
NE DD LC NT VU EN CR EW EX

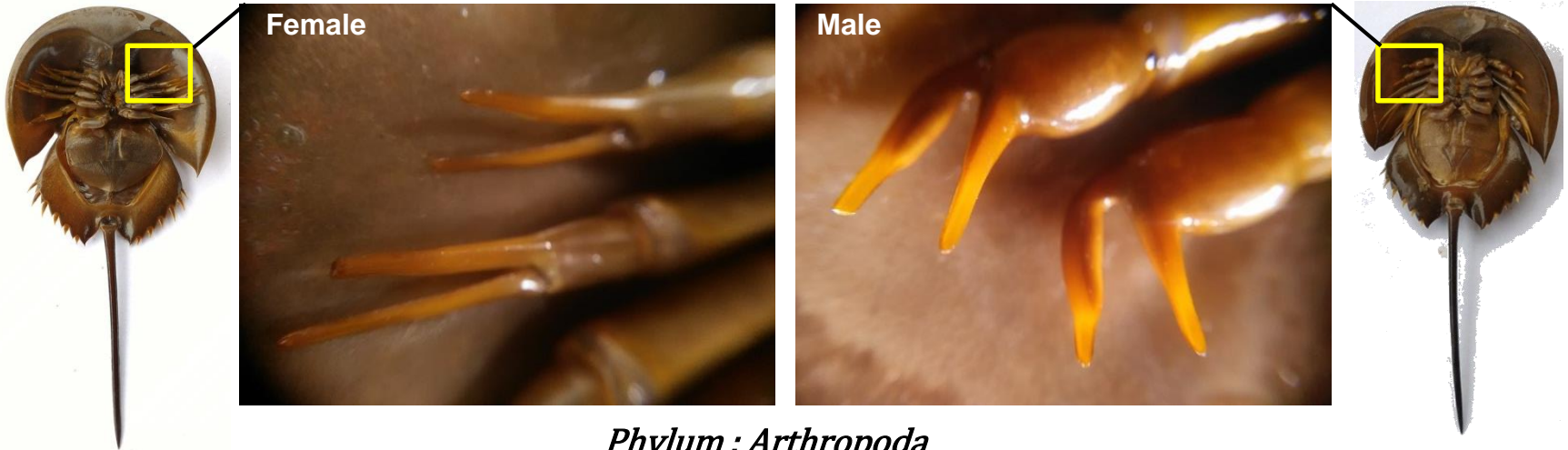


< VULNERABLE >
VU



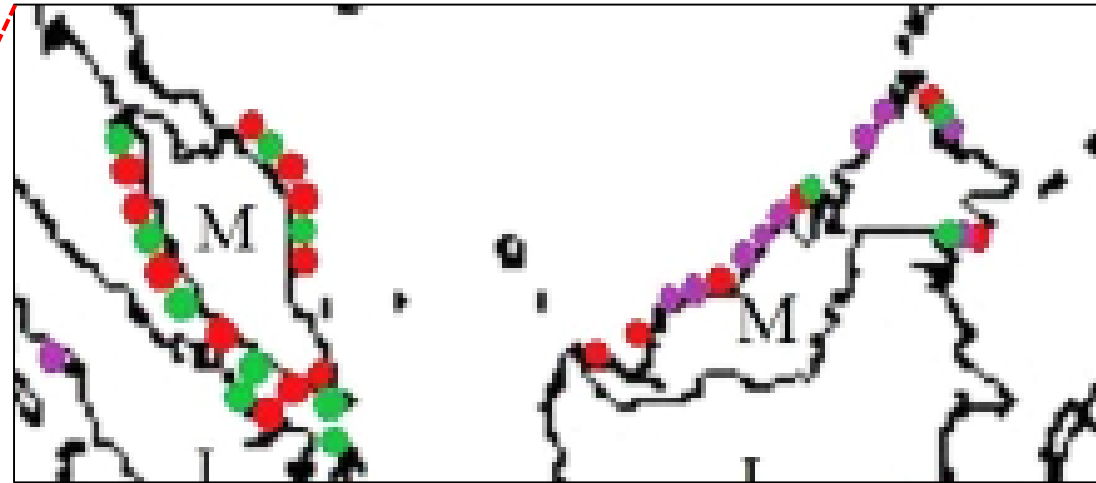
Brockmann et al., 2018
(Animal Behaviour 143: 177-191)

- ❖ Besides the similarities in living / nesting behaviour close to beach and estuarine habitats, only *C. rotundicauda* is known as mangrove horseshoe carb.



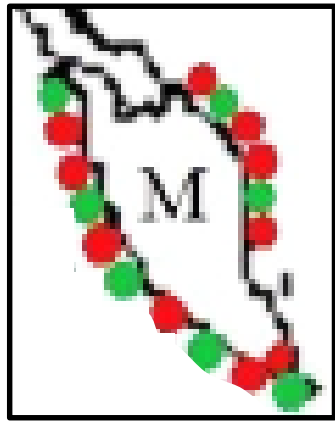
Phylum : Arthropoda
Class : Merostomata
Order : Xiphosura
Family : Limulidae
Genus : Carcinoscorpius
Species : rotundicauda

Geographic distribution of the Asian horseshoe crabs



- *Carcinoscorpius rotundicauda*
- *Tachypleus gigas*
- *Tachypleus tridentatus*

Scientific concerns



- ❖ Population status and spawning behaviour of the horseshoe crabs remain unclear for several locations, to judge its role as nesting or feeding ground.

● *Carcinoscorpius rotundicauda*
● *Tachypleus gigas*

- ❖ Physical infrastructure developments along the coast are posing a serious threat to the horseshoe crab population.

- ❖ For *C. rotundicauda*, the loss of mangrove has been a severe problem.



Wave breaker and parking lots



Discarded fishing nets



Biomedical research



Exotic delicacy



**No
edible
preference
for
*C. rotundicauda***



❖ **Monkeys are fond of eating these crabs**

❖ **Ecotourism attraction**



Knowledge-gaps



ELSEVIER

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco



The Malay Peninsula as a barrier to gene flow in an Asian horseshoe crab species, *Carcinoscorpius rotundicauda* Latreille

A.B. Adibah, W.L. Ng, S.G. Tan*

Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Selangor, Malaysia



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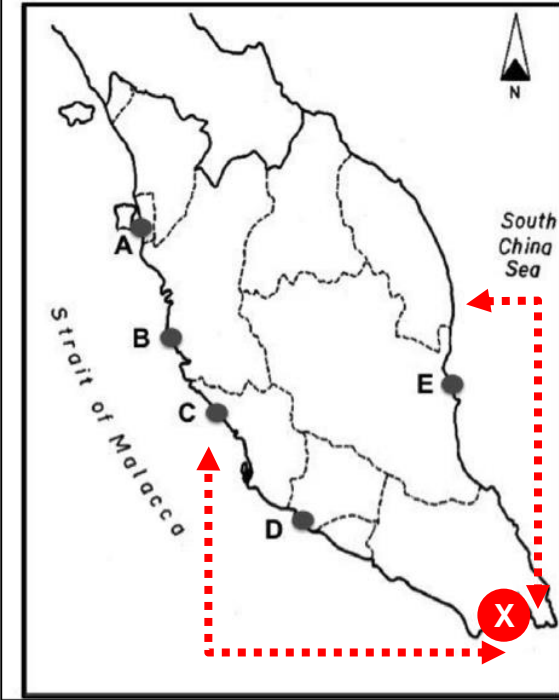
Available online 15 May 2015

Keywords:

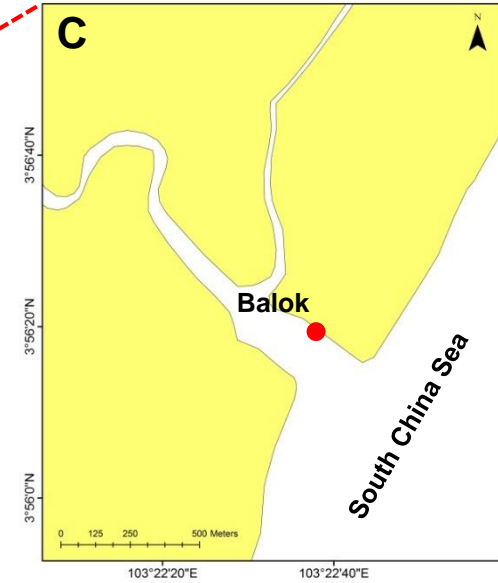
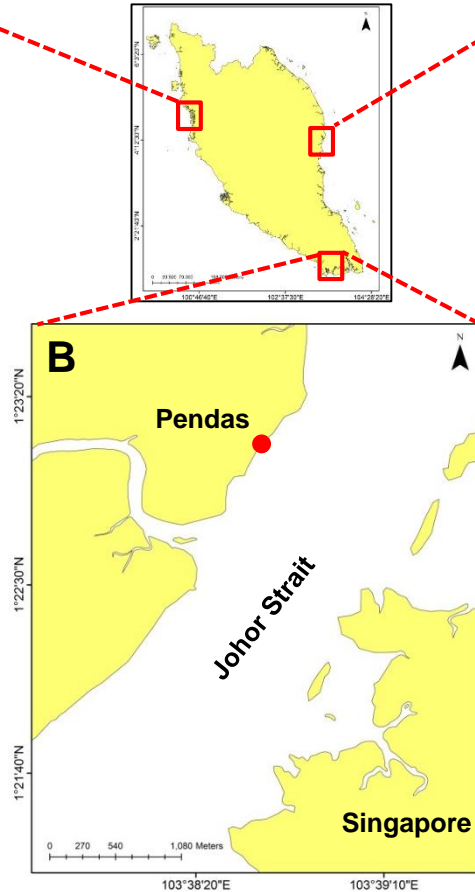
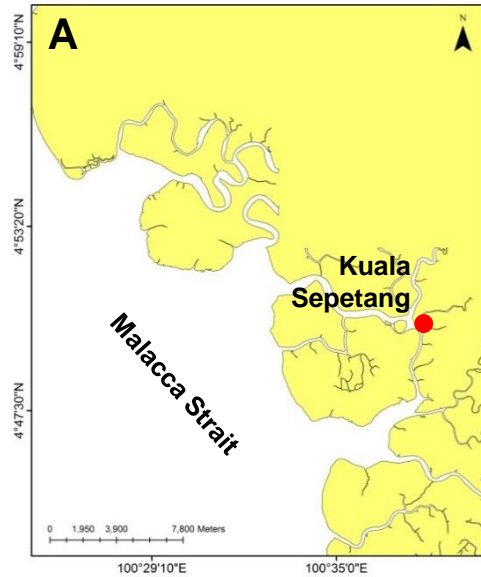
Population structure

ABSTRACT

Horseshoe crabs are marine arthropods that are amongst the oldest living creatures that still exist today. Among the four extant species of horseshoe crabs, *Carcinoscorpius rotundicauda* differs from the other species by having poisonous eggs and lays its eggs in sandy-mud areas near river mouths. With the rapid development of coastal areas worldwide, *C. rotundicauda* habitats are decreasing. Until now, however, there has not been any study on the species' genetic variation. Simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) markers were employed to study the genetic variation in



Study areas



❖ Chosen at least 3 sampling sites from each area

❖ Investigation: Jan 2016 - Jan 2017, every month during full moon and new moon

Sampling protocol



- ❖ Gill net (200 m length \times 3 m height with a mesh size of 12.7 cm) was used to collect the spawning crabs.

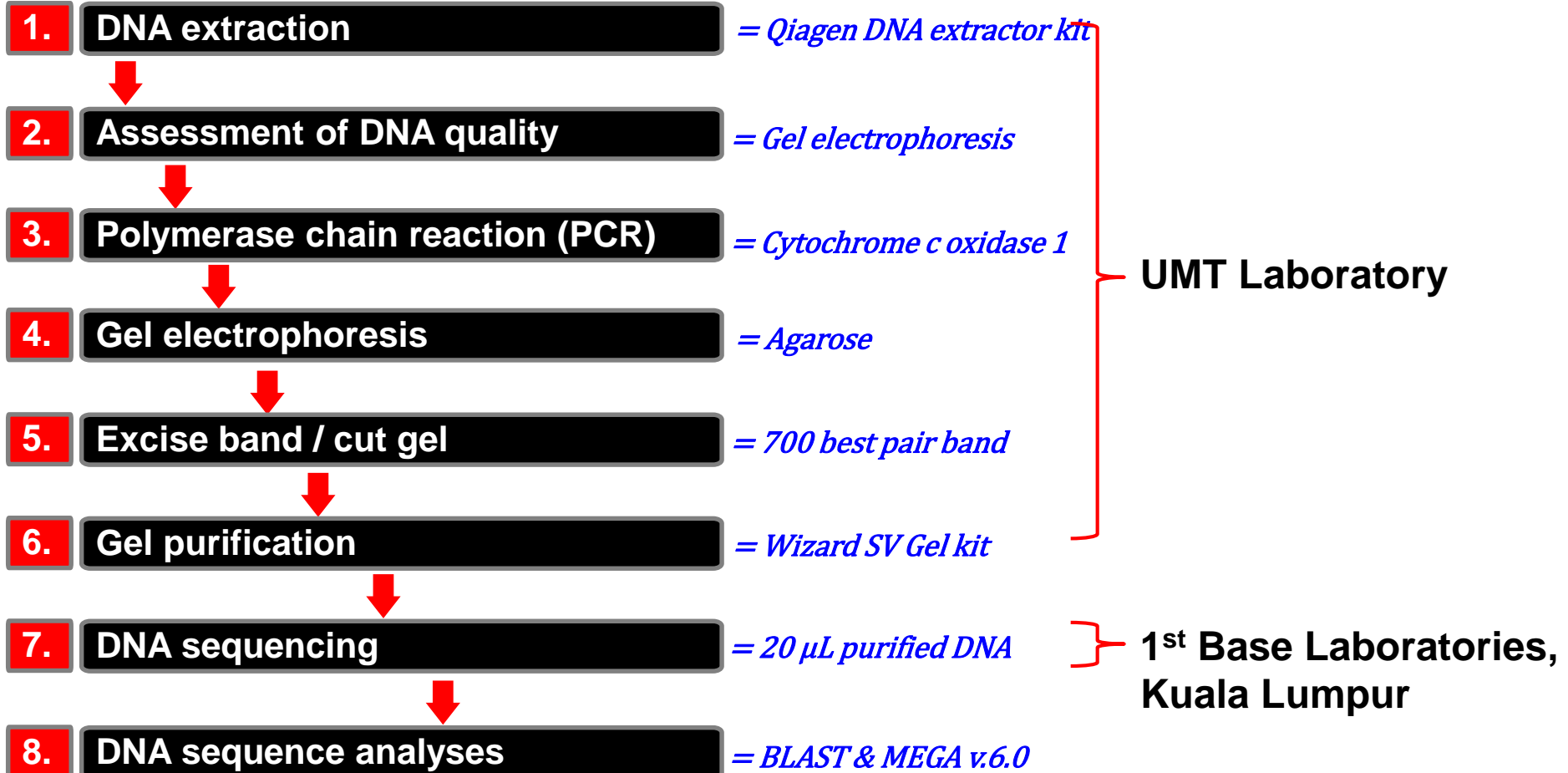


- ❖ Surface sediment in the intertidal area was gently excavated to find nests/eggs of *C. rotundicauda*.



- ❖ Freshly deposited eggs (6-8 nos.), and female crab's blood (0.5 ml) were collected.
- ❖ Due to no observed nesting activity at Perak, only blood samples - from the crabs found as bycatch by local fishermen, were considered.
- ❖ In total, 186 egg samples and 162 blood samples were tested.

Gene isolation & analyses



CO1 for DNA barcoding

- ❖ **Mitochondrial gene, cytochrome c oxidase subunit 1 (CO1) able to -**
 - ✓ ***discriminate the closely allied species***
 - ✓ ***possess a greater range of phylogenetic signal than other genes***
 - ✓ ***identify distinct phylogeographic groups within a species***

Hebert et al. (2003)

NCBI - GenBank®

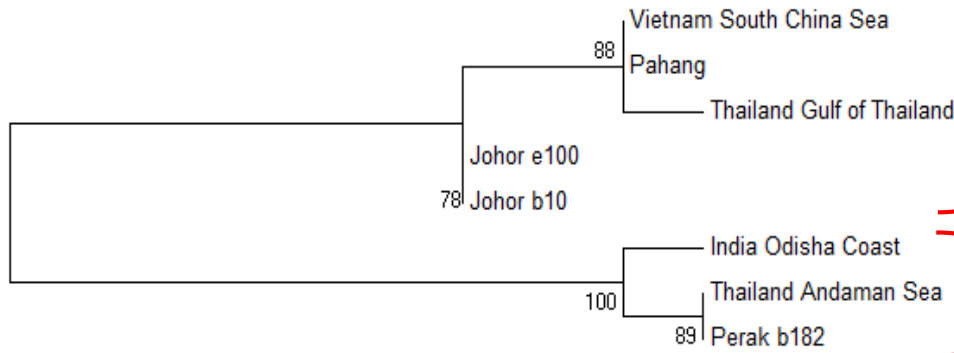
	Code	Region	Country	GPS	Collection date	Accession number
1.	INDEC1	Odisha (Bay of Bengal)	India	N: 21°07'00.00" E: 87°04'00.00"	30 Sep 2015	KM350551
2.	CR_T5_3F	Andaman Sea	Phuket, Thailand	N: 7°56'53.74" E: 98°29'43.31"	25 Jul 2016	HQ588754
3.	CR_T3_2F	Gulf of Thailand	Bang Pu, Thailand	N: 6°54'43.28" E: 101°16'32.49"	25 Jul 2016	HQ588750
4.	CR_V1_	South China Sea	Bac Lieu, Vietnam	N: 9°22'37.30" E: 106°13'43.70"	25 Jul 2016	HQ588746
5.	AKB124	Sungai Pahang Tua	Pahang, Malaysia	N: 3°36'09.40" E: 103°23'57.10"	25 Jul 2016	JF896106
6.	Pahang	Balok	Pahang, Malaysia	N: 3°36'09.40" E: 103°23'57.10"	2016	MF469060
7.	Perak_b20	Kuala Sepetang	Perak, Malaysia	N: 4°50'38.36" E: 101°15'38.36"	2016	MF469061

Present study

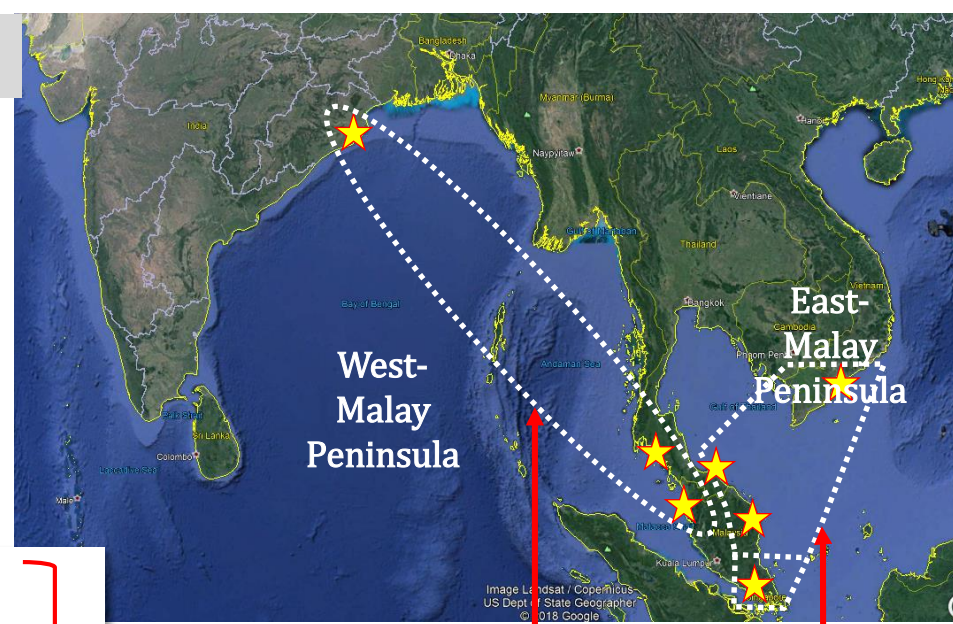


Phylogenetic tree

Maximum likelihood tree
based on
CO1 sequence
(levels of statistical support are based on 1000
bootstrap replicates)

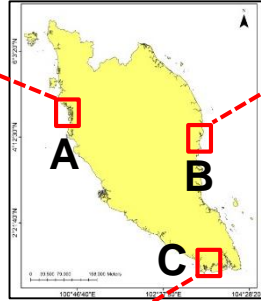


0.005



Two major clades

Sediment matters for nesting



	A - MMFR	B - Pahang	C - Johor
Gravel (%)	0	0.85	3.6
Sand (%)	26.8	93.60	94.3
Silt & Clay (%)	73.2	5.55	2.1

Matang Mangrove Forest Reserve - as Feeding ground



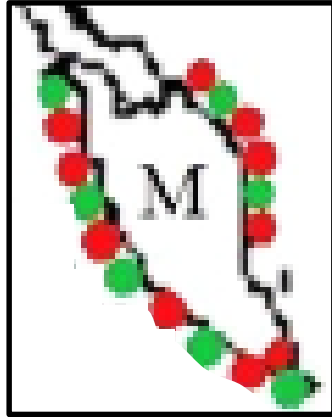
- ❖ Mangrove area: 40,288 ha
- ❖ Length: ~51 km along the coastline
- ❖ Under the silvicultural management since 1902
- ❖ Production of mangrove poles and charcoal

Conclusions



- ❖ Life history/habitat preference of the horseshoe crabs show a restricted dispersal along shorelines.
- ❖ Two distinct populations of *C. rotundicauda* were observed along the West- (up to India) and East- (up to Vietnam) Malay Peninsula.
- ❖ Despite the ancestral mix of crabs on the east coast of P. Malaysia, the lower phylogenetic support at Johor indicates an isolated population.
- ❖ Matang Mangrove Forest Reserve on the west coast of P. Malaysia is an active feeding ground for *C. rotundicauda*.
- ❖ P. Malaysia is acting as a land barrier and responsible for a low gene flow for the horseshoe crabs.

Still to ...



- *Carcinoscorpius rotundicauda*
- *Tachypleus gigas*



- ❖ Distinguish nesting and feeding grounds of *C. rotundicauda*
- ❖ Create awareness on the importance of horseshoe crabs
- ❖ Educate local fishermen to release the entangled crabs into water
- ❖ Include horseshoe crabs into the Wildlife Conservation Act of Malaysia



Thank you

Extra Information

Gene isolation & analyses

1. DNA extraction

= *Qiagen DNA extractor kit*

2. Assessment of DNA quality

= *Gel electrophoresis*

3. Polymerase chain reaction (PCR)

= *Cytochrome c oxidase 1*

4. Gel electrophoresis

= *Agarose*

5. Excise band / cut gel

= *700 best pair band*

6. Gel purification

= *Wizard SV Gel kit*

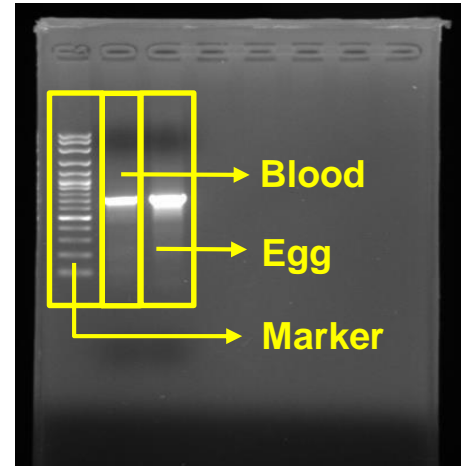
7. DNA sequencing

= *20 μ L purified DNA*

8. DNA sequence analyses

= *BLAST & MEGA v.6.0*

Central Laboratory,
UMT



1st Base Laboratories,
Kuala Lumpur

Sample size

	Eggs	Blood	Total
Perak	-	6	6
Johor	162	64	226
Pahang	24	92	116
	186	162	