



# Conservation Status of Globally Testudines River Terrapins Based on COI Mitochondrial Markers

Mohd Hairul Mohd Salleh <sup>1,2</sup>, and Yuzine Esa <sup>1,3</sup>, \*

<sup>1</sup> Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia;

<sup>2</sup> Royal Malaysian Customs Department, Persiaran Perdana, Presint 2, 62596 Putrajaya, Malaysia;

<sup>3</sup> International Institute of Aquaculture and Aquatic Sciences, Universiti Putra Malaysia, Lot 960 Jalan Kemang 6, 71050 Port Dickson, Negeri Sembilan, Malaysia;

\*Correspondence: yuzine@upm.edu.my; Tel.: +60397694933

## ABSTRACT

Terrapins inhabit brackish water and coastal salt marshes. To date, 12 species of terrapin have been reported worldwide. The present study aims to determine the global utility of terrapin DNA barcoding using novel COI sequences and compare them to other COI sequences previously published in BOLD systems and GenBank. A total of 26 COI sequences of worldwide terrapins were assessed in this study, including four COI sequences generated from field sampling on the east and west coasts of Peninsular Malaysia. The assessment of the COI sequences with the UPGMA tree identified three families, with 33% of terrapins being classified as least concern (LC) and 25% of them being classified as critically endangered (CR). In this study, we looked at the genome and bioinformatics of terrapins, which could be used as a starting point for future research on terrapin species all

## OBJECTIVE

To define the global utility of terrapin DNA barcoding via novel COI sequences and compare them to other COI sequences earlier available in BOLD systems and GenBank.

## MATERIALS AND METHODS

- There were four *B. affinis* individuals in this study who came from two different population regions that crossed the east and west coasts of the Malaysia Peninsula (Figure 1).
- Venipuncture techniques were used to draw 1.5ml blood from each individual through the subcarapacial venous plexus (SVP) and another through the internal jugular vein.
- The research and field permit approval number is B-00335-16-20, issued by the Department of Wildlife and Parks, peninsular Malaysia.
- We use the COI Tuntong set primers 5'-CGCGGAATTAAGCCAACCAG-3' (forward) and 5'-TTGGTACAG-GATTGGGTCGC-3' (reverse) (Guntoro and Riyanto, 2020).
- 17 sequences were downloaded from GenBank, and five were mined from BOLD Systems, yielding 26 sequences for this work.
- MEGAX (Kumar et al., 2018) was used to align the sequences that were made and those that were found.
- Phylogenetic analysis was done using MEGAX with 1000 bootstrap replicates and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as the best method (Sneath and Sokal 1973).

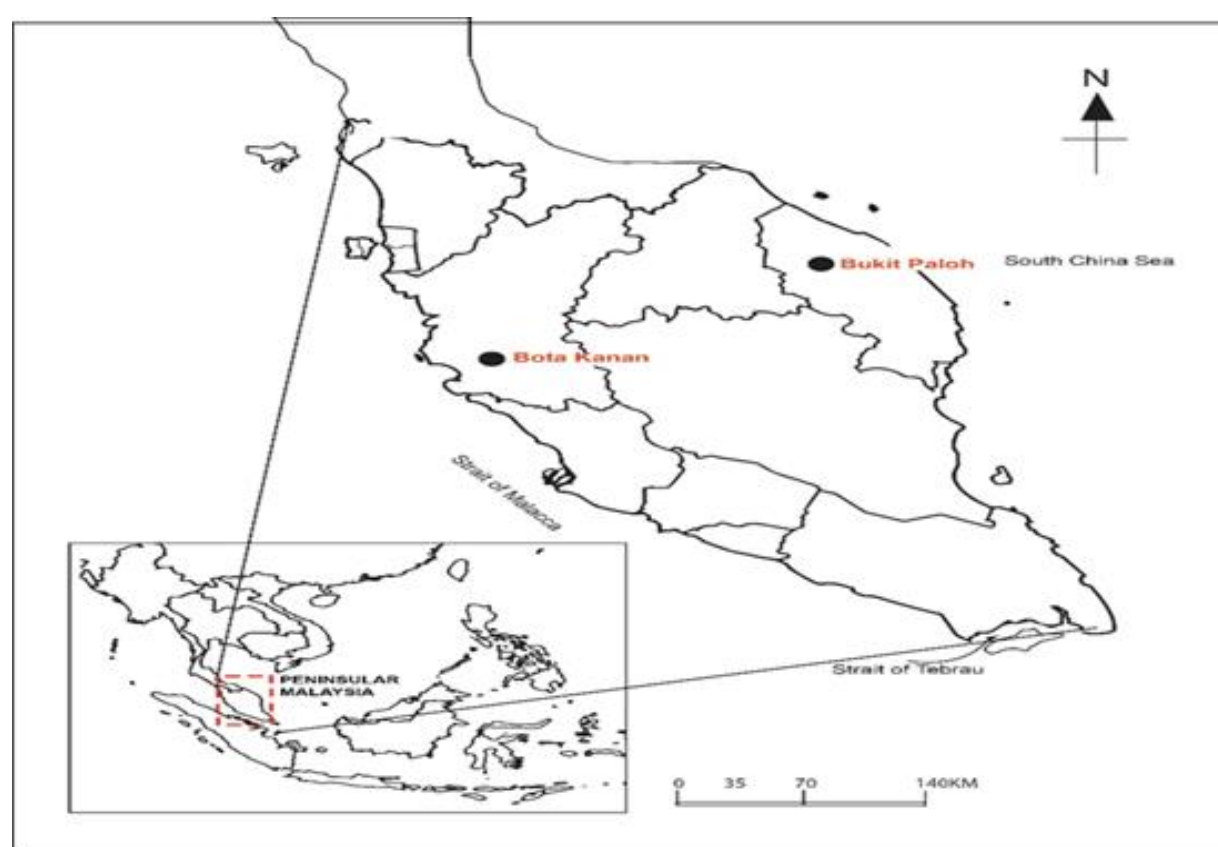


Figure 1. Sampling sites of *Batagur affinis* in Peninsular Malaysia.

Table 1. List of terrapins species studied through DNA barcoding with the BOLD IDs of their respective COI sequences and the GenBank accession of each species.

Scientific Name	English Name	GenBank Accession	BOLD ID	IUCN Red List	Locality
<i>Batagur baska</i>	Northern river terrapin	KF894752	GBGCR2852-19	CR	India
<i>Batagur baska</i>	Northern river terrapin	HQ329671	GBGCR2716-19	CR	India
<i>Batagur borneoensis</i>	Painted terrapin	HQ329672	GBGCR2717-19	CR	Indonesia
<i>Batagur borneoensis</i>	Painted terrapin	None	BENT109-08	CR	Indonesia
<i>Morenia ocellata</i>	Bengal eyed terrapin	HQ329690	GBGCR2724-19	EN	Myanmar
<i>Morenia ocellata</i>	Bengal eyed terrapin	None	BENT264-09	EN	Myanmar
<i>Malaclemys terrapin</i>	Diamondback terrapin	HQ329654	GBGC11262-13	VU	America
<i>Malaclemys terrapin</i>	Diamondback terrapin	KX559038	GBGCR2938-19	VU	America
<i>Emys orbicularis</i>	European pond terrapin	HQ329643	GBGC11273-13	NT	Unknown
<i>Emys orbicularis</i>	European pond terrapin	KP697925	None	NT	Germany
<i>Melanochelys trijuga</i>	Indian pond terrapin	KC354725	GBGC11418-13	LC	India
<i>Melanochelys trijuga</i>	Indian pond terrapin	KC354724	GBGC11419-13	LC	India
<i>Rhinoclemmys rubida</i>	Mexican spotted terrapin	HQ329701	GBGCR2766-19	NT	Mexico
<i>Trachemys scripta elegans</i>	Red-eared terrapin	KX559044	GBGCR1038-18	LC	America
<i>Trachemys scripta elegans</i>	Red-eared terrapin	KM216748	GBGCR1008-15	LC	America
<i>Pelusios sinuatus</i>	Serrated hinged terrapin	None	BENT174-08	LC	Southern Africa
<i>Pelusios sinuatus</i>	Serrated hinged terrapin	HQ329735	GBGC11221-13	LC	Southern Africa
<i>Siebenrockiella crassicolis</i>	Smiling terrapin	HQ329704	GBGCR2769-19	EN	Unknown
<i>Siebenrockiella crassicolis</i>	Smiling terrapin	None	BENT190-08	EN	Unknown
<i>Mauremys caspica</i>	Striped-neck terrapin	AY337348	GBGC0806-06	LC	Iran
<i>Mauremys caspica</i>	Striped-neck terrapin	AY337347	GBGC0805-06	LC	Bahrain
<i>Batagur affinis</i>	Southern river terrapin	None	MTD042-21	CR	Malaysia
<i>Batagur affinis affinis</i>	Southern river terrapin	OL658844	HYT001-21	CR	Malaysia
<i>Batagur affinis edwardmollii</i>	Southern river terrapin	OL658845	HYT002-21	CR	Malaysia
<i>Batagur affinis edwardmollii</i>	Southern river terrapin	OL658846	HYT003-21	CR	Malaysia
<i>Batagur affinis edwardmollii</i>	Southern river terrapin	OL658847	HYT004-21	CR	Malaysia

## RESULTS AND DISCUSSION

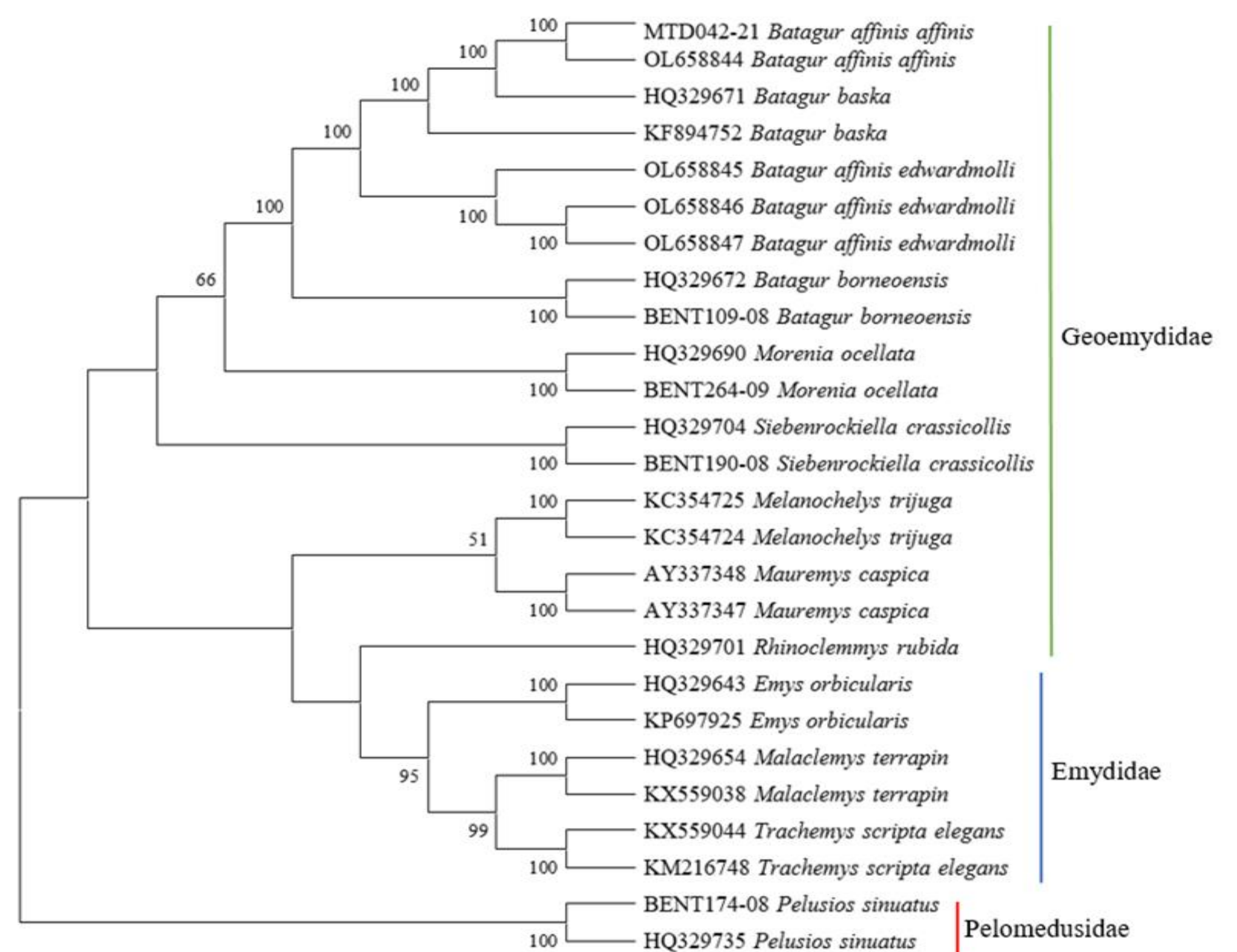


Figure 2. UPGMA tree constructed with MEGAX based on COI sequences belonging to order Testudines.

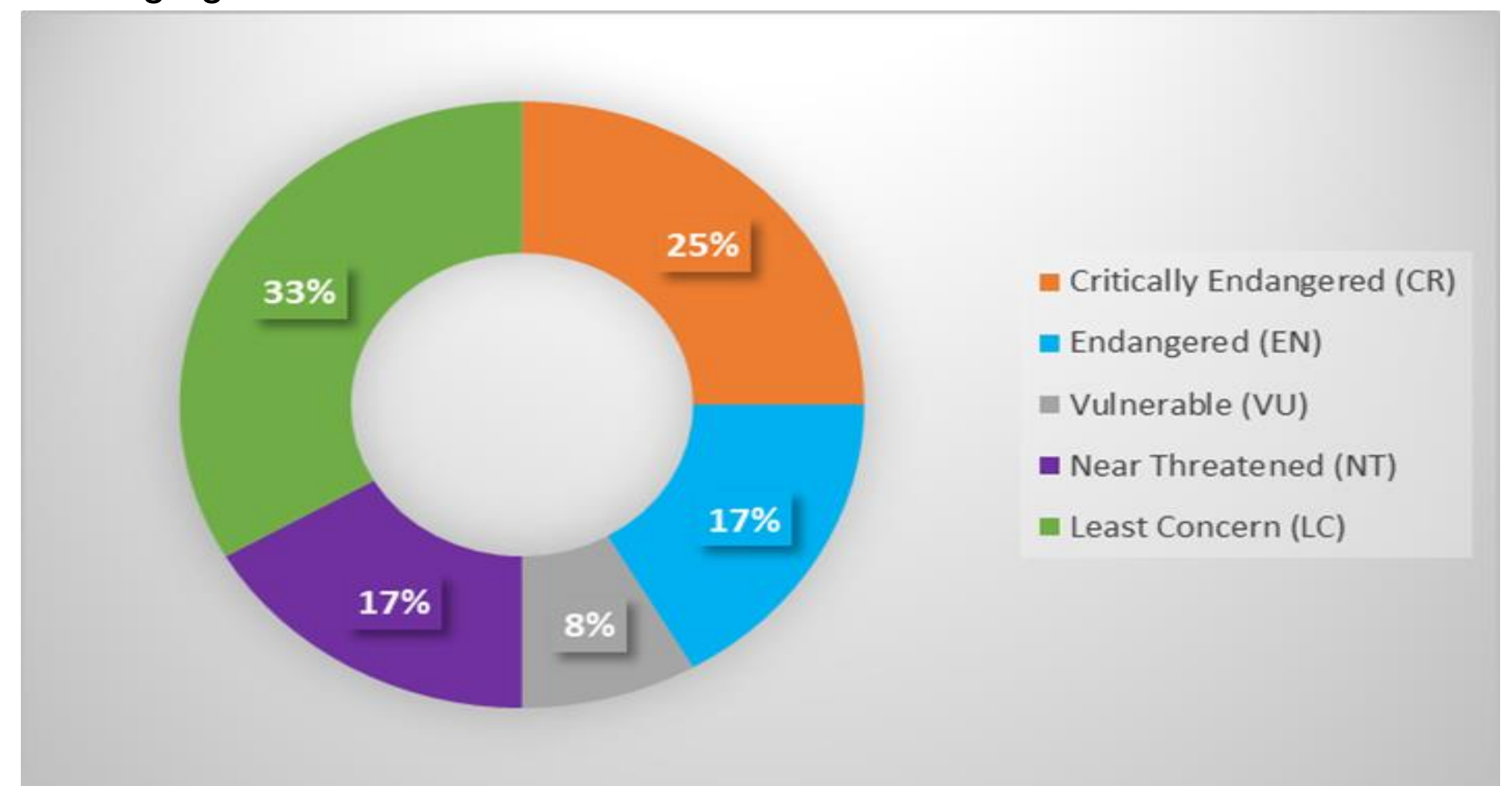


Figure 3. The conservation status of the terrapins is based on the IUCN Red List.

## CONCLUSION

In conclusion, the COI marker continues to be an effective barcode marker for terrapin species, providing vital evidence that may be utilised to distinguish and recognise genera and species of these Testudines organisms. The genomic and bioinformatics analyses of terrapins reported here may serve as a foundation for future research on this species throughout the world, allowing for more practical conservation work for this threatened species.

## REFERENCES

- ❖ Guntoro, J., & Riyanto, A. (2020). The very low genetic variability on Aceh Tamiang's (Indonesia) population of Painted Terrapin (*Batagur borneoensis*) inferred by cytochrome oxidase I (CO I) and D-loop (control region). *Biodiversitas Journal of Biological Diversity*, 21(6).
- ❖ Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, 35(6), 1547.
- ❖ Sneath, P.H.A. and Sokal, R.R. (1973). *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. WF Freeman & Co., San Francisco; 573 p.

## ACKNOWLEDGEMENTS

We want to acknowledge the Universiti Putra Malaysia for funding my study through a Graduate Research Fellowship (GRF). In addition, we would also like to express our gratitude to the Universiti Putra Malaysia Research Committee for their assistance and support of this research project. Furthermore, we thank the Turtle Conservation Society of Malaysia and the Department of Wildlife and National Parks, Peninsular Malaysia, for their collaboration.