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# Geographic structure of masked stingray in the Indo-Malay-Papua archipelago

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The blue-spotted or masked stingray, *Neotrygon kuhlii* (Myliobatiformes: Dasyatidae), is one of the most common elasmobranch species from the Indo-Malay-Papuan waters, where it occurs in reef-associated habitats. For rationally managed exploitation, it is important to acquire fundamental knowledge of its stock structure.

Another issue is the origin of the high marine biodiversity in the Indo-Malay-Papuan archipelago at the core of the Coral Triangle, which is the global epicentre for marine biodiversity (P.H. Barber, J. Biogeogr. 2009). Does speciation occur within the Coral Triangle?

We used the mitochondrial DNA marker to investigate (1) the population geographic structure and (2) the phylogeography of *Neotrygon kuhlii* in the central Indo-West Pacific seas.

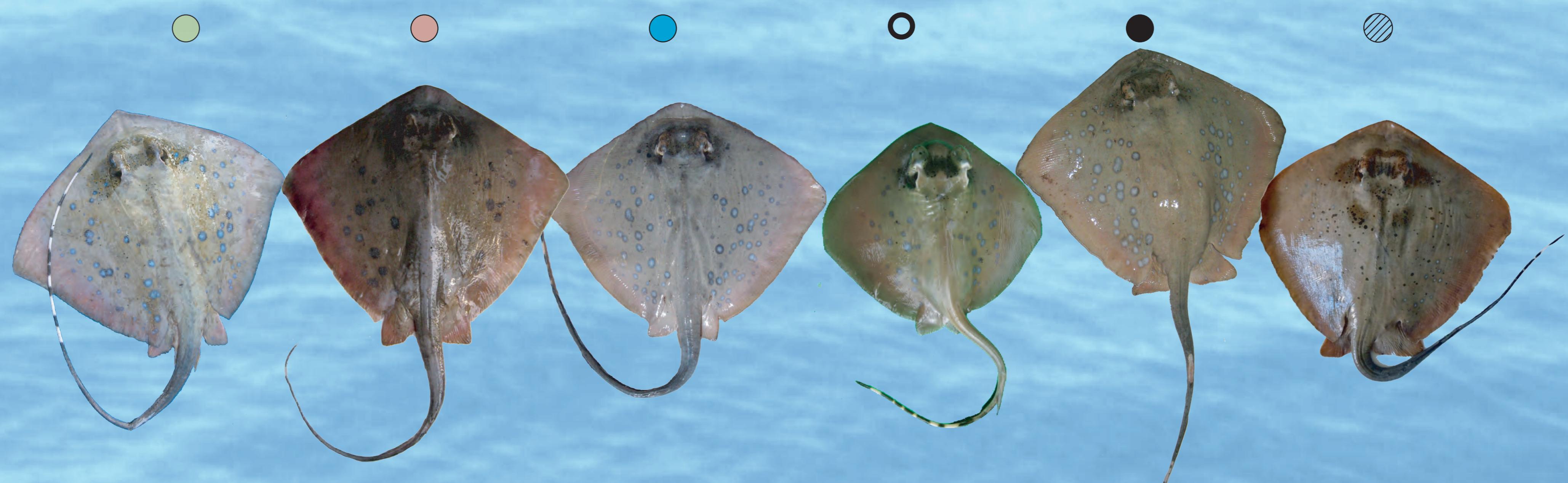
## Methods

*Neotrygon kuhlii* specimens were collected from 21 local fish-landing places throughout Indonesia, in West Papua, and in New Caledonia. Tissue samples of a total  $N = 102$  individuals were collected. The nucleotide sequences of a fragment (611 bp) of the COX-I gene were produced. Homologous sequences from GenBank (*Neotrygon kuhlii* from Java, Bali, Taiwan, Japan and Queensland) were added to the dataset. A neighbor-joining tree of haplotypes (Kimura-2 parameter distances) was produced.

## Results

Six deeply divergent lineages were observed. Separate lineages corresponded to separate geographic regions, with no, or very little, overlap. Further geographic partition was evident, with some populations or groups of populations within a region being characterized by private haplogroups.

The topology of the tree suggests that the ancestral form that gave rise to the present-day *Neotrygon kuhlii* populations was in the West Pacific rather than the Coral Triangle. Bootstrap scores were not sufficient yet to provide a watertight support to this hypothesis, which makes increasing the length of the assayed marker our priority.



## Conclusions

Unexpectedly strong geographic composition was uncovered in *Neotrygon kuhlii*. It is sensible to assume that the shallow-benthic habits and the lack of egg and juvenile dispersal enhance geographic isolation in this species.

It has been argued that deeply divergent mitochondrial lineages in *Neotrygon kuhlii* may indicate cryptic species (R.D. Ward et al., Mar. Freshw. Res. 2008). Testing that hypothesis requires the use of nuclear-DNA markers on adequately chosen samples.

**Table S1.** Variable nucleotide sites in *Neotrygon kuhlii* haplotypes (*COI* gene fragment, 519 bp). Asterisks indicate sequences that were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/>).

Location	Nucleotidesite no.
	1111111111111111111122222222222222222222233333333333333344444444444555 112222233556678890112223334445566788900112223334445566889900223345556778802345567899001 9691346703456727835171392584573625505920436269157349382472857037780321243351420253927725259
Zanzibar*	cgcagcaaacaaaaccgaaggatattcagagagtgtgacgcgtactacaagagcgcatcgtttagctccatcaccttgttaccatgt
Breueh	.....G.C.....G.C.....TA.....G.....G.....C..
Breueh	.....G.....G.....G.C.G.GA.....TA.....G.....T.....C.....G.....G.....CAC
Breueh	.....G.....A.G.....G.C.G.GA.....C.....TA.....G.....G.....G.....G.....C.C
Breueh	.....T.....G.....G.C.G.GA.....C.....TA.....G.....G.....A.....G.....A.....G.....C.C
Breueh	.....G.....G.....G.C.G.G.....TA.....G.....G.....G.....C.....G.....C.C
Breueh	.....G.C.....G.C.....TA.....G.....GT.....C..
Meulaboh	.....G.....G.C.G.G.....TA.....G.....G.....G.....G.T.C.C
Meulaboh	.....G.....G.C.G.G.....TA.....G.....G.....G.....G.T.C.C
Sibolga	.....G.....T.....G.C.....G.....TA.....TG.....G.....C.....C
Sibolga	.....G.....G.....G.C.....G.....TA.....TG.....A.....G.....C.....C
Sibolga	.....G.....G.....G.C.....G.....TA.....TG.....A.....G.....C.....C
Sibolga	.....G.....G.....G.C.....G.....TA.....TG.....G.....C.....C
Perbaungan	.....G.....G.....G.C.....G.....TA.....TG.....T.....TG.....A.....C
Pagurawan	.....G.....G.....G.C.....G.....TA.....TG.....T.....TG.....A.....C
Pagurawan	.....G.....G.....G.C.....G.....TA.....TG.....T.....TG.....A.....C
Pagurawan	.....G.....G.....G.C.....G.....TA.....TG.....T.....G.....A.....C
Pagurawan	.....A.G.....G.C.....G.....TA.....TG.....T.....TG.....A.....C
Pagurawan	.....G.....G.....G.C.....G.....TA.....TG.....T.....TG.....A.....C
Pagurawan	.....G.....G.....G.C.....G.....TTA.....TG.....T.G.....TG.....A.....C
Pagurawan	.....G.....G.....G.C.....G.....TAC.....TG.....T.....TG.....A.....C
Pagurawan	.....A.G.....G.C.....G.....TA.....A.....T.....TG.....A.....C
Padang	.....G.....G.....G.C.....G.....TA.....TG.....A.....G.....C.....C
Pabelokan	.....C.....CG.....G.....T.TA..T.....T.....G.T.CA.....T..C
Karangatu	.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Karangatu	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Karangatu	.....C.....CG.....G.....T.TA..T.....AT.....G.T.CA.....T..C
Karangatu	.....C.....CG.....G.....T.TA..T.....T.....G.T.CA.....T..C
Karangatu	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Karangatu	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
PulauPari	T.....G.....CG.....G.....T.TA..T.....G.A.T.....G.T.CA.....T..C
Peniki	.....C.....CG.....G.....T.TA..T.....T.....G.T.CA.....T..C
Peniki	.....C.....CG.....G.....T.TA..T.....T.....G.T.CA.....T..C
Peniki	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..T
Peniki	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Peniki	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..T
Peniki	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Selakau	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Selakau	.....TT.....CG.....G.....T.TA..T.....T.....C.....T.....G.TC.....T..C
Selakau	.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Selakau	.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CAC.....T..C
Selakau	.....T.G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Selakau	.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Selakau	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Selakau	T.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Batang	.....C.CG.....CG.....G.....A.TTTA..T.....T.....T.....G.T.CA.....T..C
Batang	.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Batang	.....G.....CG.....G.....T.TA..T.....G.....T.....G.T.CA.....T..C
Batang	.....C.....CG.....G.....C.....T.TA..T.....CT.....G.T.CA.....T..C

Batang	T.....	G....CG....G.....T.TA..T...G....T.....	G.T.CA....T..C..
Batang	.....C.	CG....G.....T.TA..T.....T.....	G.T.CA....T..C..
Batang	T.....G....CG....G.....T.TA..T...G....T.....	G.T.CA....T..C..	
Paotere	.....CG....G....C.....T.TA..T.....T.....C.....	G.T..A.....C..	
Paotere	.....CG....G.....T.TA..T.....T.....	G.T.CA....T..C..	
Tarakan	.....CG....TG.....T.TA..T.....T.....C..A.....	G.T.CA.....C..	
Tarakan	.....CG....G.....T.TA..T.....T.....C.....	G.T.CA.....C..	
Tarakan	.....CG..T..G.....T.TA..T.....T.....C.....	G.T.CA....T..C..	
Tarakan	.....CG..T..G.....T.TA..T.....T.....C.....	G.T.CA....T..C..	
Tarakan	.....CC.....CG....G.....T.TA..T.....T.....C.....	G.T.CA.....C..	
Tonra	.....T.....CGC....G..C.....T.TA..T.....T.....	G.T..A.....C..	
Sinjai	.....T.....T.....G....G.....T.C.....T.....T.....G..G.....C..		
Sinjai	.....T.....T.....G....G.....T.C.....T.....T.....G..G.....C..		
Ambon	.....T.....GAG.....A.....T.C.....T.....T.....C..		
Ambon	.....T.....GAG.....A.....T.C.....A..T.....T.....C..		
Ambon	.....T.....GAG.....A.....T.C.....T.....		
Ambon	.....C.C.....T.....GAG.....A.....A..T.C.....T.....		
Ambon	.....C.....T.....GAG.....A.....CT.C.....T.....T.....C..		
Ambon	.....T.....GAG.....A.....T.C.....T.....		
Kei	.....C.C.....G..T..G.G.....A.....CTG.....C.....T.....C..		
Kei	.....G..T..G.G.....A.....TG.....T.....		
Kei	.....G..T..G.G.....A.....TG.....T.....		
Kei	.....G..T..G.G.....A.....TG.....T.....		
Kei	.....G..T..G.G.....A.....TT.....T.....		
Kei	.....G..T..G.G.....A.....TG.....T.....		
Kei	.....G..T..G.G.....A.....TG.....T.....		
Kei	.....G..T..G.G.....A.....TG.....T.....		
Biak	.....G..T..A.....G..T..C.....G.....A.....G.....C..		
Biak	.....C.....T.....T.G..T..A.....G..T..C.....G.....G.....C..		
Biak	.....G..T..A.....G..T..C.....G.....G.....C..		
Biak	.....G.....G..T..A.....G..T..C.....G.....C.G.....C..		
Bali	.....GC.....G..C..G..TA.....T..G.....C..C..		
Bali*	.....GC.....G..C..G..TA.....T..G.....C..C..		
Java*	.....G.....CG.....G..C..T.TA..T...G....T.....G.T.CA....T..C..		
Java*	.....G.....CG.....G.....T.TA..T...G....T.....G.T.CA....T..C..		
Java*	.....C.....CG.....G.....T.TA..T.....T.....G.T.CA....T..C..		
Java*	.....T.....G.....CG.....G.....T.TA..T...G....T.....G.T.CA....T..C..		
Java*	.....T.....G.....CG.....G.....T.TA..T...G....T.....C.....G.T.CA....T..C..		
Penghu*	.....TT.....CG.....G.....T.TA..T.....T.....C.....T.....G..TC.....T..C..		
Penghu*	.....TT.....CG.....G.....T.TA..T.....T.....C.....T.....G..TC.....T..C..		
Penghu*	.....TT.....CG.....G.....T.TA..T.....T.....C.....T.....G..TC.....T..C..		
Queensland*	.....T..T.T.....G....G.....T.C.....T.....C.....T.....G.....C..		
Japan*	.....G.....A..G....G..A.....T.TA..TC..G.....T.....G.....G.T.....GC..		
New Caledonia	.....G.....CG.....GA.....TA.C.CC.....T.T.....C.G.....C..C..		