

***Neotrygon indica* sp. nov., the Indian-Ocean blue spotted maskray (Myliobatoidei, Dasyatidae)**

Annam Pavan-Kumar<sup>1</sup>, Rajan Kumar<sup>2</sup>, Philippe Borsa<sup>3</sup>

<sup>1</sup> *Fish Genetics and Biotechnology Division, ICAR-Central Institute of Fisheries Education, Mumbai – 61, India*

<sup>2</sup> *Crustacean Fisheries Division, ICAR-Central Marine Fisheries Research Institute, Regional centre, Tuticorin, India*

<sup>3</sup> *Institut de recherche pour le développement (IRD), UMR 250 “Ecologie marine tropicale des océans Pacifique et Indien”, BP A5, 98848 Nouméa, New Caledonia*

## Abstract

The blue-spotted maskray, previously *N. kublii*, consists of up to eleven lineages representing separate species. Nine of these species (*N. australiae*, *N. bobwardi*, *N. caeruleopunctata*, *N. malaccensis*, *N. moluccensis*, *N. orientale*, *N. vali*, *N. varidens*, *N. westpapuensis*) have already been formally described. Here the Indian-Ocean maskray is described as a new species, *Neotrygon indica* sp. nov. A diagnosis based on its distinct colour patterns and nucleotide sequence at the *CO1* locus is proposed.

## Keywords

Taxonomy; Diagnosis; Distribution

## Introduction

The blue-spotted maskray, previously *N. kublii*, consists of up to eleven lineages representing separate species (Arlyza et al. 2013; Puckridge et al. 2013; Borsa et al. 2016a, 2016b, 2017b) of which nine (*N. australiae*, *N. bobwardi*, *N. caeruleopunctata*, *N. malaccensis*, *N. moluccensis*, *N. orientale*, *N. vali*, *N. varidens*, *N. westpapuensis*) have already been formally described. Two other species, the nominal *N. kublii* from Vanikoro and *N. trigonoides* possess distinctive spot patterns (Borsa et al. 2013; Borsa and Béarez 2016; Borsa 2017a) that tell them apart from the blue spotted maskray as it was originally described by Müller and Henle (1841). The Indian-Ocean maskray reported in the recent genetic literature (Puckridge et al. 2013; Borsa et al. 2017) remains undescribed. The objective of the present paper is to formally describe this species, based on fresh material from the eastern coast of India.

## Materials and Methods

*Material examined.* Seven specimens of the new species that were examined for spot patterns are listed in Table 1. Comparative material consisted of a total of 25 specimens from *N. australiae*, *N. bobwardi*, *N. caeruleopunctata*, *N. malaccensis*, *N. moluccensis*, *N. orientale*, *N. vali* and *N. westpapuensis*, plus a specimen from Zanzibar tentatively assigned to the Indian-Ocean maskray (Arlyza et al. 2013a). Specimen NKGMF-3, to be designated as the holotype of the new species, was deposited at the Marine Biology Regional Centre (MBRC) in Chennai, India. The MBRC is part of the network of museums managed by, and an official repository of the Zoological Survey of India. Voucher material for the new species also includes a specimen collected by APK and colleagues in Visakhapatnam, Andhra Pradesh state of India, Bay of Bengal on 15 August 2011. The specimen was discarded but a sub-sample of tissue has been registered at the Central Institute of Fisheries Education of Visakhapatnam under no. VIZNK-01. Its partial *CO1* gene sequence has GenBank accession no. JX978329. A photograph of this individual is available from the Barcoding of Life Datasystems database (BOLD; <http://www.barcodinglife.com/>; Ratnasingham and Hebert, 2007) under accession no. BOLD:ACB9305.

*Spot pattern analysis.* The diameter of ocellated blue spots on the dorsal side of the left and right pectoral fins, relative to disk width, was measured from photographs. Ocellated blue spots were qualified as “small” when their maximum diameter was  $\leq 2\%$  disk width (DW), “medium” when  $\leq 4\%$  DW and “large” when  $> 4\%$  DW (Borsa et al. 2013a). Dark speckles ( $\leq 1\%$  DW) and dark spots ( $> 1\%$  DW) were also counted on the dorsal surface of the disk. The counts did not include those speckles and spots located within the dark band around eyes that forms the mask (Borsa et al., 2013a). The presence or absence of a darker occipital mark at the rear end of the neurocranium was also checked.

*Barcode index number.* The BOLD datatypes distinguishes clusters of sequences that qualify as operational taxonomic units i.e., putative species using the Refined Single Linkage algorithm (Ratnasingham and Hebert 2013). The latter “clusters sequences with high similarity and connectivity and separates those with lower similarity and sparse connectivity”. Each putative species thus flagged is allocated a unique barcode index number (BIN) in BOLD. Borsa et al. (2017) have established the homology of BIN numbers with cryptic blue-spotted maskray lineages by visual inspection of the placement of the corresponding *CO1* gene sequences retrieved from BOLD in a reference maximum-likelihood tree rooted by *N. trigonoides*.

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## Results

Spot patterns of seven specimens of the Indian-Ocean maskray are summarized and compared to eight other species of the blue-spotted maskray complex (Table 1). Indian-Ocean maskray specimens were characterized by a moderately large number of small ocellated blue spots ( $N = 15-57$ ), by a low number of medium-sized ocellated blue spots ( $N = 0-16$ ), by a total absence of large ocellated blue spots, by a high number of dark speckles ( $N = 23-176$ ), generally by a few dark spots ( $N = 0-11$ ; only exceptionally present in the other species examined), and by a conspicuous occipital mark. These features are visible on the holotype of the new species (Fig. 1).

## Taxonomy

*Neotrygon* Castelnau 1873. *Neotrygon indica* sp. nov. urn:lsid:zoobank.org:act:99F4F1A4-D5F6-4379-BC4E-7FB1E74CFA58. Indian-Ocean maskray (Borsa et al. 2017); Clade I (Borsa et al. 2016b); *Neotrygon kublii* haplogroup I (Arlyza et al. 2013a; Borsa et al. 2016a); *Neotrygon kublii* clade 8 (Puckridge et al. 2013); presumably clade *Neotrygon kublii* 3 of Naylor et al. (2012); *Neotrygon kublii* (Borsa et al. 2013a). Also BIN number BOLD:AAA5611 in BOLD.

*Holotype*. A female specimen 174 mm disk length from the Gulf of Mannar, Tamil Nadu (9.12°N 79.46°E) (Fig. 1), collected at the Inico Nagar, Tuticorin fish landing centre by RK and Satish on 13 January 2017 is here designated as the holotype of *Neotrygon indica* sp. nov. The specimen, which has registration no. ZSI/MBRC/F.1495 was deposited by APK at the MBRC in Chennai, India on 12 May 2017.

*Paratype*. A female specimen 182 mm disk length fished from the Gulf of Mannar, Tamil Nadu (9.12°N 79.46°E) using bottom-set gillnets, collected by RK on 05 May 2017 is here designated as the single paratype of the new species. This specimen, labelled CIFEFG/NKGM1 was subsequently deposited by APK at the Fish Genetics and Biotechnology laboratory, ICAR-Central Institute of Fisheries Education, Mumbai, India.

*Diagnosis*. Differentiated from all species of the blue-spotted maskray described so far (*N. australiae*, *N. bobwardi*, *N. caeruleopunctata*, *N. malaccensis*, *N. moluccensis*, *N. orientale*, *N. vali*, *N. varidens* and *N. westpapuensis*) by a combination of low number of medium-sized ocellated blue spots, total absence of large ocellated blue spots, high number of dark speckles, frequent occurrence of a few dark spots, and conspicuous occipital mark. Distinct from *N. kublii* and *N. trigonoides* by the absence of conspicuous scapular blotches, although specimens of *N. indica* sp. nov. often possess a pair of diffuse darker marks in the nuchal region. Twelve out of 14 individuals sequenced at the *CO1* locus and previously assigned to the Indian-Ocean maskray haplogroup possessed T at nucleotide site 607 of the *CO1* gene, a character that was otherwise present in only two out of 130 *N. orientale* individuals and absent in all other cryptic species of the blue-spotted maskray (Borsa et al. 2017: supplementary table S1).

*Distribution*. The type locality of *N. indica* sp. nov. is the Gulf of Mannar in Tamil Nadu on the northeastern coast of the Indian sub-continent. Based on the collection of voucher specimens from present study, the distribution of *N. indica* sp. nov. includes the coasts of Andhra Pradesh and Tamil Nadu states of India, from approximately 17.7°N to 8.8°N. Based on the material genetically examined thus far, the distribution of *N. indica* sp. nov. includes the Indian coast of the Bay of Bengal (Visakhapatnam, Chennai), and may also include the Indian coast of the Laccadives Sea (Kerala). More research is necessary to understand the genetic

relationship of the blue-spotted maskrays from eastern Africa (Arlyza et al. 2013a; Puckridge et al. 2013; Borsa et al. 2016b, 2017) to *N. indica* sp. nov.

*Etymology.* Named for the country of type locality, India. Epithet *indica* is the latin feminine adjectival form of the name of the country.

*Proposed vernacular names.* Indian-Ocean blue-spotted maskray (English); Neeli Nishan Pakat (Hindi); Pulli Thirukhai (Tamil); Raie pastenague masquée à points bleus de l'océan Indien (French)

## References

- Arlyza I S, Shen K-N, Durand J-D, Borsa P. 2013a. Mitochondrial haplotypes indicate parapatric-like phylogeographic structure in blue-spotted maskray (*Neotrygon kublii*) from the Coral Triangle region. *J. Hered.* 104: 725-733.
- Borsa P. 2017a. Comments on "Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity" (Weigmann, 2016). *J. Fish Biol.* 90: 1170-1175.
- Borsa P. 2017b. *Neotrygon vali*, a new species of the blue-spotted maskray complex (Myliobatoidei: Dasyatidae). *bioRxiv* doi: 10.1101/106682.
- Borsa P, Arlyza I S, Chen W-J, Durand J-D, Meekan M G, Shen K-N. 2013a. Resurrection of New Caledonian maskray *Neotrygon trigonoides* (Myliobatoidei: Dasyatidae) from synonymy with *N. kublii*, based on cytochrome-oxidase I gene sequences and spotting patterns. *C. R. Biol.* 336: 221–232.
- Borsa P, Arlyza IS, Hoareau TB, Shen KN. 2017. Diagnostic description and geographic distribution of four new cryptic species of the blue-spotted maskray species complex (Myliobatoidei: Dasyatidae; *Neotrygon* spp.) based on DNA sequences. *Chin. J. Oceanol. Limnol.* doi: 10.1007/s00343-018-7056-2.
- Borsa P, Béarez P. 2016. Notes on the origin of Müller and Henle's illustration and type material of the blue-spotted maskray *Neotrygon kublii* (Myliobatoidei: Dasyatidae). *Cybium* 40: 255-258.
- Borsa P, Durand J-D, Chen W-J, Hubert N, Muths D, Mou-Tham G, Kulbicki M. 2016a. Comparative phylogeography of the western Indian Ocean reef fauna. *Acta Oecol.* 72: 72-86.
- Borsa P, Durand J-D, Shen K-N, Arlyza I S, Solihin D D, Berrebi P. 2013c. *Himantura tutul* sp. nov. (Myliobatoidei: Dasyatidae), a new ocellated whipray from the tropical Indo-West Pacific, described from its cytochrome-oxidase I gene sequence. *C. R. Biol.* 336: 82-92.
- Borsa P, Shen K-N, Arlyza I S, Hoareau T B. 2016b. Multiple cryptic species in the blue-spotted maskray (Myliobatoidei: Dasyatidae: *Neotrygon* spp.): an update. *C. R. Biol.* 339: 417-426.
- International Commission on Zoological Nomenclature. 2012. Amendment of Articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. *Bull. Zool. Nomencl.* 69: 161-169.
- Müller J, Henle F G J. 1841. Systematische Beschreibung der Plagiostomen, mit sechzig Steindrucktafeln. Veit und Comp, Berlin, xxii+200 p., 60 pl.

- Naylor G J P, Caira J N, Jensen K, Rosana K A M, White W T, Last P R. 2012. A DNA sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bull. Am. Mus. Nat. Hist.* 367: 1-262.
- Puckridge M, Last P R, White W T, Andreakis N. 2013. Phylogeography of the Indo-West Pacific maskrays (Dasyatidae, *Neotrygon*): a complex example of chondrichthyan radiation in the Cenozoic. *Ecol. Evol.* 3: 217-232.
- Ratnasingham S, Hebert P D N. 2007. BOLD: the Barcode of Life Data System ([www.barcodinglife.org](http://www.barcodinglife.org)). *Mol. Ecol. Notes* 7: 355–364.
- Ratnasingham S, Hebert P D N. 2013. A DNA-based registry for all animal species: the barcode index number (BIN) system. *PLoS One* 8: e66213.

**Table 1.** Spot patterns in 33 blue-spotted maskray (*Neotrygon* spp.) specimens sorted by species. *L*, left pectoral fin; *R*, right pectoral fin. *Occipital mark*, dark blotch at rear end of neurocranium that was categorized as either absent (0), weak (1) or conspicuous (2)

Species, Specimen no. (field no.)	Locality of collection	N ocellated blue spots			N dark speckles	N dark spots	Occipital mark
					(<1% DW)	(>1% DW)	
		Small L, R	Medium L, R	Large L, R	L, R	L, R	
<i>N. australiae</i>							
CSIRO 7016-01 <sup>H</sup>	Weipa, G. Carpentaria	14, 12	17, 20	3, 0	8, 9	0, 0	0
MZB-20863 (ENTTJS2)	Tanjung Sulamo	25, 25	28, 32	1, 0	13, 6	0, 0	0
<i>N. bobnardi</i>							
MZB-20843 (ME3) <sup>H</sup>	Meulaboh	28, 29	13, 11	0, 0	16, 16	0, 0	0
20150524-D	Padang	5, 11	4, 2	0, 0	6, 6	0, 0	0
20150524-E	Padang	16, 11	9, 8	0, 0	8, 15	0, 0	2
20150524-F	Padang	20, 36	14, 8	0, 0	3, 0	0, 0	1
20150524-11	Padang	8, 8	2, 1	0, 0	6, 13	0, 0	2
<i>N. caeruleopunctata</i>							
CSIRO H 7850-01 <sup>P</sup>	Sadeng	25, 22	4, 1	0, 0	17, 30	0, 0	1
20080131_BL	Bali	21, 24	6, 5	0, 0	43, 54	0, 0	1
MZB-22131	Bali	31, 22	5, 9	0, 1	0, 1	0, 0	1
<i>N. indica</i> sp. nov.							
ZSI/MBRC/F.1495 (NKGMF-3) <sup>H</sup>	Gulf of Mannar	9, 10	0, 0	0, 0	19, 34	3, 2	2
VIZNK-01 (NKVSKP-1)	Visakhapatnam	3, 12	1, 9	0, 0	13, 10	0, 0	2
NKTTK-1	Tuticorin	30, 27	3, 3	0, 0	90, 86	0, 1	2
NKTTK-2	Tuticorin	20, 8	0, 0	0, 0	24, 32	0, 1	2
NKGMM-2	Gulf of Mannar	24, 26	7, 4	0, 0	30, 33	4, 1	2
CIFEFGB/NKGM1 (NKGMF-1)	Gulf of Mannar	27, 26	0, 0	0, 0	37, 22	4, 1	2
NKGMM-4	Gulf of Mannar	14, 18	8, 4	0, 0	40, 38	5, 6	2
<i>N. cf. indica</i>							
ZAN 1 left	Zanzibar	44, 51	29, 21	2, 2	10, 15	0, 0	0
<i>N. malaccensis</i>							
MZB-20847 (MSKL3) <sup>H</sup>	Kuala Lama	2, 4	14, 10	0, 1	5, 3	0, 0	0
<i>N. moluccensis</i>							
MZB-20866 (ARA1) <sup>H</sup>	Tual	36, 30	9, 9	0, 0	53, 53	0, 1	0
MZB-20864 (AM1) <sup>P</sup>	Ambon	9, 2	7, 12	0, 0	5, 2	0, 0	1
<i>N. orientale</i>							
CSIRO H7858-01 <sup>H</sup>	Muara Kintap	20, 14	15, 17	1, 1	2, 1	0, 0	0
PB2	Pabelokan	5, 9	8, 3	1, 0	21, 17	0, 0	0
PR	Pulau Pari	22, 18	9, 6	2, 2	12, 8	0, 0	0
PN5	Pulau Peniki	12, 22	12, 11	2, 0	2, 1	0, 0	1
BO424	Tanjung Manis	35, 41	22, 6	0, 0	16, 13	0, 0	0
WJC-5367	Kota Kinabalu	17, 19	10, 8	0, 1	8, 4	0, 0	0
WJC-5368	Kota Kinabalu	19, 20	6, 12	0, 0	6, 5	0, 0	0
WJC-5369	Kota Kinabalu	20, 23	14, 10	0, 0	3, 2	0, 0	0
<i>N. valē</i>							
CSIRO H7723-01 <sup>H</sup>	Honiara	2, 4	1, 1	0, 0	3, 6	0, 0	0
Randall (2005: 18) <sup>L</sup>	Solomon Islands	11	4	0	6	0	0
Rosenstein <sup>L</sup>	Mbike Wreck	21	6	0	7	0	0
<i>N. westpauensis</i>							
MZB-20867 (BK5) <sup>H</sup>	Biak	41, 37	25, 13	0, 2	10, 7	0, 0	1

<sup>H</sup> Holotype; <sup>P</sup> Paratype; <sup>L</sup> Left side only



**Fig. 1.** Female blue-spotted maskray specimen, 174 mm disk length, from the Gulf of Mannar, Tamil Nadu, India (9.12°N 79.46°E) registered under no. ZSI/MBRC/F.1495 at the Marine Biology Regional Centre in Chennai, India and designated as holotype of *Neotrygon indica* sp. nov. (photograph by RK).