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Philippe Borsa, I S Arlyza, P H Barber. The phylogeography and taxonomy of a model-species complex, the blue-spotted maskray (formerly *Neotrygon kuhlii*): a short review. IOP Conference Series: Earth and Environmental Science, 2019, 348, pp.012055. 10.1088/1755-1315/348/1/012055 . ird-02380855

HAL Id: ird-02380855

<https://ird.hal.science/ird-02380855>

Submitted on 26 Nov 2019

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Citation:

Borsa P, Arlyza IS, Barber PH (2019) *The phylogeography and taxonomy of a model-species complex, the blue-spotted maskray (formerly Neotrygon kuhlii): a short review*. IOP Conference Series: Earth and Environmental Science 348, 012055.

The phylogeography and taxonomy of a model-species complex, the blue-spotted maskray (formerly *Neotrygon kuhlii*): a short review

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Abstract

The present paper reviews the phylogeography of an emblematic Indo-West Pacific stingray and aims at linking its taxonomic implications with conservation management. The blue spotted maskray was once understood to be a single species widely distributed in the Indo-West Pacific, from the Red Sea to Fiji and from southern Japan to New South Wales. Genetics have shown that it actually consists of a number of distinct lineages with parapatric distribution. Phylogenetic data suggest an evolutionary origin of this species complex in the southwestern Pacific in the Miocene. The blue-spotted maskray progressively colonized the Indo-West Pacific from the East to the West. The lineages in the western Coral Triangle and the Indian Ocean are the most recent. Against this updated taxonomic and phylogeographic background, there remains a need for research on the population dynamics and fisheries management of this increasingly threatened species complex. This paper is dedicated to the memory of Dr Irma S. Arlyza.

Keywords: Population genetics; phylogeny; stepwise colonization; Indo-West Pacific; conservation; obituary.

1. Obituary: Dr Irma S. Arlyza (1974-2019)

Dr. Irma S. Arlyza prematurely left us this year, and we would like to dedicate this paper to her memory. When one of us (P. Borsa) first met Irma at LIPI-P2O in Ancol in February 2008, she was a young researcher interested in the biology of the sharks and rays landed at ports throughout Indonesia. She desired to pursue a PhD program, to learn the theory and practice of molecular population genetics, and apply them to the phylogeographic study of Indonesia's sharks and rays. Irma subsequently began her PhD studies with P. Borsa focused on a limited number of stingray species, including the blue-spotted maskray *Neotrygon kuhlii* (then still a single nominal species assigned to the genus *Dasyatis*). This stingray is a geographically widespread, rather common shallow-water species, and is among the most commonly landed by shark and ray fishermen across Indonesia [1, 2], making it an ideal study organism. Moreover, populations from the adjacent Java and Bali islands strongly differed in body size at first reproduction [1, 2], and Australian geneticist R.D. Ward and co-authors had just reported an unusually high level of genetic divergence at the

CO1 locus among populations of the blue-spotted maskray, indicating limited gene flow and potential cryptic species [3].

In the laboratory (Fig. 1) Irma was soon confronted to the problem of scoring a sufficient number of nuclear markers that would be reasonably polymorphic but also consistently scored across the geographic distribution of the species. It turned out that such ideal markers were excessively rare among the exon-primed intron markers she tested in the blue-spotted maskray. These difficulties led us to switch to molecular taxonomic investigations of the *Himantura uarnak* species complex as the core of her PhD project [10]. Irma had registered for S3 (the Indonesian PhD program) at Institut Pertanian Bogor (IPB). She obtained her S3 doctorate at IPB in 2013, based on her memoir entitled “Molecular identification of *Himantura uarnak* species complex (Batoidea: Dasyatidae) in Indonesia” [11]. Meanwhile, the genetic data already produced in the blue-spotted maskray helped sorting out its intricate taxonomy. Deeply diverged lineages within the blue-spotted maskray species complex were confirmed, explaining in part the difficulties encountered with PCR-amplified intron markers.

Dr. Irma S. Arlyza was committed to understanding Indonesian marine biodiversity through field studies as well as the application of modern molecular tools. Her passion was the evolution and conservation of stingrays, the topic of her S3 thesis. While Irma largely studied marine fishes, she was also involved in international collaborations focused on understanding Indonesian marine biodiversity ranging from sea stars to snails and clownfish, and worked with a diversity of researchers, but particularly the late S.A. Karl and B.J. Wainwright, then S.A. Karl's PhD student at the Hawaii Institute of Marine Biology, and P.H. Barber of the University of California, Los Angeles. Nuclear-DNA markers were used on a series of shallow-water marine species collected throughout the geologically and ecologically complex Indo-Malay archipelago, to test hypotheses on the causes of genetic differentiation (e.g. [12]). Irma used these international collaborations to help secure graduate training opportunities for other LIPI researchers, leaving a legacy of marine research in Indonesia that will hopefully continue well into the future.

2. Objectives

The Coral Triangle region has a high diversity of sharks and rays, including stingrays. Indonesia, the largest and most populous country in this region is also the world's top producer of sharks and rays [13]. Stingrays (Dasyatidae) represent nearly 40 000 tons annually, comprising over 40% of the total catch of sharks and rays in Indonesian waters, exceeding that of any other shark and ray family [14]. It is unlikely that such levels of exploitation, mostly unregulated and unmanaged, are sustainable [14-16]. Nearly two thirds of the shark and ray species occurring in the Indonesian seas are of conservation concern [17]. The biology and ecology of stingray species in this region of the world remain poorly studied, hampering effective management efforts. Yet, the blue-spotted maskray might be an exception. Drawing on previous work [1-4, 6, 8, 9, 18-24], the present paper aims at reviewing our current knowledge of the molecular phylogeography and taxonomy of the blue-spotted maskray. This should provide managers with some of the knowledge required to improve the conservation and fisheries management of this model-species complex.

3. Phylogeography

The blue-spotted maskray species complex has a wide geographic distribution. It occupies the entire tropical Indo-West Pacific, from the Red Sea to Fiji. The distribution includes all the eastern African coast from Djibouti to Mozambique, Madagascar, the Arabian Sea, the Sea of Oman and the Persian Gulf, the periphery of the Indian peninsula, the Gulf of Bengal and the Andaman Sea, southeastern Asia, the whole Indo-Malay-Philippine archipelago, Taiwan, the Japanese archipelago, the Micronesian islands and the whole Melanesia including West Papua, Papua New Guinea, the Solomon Islands, New Caledonia, Vanuatu, and Fiji. Around Australia, the blue-spotted maskray occurs all along the tropical coastline, from Western Australia to New South Wales.

Phylogenetic analyses provide important insights into the evolutionary relationships among the many lineages within this species complex. In a rooted phylogenetic tree, each node with descendants represents the inferred most recent common ancestor of those descendants. Assuming a molecular clock, the depth of the node can be interpreted as an estimate of time since divergence. M. Puckridge and co-authors proposed a Bayesian time-tree of species and lineages of the stingray family (Dasyatidae) based on *CO1*, *16S* and *RAG-1* sequences. The tree was calibrated using the oldest known dasyatid fossil, which was dated from the early Cretaceous [19]. The authors inferred the age of the last common ancestor to all blue-spotted maskrays to be in the Miocene, approximately 15.7 My (22-8 My) ago. This date coincides with the development of modern coral reef communities in the central Indo-West Pacific, including the Coral Sea [25]. Puckridge et al. [19] identified nine deeply divergent clades within the blue-spotted maskray, five of which had been detected previously [3, 18]). Additional clades were found by Arlyza et al. [6, 21]. The first split within the blue-spotted maskray species complex separated the New Caledonian maskray *N. trigonoides* from the remaining clades [21]. The next deeper node separated the haplogroup from West Papua, which is northwest to the Coral Sea, followed by a Moluccan lineage (immediately west of West Papua) from the remaining clades. The remainder of the lineages combined into a single sub-tree that includes all lineages west of the Moluccas. This phylogenetic pattern suggests that this lineage originated in the easternmost part of the species' distribution, followed by a repeated pattern of westward range expansion and divergence (Fig. 2) giving rise to its present Indo-West Pacific distribution. Differentiation among these groups was likely heightened through genetic drift, resulting from repeated population crashes and expansions [21, 24] associated with Pleistocene sea level fluctuations.

Integrating the phylogenetic results with the geographic distribution of the different lineages shows a parapatric distribution (Fig. 2). "Parapatry" is the term used to describe a situation where pairs of taxa present separate but contiguous geographic distributions [27], and in the case of this species complex indicates reproductive isolation [27]. This result in turn confirms that the different lineages are distinct species. Indeed, reproductive isolation is one of the most obvious properties of a species, which distinguishes it from another species [28].

4. Molecular taxonomy

In their description of the blue-spotted maskray (as *Trygon kublüi*), Müller and Henle included the drawing of an individual which they may have believed (erroneously so) was from Java, part of the East Indies then confused with India, which they designated as the type locality of the species [26]. The type series comprised four specimens: one presumably from Java, whose whereabouts are to this date uncertain; one from West Papua collected during the 1826-1829 *Astrolabe* expedition; and two from Vanikoro, also collected by the *Astrolabe* expedition but already described by J.R.C. Quoy and J.P. Gaimard (as the "blue ray" *Raia coerulea*), something Müller and Henle were apparently unaware of [20]. The one syntype of *N. kublüi* that was eventually chosen as lectotype by P.R. Last and co-authors [22] was one of the two specimens from Vanikoro. Both the lectotype and a specimen represented in watercolour by Quoy possess colour patterns that designate a species different from Müller and Henle's [26] original description. Quoy and Gaimard's blue ray differs from Müller and Henle's description by the presence of a pair of darker blotches in the scapular region and a number of dark speckles all over the dorsal side. A detailed account of the taxonomic history of the species, which is marked with neglect, confusion and error, has been published [20, 24].

Molecular genetics have provided evidence that the blue-spotted maskray consists of a complex of cryptic species [3, 4, 6, 8, 9, 17, 18, 20, 22]. Presently, the species complex nominally includes *N. trigonoides*, which inhabits the Coral Sea, and *N. kublüi*, which might be a synonym because its colour patterns are similar to those of *N. trigonoides* [9, 29]. The distribution of *N. kublüi* is presently considered to be restricted to the single island of Vanikoro in the Santa Cruz archipelago. Three other species were described in 2016 [22]. These are *N. australiae* from northern Australia, *N. caeruleopunctata* from southern Bali and southern Java, and *N. orientale* whose distribution spans a wide archipelagic region that extends from the Java Sea to the Philippines (Fig. 2). While there is clear genetic support to recognizing these three species, their authors

chose to describe them based on a series of morphological traits [22], none of which were diagnostic [8, 23]. One species, *N. varidens* from the South China Sea earlier described by S. Garman [30] was resurrected [22]. Six additional species were described subsequently, including *N. bobwardi* from western Sumatra and Aceh, named after R. D. Ward as an acknowledgement of his early contribution to the taxonomy of the blue-spotted maskray [8], *N. indica* from the Indian Ocean [31], *N. malaccensis* from the Malacca strait [8], *N. moluccensis* from the Banda Sea [8], *N. vali* from the Solomon archipelago [23], and *N. westpapuensis* from Biak [8].

The latter six species were described mainly based on their DNA sequences. We also used spot patterns as a proxy of genetics, to distinguish *N. vali* from *N. kublii*. The other species were similarly re-described based on DNA sequences, except *N. kublii* for which no DNA sequence is yet available. Given that morphological data used to date have been unreliable and non-diagnostic (e.g. [22]), further advancing our understanding of the taxonomy of this group would be aided greatly by DNA sequence data [8, 23, 31].

5. Perspectives

Future genetic analyses may help uncover the genetic relationships of *N. kublii* to *N. trigonoides* and to other blue-spotted maskray lineages occurring in adjacent regions, including *N. vali* [23], whose holotype had been erroneously assigned to *N. kublii* by Last et al. [22] despite clear-cut morphological differences [20, 23]. Sequencing the DNA of the alcohol-preserved lectotype of *N. kublii* might be helpful in this respect.

Suggestions for future research on the blue-spotted maskray species complex include the role played by geological and palaeoceanographic events in the differentiation of species and the demographic history of each of the species in the complex. The parapatric distribution of species, to be maintained, implies some reproductive isolation mechanism that deserves more scrutiny. Pre-zygotic isolation mechanisms may include interspecific differences in male clasper distal length and shape and possible matching differences in the morphology of the female urogenital apparatus; and interspecific differences in colour patterns, e.g. the number, relative size and colour tones of the blue spots.

Few data are available on either the biology or the fisheries of sharks and rays in the Indonesian seas [2, 14, 15], but the data available warn us that if unsustainable exploitation processes further, it will likely cause severe depletion of fisheries stocks, potentially leading to fisheries collapse and the ecological extinction of the most vulnerable species. For the species that may survive, over-exploitation will erode genetic variability thus hampering the ability of populations to cope with future environmental change. As stated by Fahmi et al. [2], "management and conservation actions are difficult to implement for such species when basic data on biology and diversity are not available in the region". In the blue-spotted maskray, individual movements have been tracked using physical tags, which indicated sedentarity of both males and females [32]. Reports of size at maturity of both males and females, a parameter essential to investigating population dynamics, indicated strong geographic variability [1, 2, 32, 33]. While these findings focused on a few populations of blue-spotted maskrays that are now recognized as separate species, the demography of the other species in the species complex remains unaddressed.

In conclusion, future research on the demographic parameters of the blue-spotted maskray should consider its updated taxonomy. Given the poor reliability of morphological characters [8], such studies should include more reliably diagnostic DNA sequence data [3, 8] or should be done in a geographically explicit framework given the parapatric distribution of species in the species complex. Such efforts are required to inform the effective management of the blue-spotted maskray. Similar efforts are required with other shark and ray species complexes similarly threatened by overfishing in the Indonesian seas and elsewhere.

Acknowledgement

This is a contribution of the PARI project, a cooperative research project between IRD, France and LIPI-P2O, Indonesia.

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Figure 1. In the course of her PhD thesis' work, Irma S. Arlyza spent six months at Institut des sciences de l'évolution, Montpellier under the supervision of P. Berrebi and P. Borsa. Irma tested a wide array of exon-primed intron markers in two stingray species then identified as *Himantura uarnak* [Gmelin (ex Forsskål) 1789] and *Neotrygon kublii* (Müller and Henle 1841). She was able to confirm that both consisted of species complexes that it subsequently became possible to describe based on their mito-chondrial DNA sequences [4-8]. Irma co-authored the description of five new stingray species [7, 8] and the resurrection of another [9]. (Credit: P. Borsa/IRD, 24 May 2011).

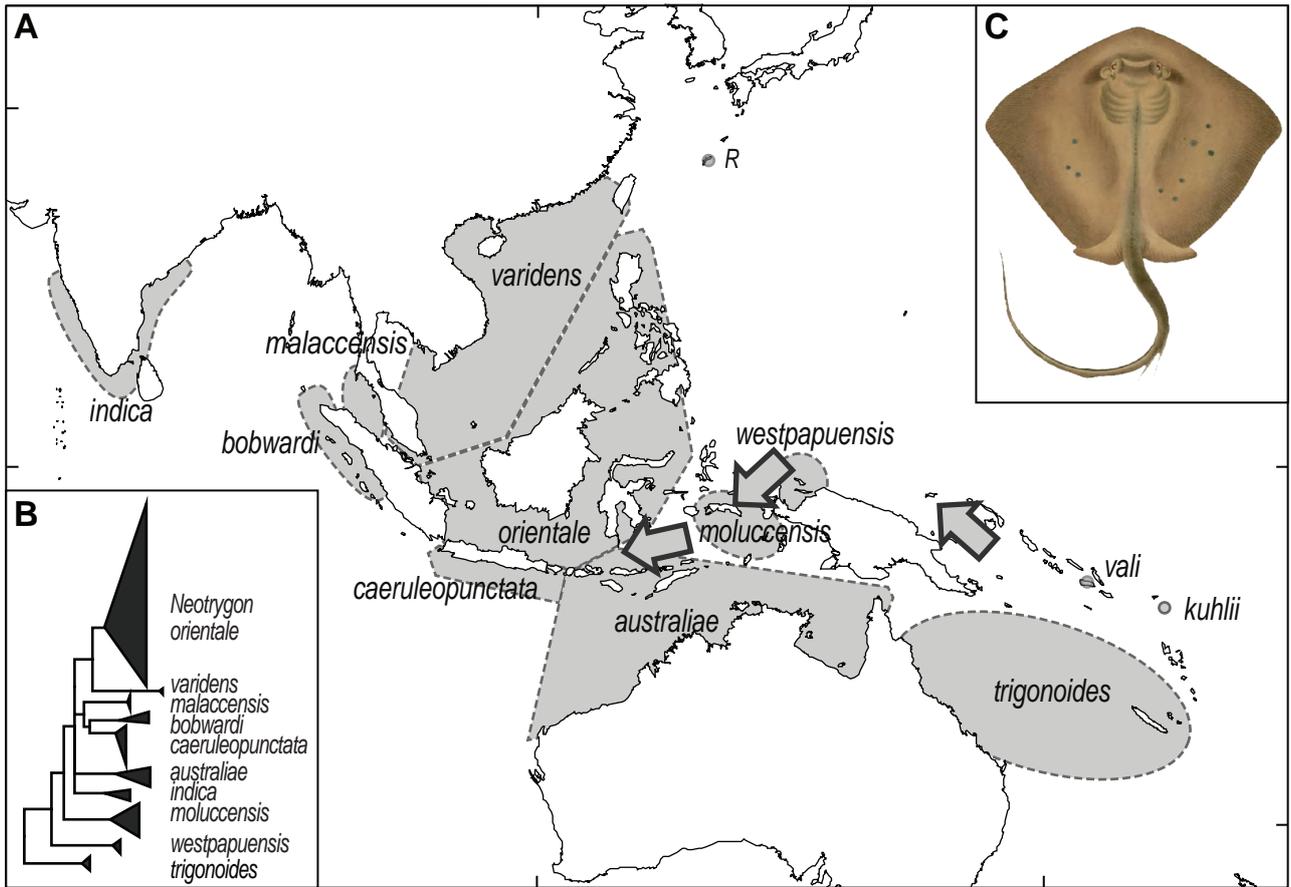


Figure 2. **A.** Parapatric distribution of species in the blue-spotted maskray species complex, *Neotrygon* spp. (modified from [8]). Dotted lines delineate the presumed extent of occurrence of each species; arrows symbolize the stepwise colonization of the Indo-West Pacific by the blue-spotted maskray, as inferred from phylogenies [19, 21, 24]; R Ryukyu maskray [21]. **B.** Simplified phylogenetic tree of the blue-spotted maskray species complex (edited from [8]). **C.** Lithography of “*Trygon kuhlii*” as published by J. Müller and F.G.J. Henle [26], which their description of the species was obviously based on. In an e-mail to P. Borsa, M.J.P. van Oijen from the Naturalis museum, Leiden revealed that this lithography was a faithful reproduction of a watercolour by Nagasaki-based Japanese artist K. Keiga ca. 1830 [20].