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

Neotrygon indica *sp. nov., la raie masquée à points bleus de l'océan Indien (Myliobatoidei, Dasyatidae)*

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ABSTRACT

The blue-spotted maskray, previously *N. kuhlii*, 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 and two (Indian-Ocean maskray and Ryukyu maskray) remain undescribed. Here the Indian-Ocean maskray is described as a new species, *Neotrygon indica* sp. nov. Specimens of the new species were generally characterized on their dorsal side by a moderately large number of small ocellated blue spots, a low number of medium-sized ocellated blue spots, the absence of large ocellated blue spots, a high number of dark speckles, a few dark spots, and a conspicuous occipital mark. The new species formed a distinct haplogroup in the tree built from concatenated nucleotide sequences at the *CO1* and *cytochrome-b* loci. A diagnosis based on colour patterns and nucleotide sequences at the *CO1* and *cytochrome b* loci is proposed. The distribution of *N. indica* sp. nov. includes the Indian coast of the Bay of Bengal, the Indian coast of the Laccadives Sea, and Tanzania. Considerable sampling effort remains necessary for an in-depth investigation of the phylogeographic structure of the Indian-Ocean maskray..

Keywords: New species; Mitochondrial DNA; Diagnosis; Distribution

RÉSUMÉ

La raie pastenague masquée à points bleus, précédemment *N. kuhlii*, comprend jusqu'à onze lignées représentant des espèces distinctes. Neuf de ces espèces (*N. australiae*, *N. bobwardi*, *N. caeruleopunctata*, *N. malaccensis*, *N. moluccensis*, *N. orientale*, *N. vali*, *N. varidens*, *N. westpapuensis*) ont déjà été formellement décrites et deux (la raie masquée de l'Océan Indien et la raie masquée des Ryukyu) restent non décrits. Ici, la raie masquée de l'Océan Indien est décrite comme une nouvelle espèce, *Neotrygon indica* sp. nov. Les spécimens de la nouvelle espèce sont généralement caractérisés sur la face dorsale par un nombre modéré de petites ocelles bleus, un faible nombre d'ocelles bleus de taille moyenne, l'absence d'ocelles bleus de grande taille, un grand nombre de petits points sombres, quelques gros points sombres et une marque occipitale visible. La nouvelle espèce forme un haplogroupe distinct dans l'arbre des séquences nucléotidiques concaténées aux locus *COI* et *cytochrome b*. Une diagnose basée sur les patrons de couleur et les séquences nucléotidiques aux locus *COI* et *cytochrome b* est proposée. La distribution de *N. indica* sp. nov. inclut la côte indienne du golfe du Bengale, la côte indienne de la mer des Laquedives et la Tanzanie. Un effort d'échantillonnage important reste nécessaire pour aborder de façon approfondie la structure phylogéographique de la raie masquée à points bleus de l'océan Indien.

Mots-clés : Nouvelle espèce ; ADN mitochondrial ; Diagnose ; Distribution

1. Introduction

Describing species is important to advancing biodiversity research at the local, regional and broader scales because species usually are the fundamental units in community ecology, biogeography, and conservation. To describe species, taxonomists typically use morphology as primary evidence and increasingly often, genetic information to support morphology [1]. Although genetics have proven essential to delineate species in many instances, genetic information such as allele frequencies or DNA sequences is still exceptionally used as primary evidence in new species descriptions, in part if not mostly because of considerable peer pressure against DNA-based descriptions [1]. However, there is no objective reason to dismiss genetics as the primary source of characters for species delineation, description, and identification [1-3].

The blue-spotted maskray, previously *N. kuhlii* (Müller and Henle 1841), has a wide Indo-West Pacific distribution, from eastern Africa to Japan [4-7]. It was hypothesized to be a species complex after a barcoding survey revealed several deeply divergent mitochondrial lineages [8]. The hypothesis of a species complex was subsequently validated [6,9]. This species complex consists of up to eleven parapatrically-distributed lineages representing separate species [6,9-12] of which nine have already been formally described [7,12-14]. These are *N. australiae* Last, White and Séret 2016, *N. bobwardi* Borsa, Arlyza, Hoareau and Shen 2017, *N. caeruleopunctata* Last, White and Séret 2016, *N. malaccensis* Borsa, Arlyza, Hoareau and Shen 2017, *N. moluccensis* Borsa, Arlyza, Hoareau and Shen 2017, *N. orientale* Last, White and Séret 2016, *N. vali* Borsa 2017, *N. varidens* (Garman 1885), and *N. westpapuensis* Borsa, Arlyza, Hoareau and Shen 2017. Two other species in the genus, the nominal *N. kuhlii* from Vanikoro and *N. trigonoides* possess distinctive spot patterns [3,15,16] that tell them apart from the blue-spotted maskray as it was originally described by J. Müller and F.G.J. Henle [4]. Based on the only available information on colour patterns, one cannot exclude that *N. kuhlii* as it has been redefined by Last and co-authors [14] and *N. trigonoides* are synonyms [16]. The genetically distinctive Indian-Ocean maskray reported in the recent phylogeographic literature [6,7,10] remains undescribed.

Some authors have attempted to use morphological characters as primary information for the description of cryptic species in the blue-spotted maskray complex [14] and a recent revision has ostensibly ignored genetic evidence (e.g., [17]) even though not a single morphological character among those utilized for the description or redescription of four species in the complex [14] was undisputably diagnostic of any of the species [7]. Also, the apportion of environmental vs. genetic determination in these morphological characters had not been evaluated [7]. Morphological diagnoses provided so far for the blue-spotted maskray [14] are therefore hardly relevant, as previously mentioned for the *Himantura uarnak* complex, another species complex among stingrays [3]. In contrast, DNA sequences offer a profusion of diagnostic characters in stingrays [3,6,9,14,18]. Species-specific haplogroups were observed in the blue-spotted maskray, based on DNA sequences [3,9].

The objective of the present paper is to formally describe the Indian-Ocean maskray, primarily based on the mitochondrial DNA sequences of fresh specimens from the eastern coast of India and of previously-reported material from India and Tanzania.

2. Materials and Methods

2.1. Material examined

Eleven specimens of the new species that were examined for spot patterns are listed in Table 1 (their pictures are provided Supplementary Fig. S1). Specimen NKGMF-3 from the Gulf of Mannar, January 2017, 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. Specimen NKGMF-1 from the Gulf of Mannar, January 2017, to be chosen as paratype of the new species, was deposited at the Fish Genetics and Biotechnology (FGB) laboratory, ICAR-Central Institute of Fisheries Education (CIFE) in Mumbai, India. Specimens NITT1, NITT2 and NITT3 were collected in Tharivaikulum, Tuticorin, India, December 2017; all three specimens are preserved in formalin at the FGB laboratory ICAR-CIFE in Mumbai. Specimen IRD-20170720-A, purchased fresh from fishermen at the Malindi fish landing place in Zanzibar, July 2017, was preserved salted; it is currently stored at the IRD-La Valette zootheque in Montpellier, France. Voucher material of the new species also includes a specimen collected by APK and colleagues in Visakhapatnam, Andhra Pradesh, Bay of Bengal on 15 August 2011. The specimen was discarded but a sub-sample of tissue was registered at the Central Institute of Fisheries Education , Mumbai under no. VIZNK-01. A photograph of this individual is available from the Barcoding of Life Data systems database (BOLD; http://www.barcodinglife.com/; [20]) under accession no. BOLD:ACB9305. Photographs of blue-spotted maskrays from India deposited in FishBase [21] were not retained for the analysis of spot patterns because of insufficient resolution. Comparative material for the characterization of spot patterns consisted of specimens from *N. australiae* (*N* = 2, including holotype), *N. bobwardi* (*N* = 5, including holotype), *N. caeruleopunctata* (*N* = 2, including a paratype), *N. malaccensis* (*N* = 1, holotype), *N. moluccensis* (*N* = 2, including holotype and single paratype), *N. orientale* (*N* = 8, including holotype), *N. vali* (*N* = 3, including holotype), *N. varidens* (*N* = 6), and *N. westpapuensis* (*N* = 1, holotype) (Table 1).

Five specimens, including four from India and one from Tanzania were measured according to [14] using a ribbon meter, a ruler, and a vernier caliper. Three of these, including the holotype and the paratype of the new species were X-ray photographed. Pectoral-fin rays were counted from the X-ray photographs. Specimens of the new species that were characterized by their nucleotide sequences at loci *CO1* and *cytochrome b* are listed in Table 2 and include 13 specimens from India and 6 specimens from Tanzania. Comparative material for the analysis of nucleotide sequences included blue-spotted maskray specimens listed in [9] and an additional specimen from Taiwan deposited in the fish collections of Academia Sinica, Taipei, and registered under no. ASIZP0805866.

2.2. Spot pattern analysis

The diameter of ocellated blue spots on the dorsal side of the left and right pectoral fins, relative to disc width (DW), was measured from photographs ([7,12,14,15]; Figs. 1 and 2; Supplementary Fig. S1). Ocellated blue spots were qualified as "small" when their maximum diameter was ≤ 2% DW, "medium" when ≤ 4% DW and "large" when > 4% DW [15]. Dark speckles (≤ 1% DW) and dark spots (> 1% DW) were also counted on the dorsal surface of the disc. The counts did not include those speckles and spots located within the dark band around eyes that forms the mask [15]. The presence or absence of a darker occipital mark at the rear end of the neurocranium was also checked and categorized as "absent", "weak", or "conspicuous". Spot patterns were compared among individuals through correspondence analysis (CA) [22]. CA was run using the FACTOMINER package [23] under R [24].

2.3. Analysis of nucleotide sequences

The DNA of six specimens of the new species and of specimen ASIZP0805866 from Taiwan was extracted using the phenol-chloroform protocol and the DNEasy extraction kit (Qiagen GmbH, Hilden, Germany), respectively. A fragment of each the *CO1* and the *cytochrome b* genes was amplified by polymerase chain reaction according to [9]. Nucleotide sequencing was done in both forward and reverse directions using the Sanger method. The consensus sequences were obtained after assembling the direct and reverse traces under BIOEDIT v. 7.1.11 [25]. Sequences were

then edited and aligned under BIOEDIT. We chose as reference for numerotating nucleotides the complete mitogenome sequence of *Neotrygon orientale* [26], accessible from GenBank (http:// www.ncbi.nlm.nih.gov) under no. KR019777. Five out of 11 *N. indica* sp. nov. individuals characterized by their spot patterns (Table 1) had their nucleotide sequences at loci *CO1* and/or *cytochrome b* examined (Table 2).

The phylogeny of concatenated *CO1*+*cytochrome b* gene haplotypes was inferred using the maximum-likelihood (ML) method under MEGA6 [27]. The most likely nucleotide-substitution model, which was determined according to the Bayesian information criterion, was the Tamura-Nei model [28] (TN93) where a discrete Gamma distribution (Γ = 0.76) was used to model evolutionary rate differences among nucleotide sites and invariable sites were allowed. The ML tree was rooted by choosing New Caledonian maskray *N. trigonoides* as outgroup [15]. The robustness of nodes in the tree was tested by bootstrap resampling.

Based on the larger number of *CO1* gene sequences available ([9]; Table 2), a parsimony network was constructed to further explore the relationships among haplotypes of the new species, and with those of the geographically adjacent species *N. bobwardi*, *N. caeruleopunctata* and *N. malaccensis*. Median-joining parsimony analysis was done using NETWORK [29] on the nucleotide sequence matrix of 57 individual *CO1* gene haplotypes compiled from the present work and from previous reports [9], using the default settings of the program. This matrix comprised sequences from blue-spotted maskrays from India (N = 11) and Tanzania (N = 5) as well as from the geographically adjacent *N. bobwardi* from the Andaman Sea and Western Sumatera (N = 13), *N. caeruleopunctata* from southern Java and southern Bali (N = 18), and *N. malaccensis* from the Malacca Strait and the eastern Andaman Sea (N = 10). Prior to median-joining analysis, the sequence matrix was trimmed to a core length of 638 bp, between nucleotide sites 68 and 705 of the *CO1* gene. The placement of the root was inferred from the maximum-likelihood tree of concatenated *CO1* + *cytochrome-b* gene sequences. Parsimony network analysis was similarly run on a matrix of 48 *cytochrome b* gene sequences, trimmed to a core length of 795 bp, between nucleotide sites 238 and 1032 of the *cytochrome b* gene. This comprised six sequences from India and five sequences from Tanzania (Table 2), as well as seven *N. bobwardi*, 17 *N. caeruleopunctata*, and 13 *N. malaccensis*.

Mean nucleotide distances within and between species were estimated from the same 57-individual *CO1* and 48-individual *cytochrome-b* sequence datasets using MEGA6. According to the Bayesian information criterion, the best substitution model for the *CO1* gene sequence dataset was K2+G (Γ = 0.07). The best substitution model for the *cytochrome b* sequence data was HKY+G (Γ = 0.05), but the model used for the calculations was the second-best (TN93+G) which, unlike the former, is available in MEGA6.

2.4. Barcode index number assignment

The BOLD datasystems distinguishes clusters of sequences that qualify as operational taxonomic units, i.e. putative species using the Refined Single Linkage algorithm [30]. 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. [7] have established the homology of BIN numbers with cryptic blue-spotted maskray lineages by visual inspection of the placement of the *CO1* gene sequences retrieved from BOLD in a reference maximum-likelihood tree rooted by *N. trigonoides*.

2.5. Notice

The present article in portable document (.pdf) format is a published work in the sense of the International Code of Zoological Nomenclature [31] or Code and hence the new names contained herein are effectively published under

the Code. This published work and the nomenclatural acts it contains have been registered in ZooBank (http://zoobank.org/), the online registration system for the International Commission on Zoological Nomenclature. The ZooBank life science identifier (LSID) for this publication is urn:lsid:zoobank.org:pub:10551DF9-4B93-40ED-8910-012C5DCF8B96. The information associated with this LSID can be viewed through any standard Internet browser by appending the LSID to the prefix "http://zoobank.org/". The online version of the present paper is archived and available from the *Comptes Rendus Biologies* (www.sciencedirect.com/science/journal/ aip/16310691), *bioRxiv*, Cold Spring Harbor NY, U.S.A. (http://www.biorxiv.org/) and *haL-IRD*, France (http://www.hal.ird.fr/) repository websites.

3. Results and Discussion

3.1. Spot patterns

Spot patterns of 11 *N. indica* sp. nov. specimens were summarized and compared to nine other species of the blue-spotted maskray complex (Table 1). *N. indica* sp. nov. specimens from India were characterized by a moderately large number of small ocellated blue spots (n = 15-57), a generally low number of medium-sized ocellated blue spots (n = 0-34), a total absence of large ocellated blue spots, a high number of dark speckles (n = 23-176), generally a few dark spots (n = 0-11; only exceptionally present in the other species examined), and a conspicuous occipital mark. These features were visible on the holotype (Fig. 1) and the paratype (Fig. 2) of the new species. One of the two individuals from Tanzania had similar features, but not the other (Table 1). Incidentally, all five *N. varidens* specimens examined here possessed at least one, to several (n = 1-23) small ocellated blue spots, at variance with Garman's [13] original description, which states: "The species resembles *D. kuhlii*, but has […] no spots." As the specimens examined here originated from Taiwan and not from Hong Kong, the type locality of *N. varidens*, it is possible that geographic variation in spot patterns exists within *N. varidens*. Four out of five *N. varidens* specimens remained distinct from all the other blue-spotted maskrays by the absence of dark speckles and spots (Table 1). *Neotrygon varidens* was also distinct from all its congeners except *N. indica* sp. nov. by the frequent absence of medium-sized ocellated blue spots (Table 1).

Correspondence analysis run on the whole matrix of individuals x characters (Table 1) plotted *N. indica* sp. nov. specimens as a cluster mostly distinct from the other species previously referred to as *N. kuhlii*, albeit with some overlap (not shown). A second run focused on the specimens from the Indian Ocean, including all nine *N. indica* sp. nov. specimens from India, the two specimens from Zanzibar, and all available specimens of *N. bobwardi*, *N. caeruleopunctata* and *N. malaccensis*. Again, *N. indica* sp. nov. formed a cluster mostly distinct from the other species (Fig. 3; Supplementary Fig. S2). *Neotrygon indica* sp. nov. specimens tended to have a higher count of dark spots and dark speckles than the other three species (Fig. 3).

As shown by our results, some overlap in spot patterns was observed between some pairs of species. Also, one expects that with increased geographic sampling, based on the current sample sizes, more variation will show up within a species, hence further blurring the distinction between species and further relativizing diagnoses based on spot patterns.

3.2. Genetic distinctness of the Indian-Ocean blue-spotted maskray

It has been recently reported that blue-spotted maskray populations from the Indian Ocean west of Bali (including *N. bobwardi* and *N. indica* sp. nov.) were "unresolved" but hypothesized that they were closely related to *N.*

caeruleopunctata [14]. Also, a single same BIN number (BOLD: AAA5611) has so far been allocated by BOLD to *N. caeruleopunctata*, *N. indica* sp. nov. and *N. malaccensis* [7] suggesting that the level of genetic differentiation at the *CO1* locus is not sufficiently clearcut to raise the three species, together with the closely related *N. bobwardi* to the level of fully distinct operational taxonomic units in the sense usually understood by the barcoding community. More in-depth investigation of the genetic relationships among populations within this group of geographically adjacent species from the Indian Ocean was warranted. Here, *Neotrygon indica* sp. nov. haplotypes formed a distinct haplogroup on the maximum-likelihood phylogenetic tree of concatenated *CO1*+*cytochrome b* gene sequences (Fig. 4), confirming previous results [9]. A close-up within this haplogroup (Supplementary Fig. S3) revealed possible within-species phylogenetic structure, as it cannot be excluded that the Indian-Ocean maskray comprises at least three subclades, including two from India and one from Tanzania. Testing this hypothesis would require more comprehensive and denser sampling of the wide Indian Ocean. Future, additional sampling effort is needed to investigate the phylogeographic structure of the Indian-Ocean maskray in more depth.

Maximum-likelihood trees based on nucleotide sequences at the *CO1* locus or the *cytochrome-b* locus grouped *N. indica* sp. nov. haplotypes into a monophyletic lineage (Supplementary Fig. S4) or two poorly resolved haplogroups, where the haplotypes sampled in Visakhapatnam formed a distinct sub-clade (Supplementary Fig. S5). These minor differences in the topologies of the two trees presumably arose because of insufficient phylogenetic information borne by either gene fragment when considered separately. Nevertheless, the *CO1* and *cytochrome-b* median-joining parsimony networks (Fig. 5) both distinguished *N. indica* sp. nov. as a haplogroup separate from the other, geographically adjacent species. It is worth mentioning that in both *CO1*- and *cytochrome b*-gene networks, haplotypes from Tanzania formed a distinct haplogroup within *N. indica* sp. nov. (Fig. 5).

Estimates of genetic distance between pairs of species in the Indian Ocean blue-spotted maskray group ranged from 0.020 to 0.025 at the *CO1* locus and from 0.020 to 0.033 at the *cytochrome b* locus (Table 3). These values were all well above the highest estimates of genetic distance within a species (respectively, 0.012 and 0.014; Table 3). Therefore, the four species albeit closely related remained distinct from one another using either the standard *CO1* barcode or the *cytochrome-b* marker.

So far, only two nuclear markers have been made available for maskrays *Neotrygon* spp. [6,9] and neither is variable enough to distinguish cryptic *Neotrygon* spp. as reciprocally monophyletic lineages. From the nuclear results synthesized by [9], it was nevertheless possible to distinguish *Neotrygon indica* sp. nov. from *N. australiae*, *N. leylandi*, *N. moluccensis*, *N. orientale*, *N. picta*, *N. trigonoides*, *N. varidens* and *N. westpapuensis*, but not from *N. caeruleopunctata* and *N. malaccensis*.

3.3. Mitochondrial DNA sequences as reliable taxonomic characters

Here, useful characters to distinguish *N. indica* sp. nov. from its blue-spotted maskray congeners derived from, in part (1) the analysis of spot patterns and, more accurately (2) molecular genetics. Although spot patterns proved somewhat helpful to tentatively characterize species including *N. indica* sp. nov., they were not fully diagnostic. Therefore, we chose to primarily base the description and diagnosis of the new species on the more powerful nucleotide sequence of its mitochondrial DNA, as done previously with other species of the genus *Neotrygon* [7,12,15]. We have shown that meristics and morphometrics the way they were used in taxonomic descriptions of cryptic species in the blue-spotted maskray complex [14] hardly provided any diagnostic character [7]. Part of the problem may stem from the fact that so far individual values of meristic and morphometric parameters have not been made available to the community except for a few type specimens [14]. Values averaged over a number of individuals [14] suppress information on inter-individual variation and are of limited interest in multivariate meristics and

morphometrics. Here we present morphometric values for four specimens from Tuticorin, India and one specimen from Zanzibar, Tanzania (Table 4), as a contribution to a future morphometric database of *Neotrygon* spp. specimens. We also present counts of pectoral-fin rays for three specimens from Tuticorin (see Supplementary Figs. S6-S8). It is hoped that in the future the database will include a sufficiently high number of specimens from this and other species in the genus *Neotrygon* so as to enable, if needed, meaningful morphometric analyses.

4. Taxonomy

Neotrygon Castelnau 1873 [32]. Characterized by a mask-shaped colour pattern around the eyes, a narrow internasal curtain, and a short tail that possesses well-developed dorsal and ventral skin folds [33]. Forms a distinct clade in the mitochondrial tree of Dasyatidae [6,18,26].

Blue-spotted maskray complex, *Neotrygon* spp., previously *N. kuhlii*. Characterized by polygonal shape and blue spots [4]. Blue-spotted maskray haplotypes cluster as a single clade in the mitochondrial phylogeny of the genus (figure 2b of [6]; [12]).

Neotrygon indica sp. nov. (Fig. 1A, B; Fig. 2; Supplementary Fig. S6); urn:lsid:zoobank.org:act:99F4F1A4-D5F6-4379-BC4E-7FB1E74CFA58. *Trygon kuhlii* [5]; presumably clade *Neotrygon kuhlii 3* of Naylor et al. [18]; *Neotrygon kuhlii* [15]; *Neotrygon kuhlii* haplogroup *I* [10,11]; *Neotrygon kuhlii* clade *8* [6]; Clade *I* [9]; Indian-Ocean maskray [7]. Also BIN number BOLD:AAA5611 in BOLD.

4.1. Material examined

All specimens examined to characterize spot patterns are listed in Table 1. Specimens characterized morphometrically are listed in Table 4. The list of specimens characterized by their nucleotide sequence at mitochondrial loci *CO1* and *cytochrome b* is provided as appendix A of [9]. Additional *N. indica* sp. nov. specimens sequenced at the *CO1* and *cytochrome b* loci are listed in Table 2.

4.2. Types

A female specimen 236 mm disc width (Fig. 1A) from the Gulf of Mannar, Tamil Nadu (9.12°N 79.46°E), 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 was deposited by APK at the MBRC in Chennai, India on 12 May 2017. The specimen has registration no. ZSI/MBRC/F.1495

A female specimen 213 mm disc width (Fig. 2) fished from the Gulf of Mannar, Tamil Nadu (9.12°N 79.46°E) using bottom-set gillnets, collected by RK on 25 January 2017 is here designated as the single paratype of the new species. This specimen, labelled CIFEFGB/NKGM1 was subsequently deposited by APK at the Fish Genetics and Biotechnology laboratory, ICAR-Central Institute of Fisheries Education, Mumbai, India.

4.2. Description of holotype

The partial *CO1* gene sequence of the holotype, comprised between nucleotide sites 51 and 722 of the gene, was
5'- C A C C C T T T A T T T A G T C T T T G G T G C A T G A G C A G G G A T A G T A G G C A C T G G
C C T C A G T T T A C T T A T C C G A A C A G A A C T A A G C C A A C C A G G C G C T T T A C T G G
G T G A T G A T C A A A T T T A T A A T G T A A T C G T C A C T G C C C A C G C C T T C G T A A T A

A T C T T C T T T A T A G T A A T G C C A A T T A T A A T T G G T G G A T T T G G T A A C T G A C T
A G T G C C C C T G A T A A T T G G G G C T C C G G A C A T A G C C T T T C C A C G A A T A A A C
A A C A T A A G T T T T T G A C T T C T A C C T C C C T C A T T C C T A T T A C T G C T A G C C T C A
G C A G G A G T A G A A G C C G G G G C C G G A A C A G G T T G A A C A G T T T A T C C C C C A T
T A G C T G G T A A T C T A G C A C A T G C C G G A G C T T C T G T A G A C C T T A C A A T C T T C
T C T C T T C A C C T A G C A G G T G T T T C C T C T A T T C T G G C A T C C A T C A A C T T T A T C
A C A A C A A T T A T T A A T A T A A A A C C A C C T G C A A T C T C C C A G T A T C A A A C C C C
A T T A T T C G T C T G A T C T A T T C T T G T T A C A A C T G T A C T T C T C C T G C T A T C C C T
A C C A G T C T T A G C A G C T G G C A T T A C T A T A C T C C T C A C A G A C C G A A A T C T T A
A T A C A A C T T T C T T T G A C C C A G C T G G G C G A G G A G A T C C C A T T C T T T A C C A A
C A C C T C T T C T G A T T C T T T G G C C A – 3'. This sequence has no. MG017600 in GenBank.

The partial *cytochrome b* gene sequence of the holotype, comprised between nucleotide sites 238 and 1032 of the gene, was 5'- A T C C G C A A T A T T C A C G C T A A C G G C G C C T C A A T A T T C T T C A T C T G T
G C T T A T C T C C A T A T T G C T C G A G G A C T T T A C T A T G G C T C C T A C C T C A A T A A
A G A A A C C T G A A A C A T C G G A G T A A T T A T C C T A G T G T T A C T A A T A G C C A C C G
C A T T T G T A G G C T A T G T T C T C C C A T G A G G A C A A A T A T C A T T C T G A G G G G C
A A C C G T T A T T A C C A A C T T G C T A T C A G C C C T C C C C T A T A T T G G A G A C A T G T
T A G T T C A A T G A A T C T G A G G A G G C T T C T C A A T T G A C A A T G C A A C A C T A A C T
C G A T T T T T C A C A T T T C A T T T T C T A T T T C C C T T T G T A A T T G C A G C T C T T A C T
A T A A T T C A C C T T C T C T T C C T T C A T G A A A C A G G T T C T A A C A A C C C A A C C G G
A C T C T C A T C C A A C A T A G A C A A A A T C C C G T T T C A T C C T T A T T A T A C A T A T A A
A G A T C T A G T A G G C T T C T T C A T C C T T C T A A T A C T A C T A A C T C T A C T T G C C T T
A T T T A C A C C A A A C C T C C T A G G A G A T A C A G A A A A C T T T A T C C C A G C C A A C C
C C C T C G T C A C A C C T C C C C A T A T T A A A C C A G A G T G A T A C T T C T T A T T T G C C T
A C G C T A T T C T A C G C T C C A T C C C C A A T A A A C T A G G A G G A G T C C T A G C C C T C
G C C T T C T C A A T C T T T A T C C T G C T A C T A G T C C C C A T T C T T C A C A C C T C C A A A
C A A C G A A G C C T C A C C T T C C G T C C A A T T A C A C A A C T C C T A T T C T G A C T C T T A
G T G G C A A A T A C A A T C A T C C T A A C A T G A A T C G G C G G C C A A C C C G T A -3'. This sequence has no. MG017605 in GenBank.

Ocellated blue-spot, dark-spot and dark-speckle counts on the dorsal side of the holotype are presented in Table 1. The meristic details of the holotype, which were scored from X-ray photographs (Supplementary Fig. S6), were the following: number of pectoral-fin radials 106-108 including propterygion 47-51, mesopterygion 12 and metapterygion 45-47. Morphometric measurements on the holotype are presented in Table 4.

4.3. Measurements on paratype

Similarly, spot pattern details on the dorsal side of the paratype are presented in Table 1 and its meristic details were scored from X-ray photographs (Supplementary Fig. S7). The number of pectoral-fin radials was 108-110 including propterygion 45-47, mesopterygion 14-15 and metapterygion 47-50. Morphometric measurements are presented in Table 4.

4.4. Diagnosis

Neotrygon indica sp. nov. from India is 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. However, this diagnosis did not apply to the two specimens sampled from Zanzibar whose spot patterns were examined. *Neotrygon indica* sp. nov. is distinct from *N. kuhlii* and *N. trigonoides* by the absence of the pair of conspicuous scapular blotches characteristic of these two species [15,16], although specimens of *N. indica* sp. nov. often possess a pair of diffuse darker marks in the scapular region.

Fifteen out of 19 individuals of *N. indica* sp. nov. sequenced at the *CO1* locus 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 (supplementary table S1 of [7]; present study). *Neotrygon indica* sp. nov. was further distinguished from other species previously under *N. kuhlii* by A at nucleotide site 70 of the *cytochrome b* gene (based on 5 individuals from Tanzania). Eight out of 11 individuals of *N. indica* sp. nov. also possessed T at nucleotide site 284 of the *cytochrome b* gene, a character that was otherwise present in only one out of 77 *N. orientale* individuals and absent in all other cryptic species of the blue-spotted maskray (supplementary table S2 of [7]; present study).

4.5. Distribution

The type locality of *N. indica* sp. nov. is the Gulf of Mannar in Tamil Nadu on the eastern 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. also includes the Indian coast of the Laccadives Sea (Kerala) and Tanzania ([6,7,9,10]; present study).

4.6. Etymology

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

4.7. 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).

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.crvi.2018.xx.xxx.

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Table 1

Spot patterns on the dorsal side of 41 blue-spotted maskray (*Neotrygon* spp.) specimens sorted by species. Specimens from Padang and Kota Kinabalu, two specimens from the Solomon Islands [12] as well as ASIZP0073604, ASIZP0073605 and ASIZP0078458 were identified to species based on sampling location [9]; all other specimens were identified to species based on their *CO1* gene sequence [9]. *L*, left pectoral fin; *R*, right pectoral fin. *Occipital mark*, diffuse 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, country	*N* ocellated blue spots			*N* dark speckles (<1% DW)	*N* dark spots (>1% DW)	Occipital mark
		1. Small L, R	2. Medium L, R	3. Large L, R	L, R	L, R	
N. australiae							
CSIRO 7016-01 [a]	Weipa, Australia	14, 12	17, 20	3, 0	8, 9	0, 0	0
MZB-20863 (ENTTJS2)	Tanjung Sulamo, Indonesia	25, 25	28, 32	1, 0	13, 6	0, 0	0
N. bobwardi							
MZB-20843 (ME3) [a]	Meulaboh, Indonesia	28, 29	13, 11	0, 0	16, 16	0, 0	0
20150524-D	Padang, Indonesia	5, 11	4, 2	0, 0	6, 6	0, 0	0
20150524-E	Padang, Indonesia	16, 11	9, 8	0, 0	8, 15	0, 0	2
20150524-F	Padang, Indonesia	20, 36	14, 8	0, 0	3, 0	0, 0	1
20150524-11	Padang, Indonesia	8, 8	2, 1	0, 0	6, 13	0, 0	2
N. caeruleopunctata							
CSIRO H 7850-01 [b]	Sadeng, Indonesia	25, 22	4, 1	0, 0	17, 30	0, 0	1
20080131_BL (NK-K-BL)	Bali, Indonesia	21, 24	6, 5	0, 0	43, 54	0, 0	1
MZB-22131 (BAL-S)	Bali, Indonesia	31, 22	5, 9	0, 1	0, 1	0, 0	1
N. indica sp. nov.							
ZSI/MBRC/F.1495 (NKGMF-3) [a]	Gulf of Mannar, India	9, 10	0, 0	0, 0	19, 34	3, 2	2
CIFEFGB/NKGM1 (NKGMF-1) [b]	Gulf of Mannar, India	27, 26	0, 0	0, 0	37, 22	4, 1	2
VIZNK-01 (NKVSKP-1)	Visakhapatnam, India	3, 12	1, 9	0, 0	13, 10	0, 0	2
NKTTK-1	Tuticorin, India	30, 27	3, 3	0, 0	90, 86	0, 1	2
NKTTK-2	Tuticorin, India	20, 8	0, 0	0, 0	24, 32	0, 1	2
NKGMM-2	Gulf of Mannar, India	24, 26	7, 4	0, 0	30, 33	4, 1	2
NKGMM-4	Gulf of Mannar, India	14, 18	8, 4	0, 0	40, 38	5, 6	2
NITT-1	Tuticorin, India	28, 28	20, 14	0, 0	68, 73	1, 1	2
NITT-3	Tuticorin, India	10, 32	9, 3	0, 0	34, 35	1, 3	2
ZAN 1	Zanzibar, Tanzania	44, 51	29, 21	2, 2	10, 15	0, 0	0
20170720-A	Zanzibar, Tanzania	32, 35	6, 6	0, 0	16, 21	1, 0	2
N. malaccensis							
MZB-20847 (MSKL3) [a]	Kuala Lama, Indonesia	2, 4	14, 10	0, 1	5, 3	0, 0	0
N. moluccensis							
MZB-20866 (ARA1) [a]	Tual, Indonesia	36, 30	9, 9	0, 0	53, 53	0, 1	0
MZB-20864 (AM1) [b]	Ambon, Indonesia	9, 2	7, 12	0, 0	5, 2	0, 0	1
N. orientale							
CSIRO H7858-01 [a]	Muara Kintap, Indonesia	20, 14	15, 17	1, 1	2, 1	0, 0	0
MZB-20852 (PB2)	P. Pabelokan, Indonesia	5, 9	8, 3	1, 0	21, 17	0, 0	0
MZB-20851 (PR)	Pulau Pari, Indonesia	22, 18	9, 6	2, 2	12, 8	0, 0	0
MZB-20850 (PN5)	Pulau Peniki, Indonesia	12, 22	12, 11	2, 0	2, 1	0, 0	1
BO424	Tanjung Manis, Malaysia	35, 41	22, 6	0, 0	16, 13	0, 0	0
WJC-5367	Kota Kinabalu, Malaysia	17, 19	10, 8	0, 1	8, 4	0, 0	0
WJC-5368	Kota Kinabalu, Malaysia	19, 20	6, 12	0, 0	6, 5	0, 0	0
WJC-5369	Kota Kinabalu, Malaysia	20, 23	14, 10	0, 0	3, 2	0, 0	0
N. vali							
CSIRO H7723-01 [a]	Honiara, Solomon Is.	2, 4	1, 1	0, 0	3, 6	0, 0	0
Randall ([19]: 18) [c]	Solomon Is.	11	4	0	6	0	0
Rosenstein [c]	Mbike wreck, Solomon Is.	21	6	0	7	0	0
N. varidens							
ASIZP0073604	Taiwan	0, 1	0, 0	0, 0	0, 0	0, 0	1
ASIZP0073605	Taiwan	3, 2	0, 0	0, 0	0, 0	0, 0	1
ASIZP0073535	Taiwan	3, 1	0, 0	0, 0	0, 0	0, 0	1
ASIZP0078458	Taiwan	7, 16	0, 0	0, 0	0, 0	0, 0	1
ASIZP0805866	Taiwan	4, 3	5, 6	0, 0	2, 7	0, 0	1
N. westpapuensis							
MZB-20867 (BK5) [a]	Biak, West Papua	41, 37	25, 13	0, 2	10, 7	0, 0	1

[a] Holotype; [b] Paratype; [c] Left side only

Table 2

Neotrygon indica sp. nov. specimens examined for nucleotide variation at mitochondrial loci *CO1* and *cytochrome b*.

Specimen no. (field no.)	Locality of collection, country	GenBank accession no.		Source
		CO1	*cytochrome b*	
NBFGR:CHN 156	Chennai, India	HM467799	-	Mined from GenBank
NBFGR:CHN:R24	Chennai, India	KF899609	-	Mined from GenBank
NBFGR:CHN:R25	Chennai, India	KF899610	-	Mined from GenBank
NBFGR:CHN:R165	Chennai, India	KF899611	-	Mined from GenBank
NBFGR:CHN:NK13	Chennai, India	KF899612	-	Mined from GenBank
NBFGR:CHN:199	Chennai, India	KF899613	-	Mined from GenBank
-	Tamil Nadu, India	KR003770	-	Mined from GenBank
ZSI/MBRC/F.1495 (NKGMF-3) [a]	Gulf of Mannar, India	MG017600	MG017605	Present study
NKGMM-2	Gulf of Mannar, India	MG017601	MG017606	Present study
NKVSKP-1 (=VIZNK-01)	Visakhapatnam, India	JX978329, MG017602	MG017607	Mined from BOLD, Present study
NKVSKP2	Visakhapatnam, India	MG017603	MG017608	Present study
NKVSKP3	Visakhapatnam, India	MG017604	MG017609	Present study
NKTTK1	Tuticorin, India	-	MG017610	Present study
ZANZ 1	Zanzibar, Tanzania	JX263421	KU497907	[10]
80611	Tanga, Tanzania	KC249906	-	[6]
KNS-TZN1-52	Pemba Island, Tanzania	KU498035	KU497908	[9]
KNS-ZAN3-86	Pemba Island	KU498036	KU497909	[9]
KNS-ZAN4-71	Pemba Island	KU498037	KU497910	[9]
KNS-ZAN5-80	Pemba Island	KU498038	KU497911	[9]

[a] Holotype

Table 3

Blue-spotted maskray *Neotrygon* spp. from the Indian Ocean and geographically adjacent Andaman Sea. Estimates (mean ± *SD*) of nucleotide distances between pairs of, and within blue-spotted maskray species at loci *CO1* and *cytochrome b* .

Marker, Species	Within-species	Species		
		N. indica	*N. bobwardi*	*N. caeruleopunctata*
CO1				
N. indica	0.012±0.003	-		
N. bobwardi	0.009±0.003	0.025±0.006	-	
N. caeruleopunctata	0.002±0.001	0.022±0.007	0.024±0.007	-
N. malaccensis	0.001±0.001	0.020±0.006	0.020±0.006	0.022±0.008
cytochrome b				
N. indica	0.014±0.004	-		
N. bobwardi	0.006±0.002	0.033±0.007	-	
N. caeruleopunctata	0.002±0.001	0.020±0.005	0.026±0.006	-
N. malaccensis	0.000±0.000	0.026±0.006	0.026±0.009	0.021±0.007

Table 4

Morphometric measurements [14] for four *Neotrygon indica* sp. nov. specimens from the eastern coast of India (holotype, paratype, NITT1, NITT2) and one specimen from Tanzania (IRD-20170720-A). All measurements, except disc width and number of caudal stings expressed as percentage of disc width.

Parameter	Specimen				
	Holotype	Paratype	NITT1	NITT2	IRD-20170720-A
Disc width (mm)	236	213	314	307	300
Total length	169.5	183.1	191.1	198.7	-
Disc length	80.5	84.5	79.6	81.4	80.4
Distance from tip of snout to pectoral fin insertion	69.9	70.4	73.9	76.5	71.9
Distance from tip of snout to eye	15.3	16.0	15.9	16.3	13.8
Mouth width	10.6	8.5	8.3	9.1	10.0
Nostril length	6.8	4.7	5.1	5.9	4.2
Distance between nostrils	11.9	14.1	9.6	10.7	7.5
Head length	40.3	39.9	39.8	42.3	40.8
Eye length	6.4	6.6	6.4	6.5	5.4
Orbit diameter	10.2	9.4	7.6	7.8	7.1
Inter orbital width	7.6	7.0	7.3	7.2	7.1
Inter ocular width	16.9	18.8	12.7	13.0	16.1
Spiracle length	7.6	8.0	7.6	7.2	7.1
Interspiracular width	14.8	15.0	12.7	14.3	15.6
Orbit and spiracle length	9.3	14.1	19.7	13.0	10.0
Orbit to pectoral fin insertion	55.9	46.9	51.0	53.7	52.2
Nasal curtain length	5.9	4.7	6.4	6.5	4.6
Nasal curtain width	9.3	8.5	8.9	9.1	9.4
Width of 1st gill slit	4.2	3.3	3.2	3.3	3.3
Width of 3rd gill slit	3.0	3.3	3.2	3.3	3.8
Width of 5th gill slit	1.7	2.3	2.2	2.3	2.1
Distance between 1st and 5th gill slits (left)	13.6	14.1	19.1	19.5	14.2
Distance between 1st and 5th gill slits (right)	13.6	14.1	13.4	13.0	14.2
Distance between 1st gill slits	16.9	16.9	14.9	16.2	17.5
Distance between 5th gill slits	11.0	9.5	8.3	8.4	9.6
Pre-nasal length of snout	9.3	5.7	9.6	9.8	5.6
Distance from tip of snout to cloaca origin	62.7	67.6	70.1	71.7	71.8
Distance from pectoral fin insertion to base of 1st sting	44.5	37.6	38.2	35.8	-
Distance from origin of cloaca to base of 1st sting	42.4	44.6	43.0	42.3	-
Cloaca length	7.2	6.6	7.3	7.5	4.6
Tail width at axil of pelvic fin	8.5	9.4	8.3	9.8	9.4
Tail width at base of 1st sting	2.3	6.6	3.8	4.6	-
Tail heighth at axil of pelvic fin	5.5	6.6	4.8	4.9	4.6[a]
Tail heighth at base of 1st sting	2.6	2.3	2.5	2.9	-
Pelvic fin (embedded) length	14.4	13.6	15.9	14.7	14.7
Width across pelvicfin base	19.9	18.8	19.1	17.9	22.1
Greatest width across pelvic fin	33.9	42.3	41.4	44.3	43.0
Clasper length from pelvic axil	-	-	15.6	16.6	-
Post-cloacal clasper length	-	-	20.4	22.8	-
Tail width at base of sting	3.4	3.8	7.6	7.8	-
Tail height at base of sting	2.5	2.8	3.8	3.9	-
Disc thickness	14.8	14.1	14.3	13.7	13.5[a]
Number of caudal stings	1	1	2	2	-
Length of 1st sting	-	14.1	16.2	15.0	-
Length of 2nd sting	-	-	14.3	18.6	-

[a] measured after specimen had been salted

Fig. 1. Holotype of *Neotrygon indica* sp. nov.: female specimen, 174 mm disc length from the Gulf of Mannar, Tamil Nadu, India (09.12°N 79.46°E) registered under no. ZSI/MBRC/F.1495 at the Marine Biology Regional Centre in Chennai, India. **A.** Dorsal side (photograph by RK). **B.** Oronasal region (photograph by APK).

Fig. 2. Paratype of *Neotrygon indica* sp. nov.: female specimen no. CIFEFGB/NKGM1, 182 mm disc length from the Gulf of Mannar, Tamil Nadu, India (9.12°N 79.46°E) (photograph by RK).

Fig. 3. Correspondence analysis: projection of individuals characterized by counts of ocellated blue spots and dark spots and speckles, and by intensity of occipital mark on the dorsal side of a half-disc. *Blue triangles* indicate the position of the variables used to characterize spot patterns: *1*. Small ocellated blue spots; *2*. Medium-sized ocellated blue spots; *3*. Large ocellated blue spots; *4*. Dark speckles; *5*. Dark spots; *6*. Occipital mark. *h* holotype; *z* sample from Zanzibar.

Fig. 4. Blue-spotted maskray *Neotrygon* spp. Simplified maximum-likelihood tree of concatenated *CO1+ cytochrome b* gene fragments, rooted by *N. trigonoides* [15] showing the placement of the *N. indica* sp. nov. lineage. Terminal branches were collapsed into triangles whose width is proportional to the number of individuals sequenced and whose depth represents the genetic variability within a species. Numbers at a node are bootstrap scores, from 600 bootstrap resampling runs under MEGA6 [27]. For the same tree with all terminal branches apparent, see Supplementary Table S3.

Fig. 5. Genetic relationships of *Neotrygon indica* sp. nov. relative to other blue-spotted maskray *Neotrygon* spp. from adjacent regions in the Indian Ocean, based on mitochondrial haplotypes. Symbols for species are the same as those of Fig. 2. **A.** Median-joining parsimony network [29] of *CO1* haplotypes (638-bp fragment of the *CO1* gene). Groups of haplotypes delineated according to genetic proximity, with indication of area of occurrence for *N. indica* sp. nov. Branch length is proportional to the number of mutational steps; circles represent individual haplotypes, their area being proportional to their frequency in the total sample. Arrow indicates possible placement of root, as inferred from the maximum-likelihood tree of concatenated *CO1* and *cytochrome-b* gene sequences (Fig. 4); numbers at nodes are bootstrap scores, obtained from the *CO1* maximum-likelihood phylogeny (Supplementary Fig. S4). Scale bar: 1 mutational step. **B.** Homologous median-joining parsimony network based on a 795-bp fragment of the *cytochrome b* gene. Bootstrap scores from Supplementary Fig. S5.

Figure 1 - Pavan-Kumar et al.

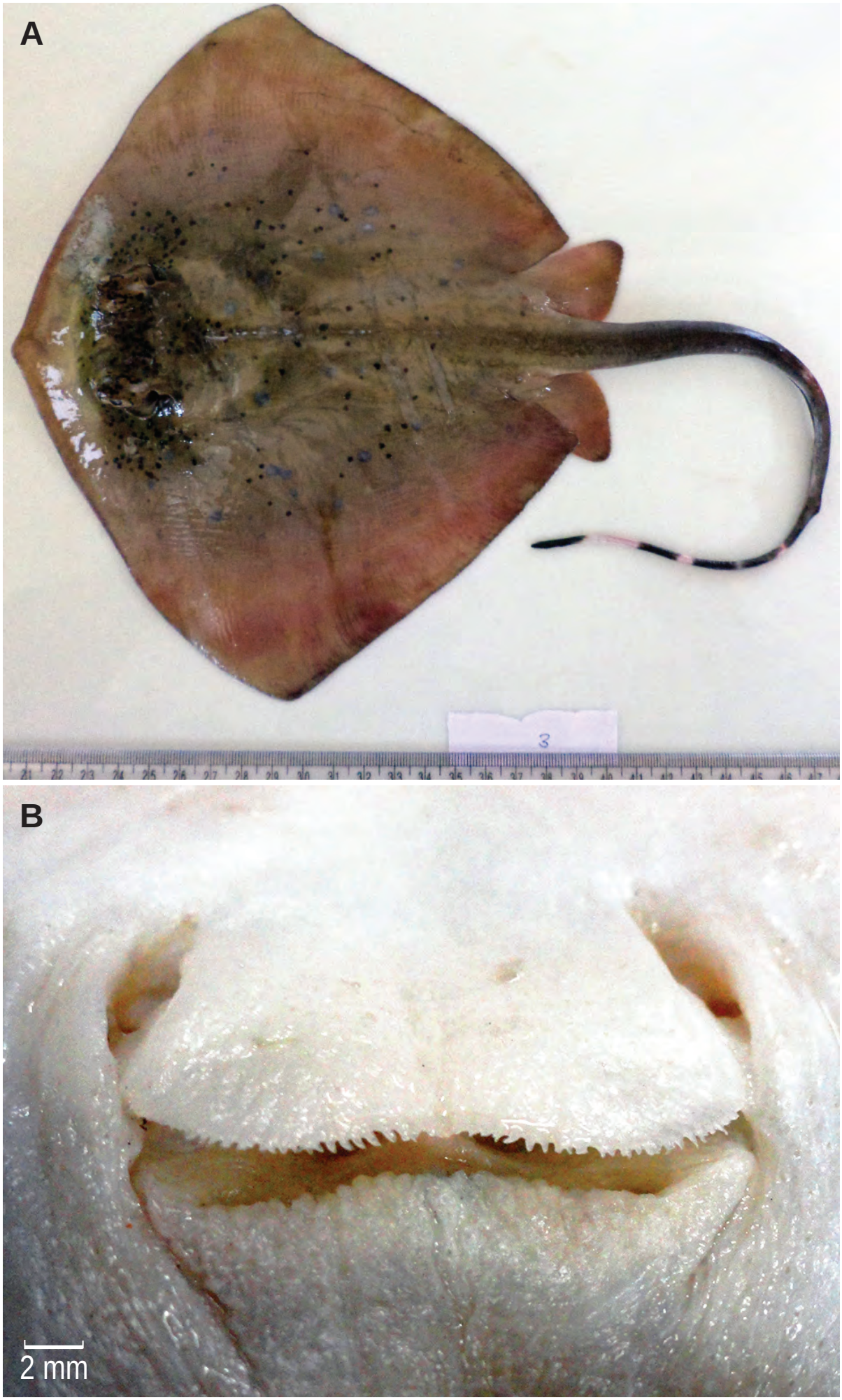


Figure 2 - Pavan-Kumar et al.

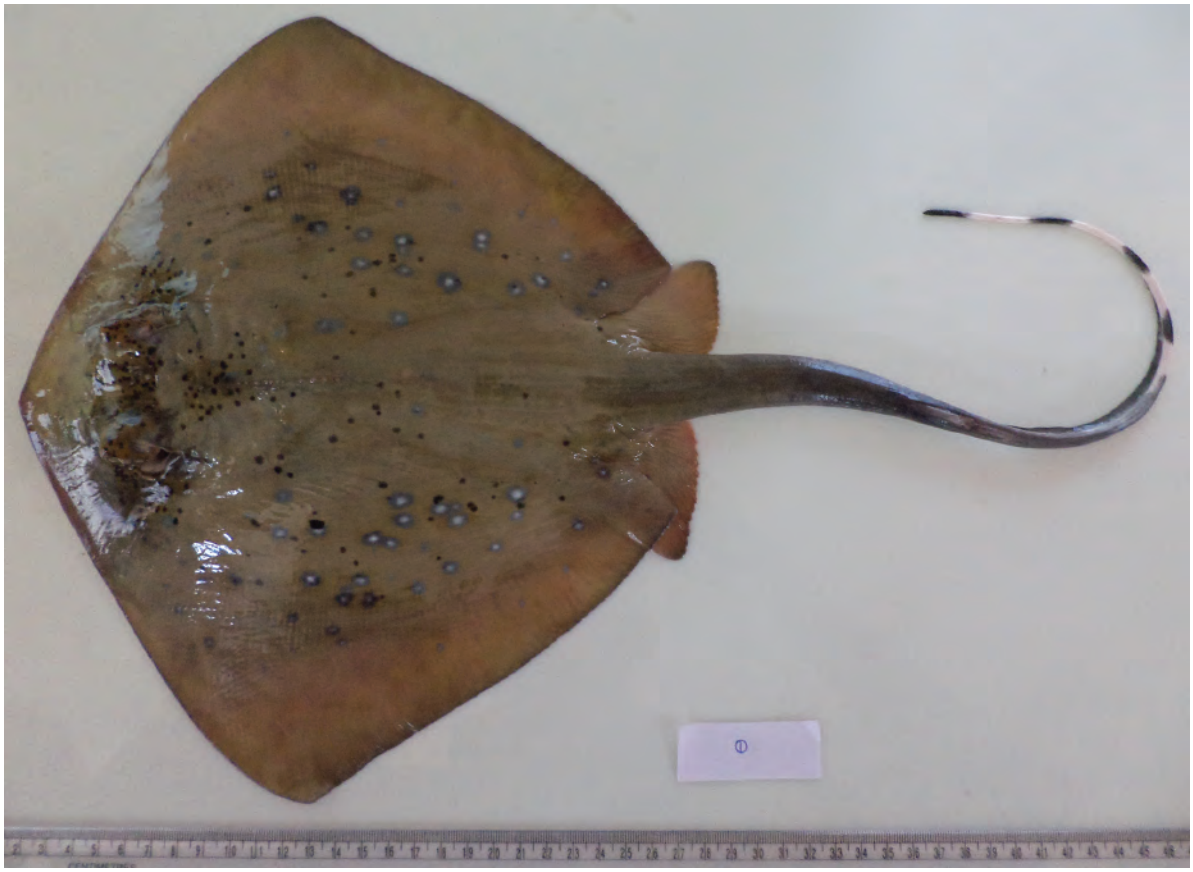


Figure 3

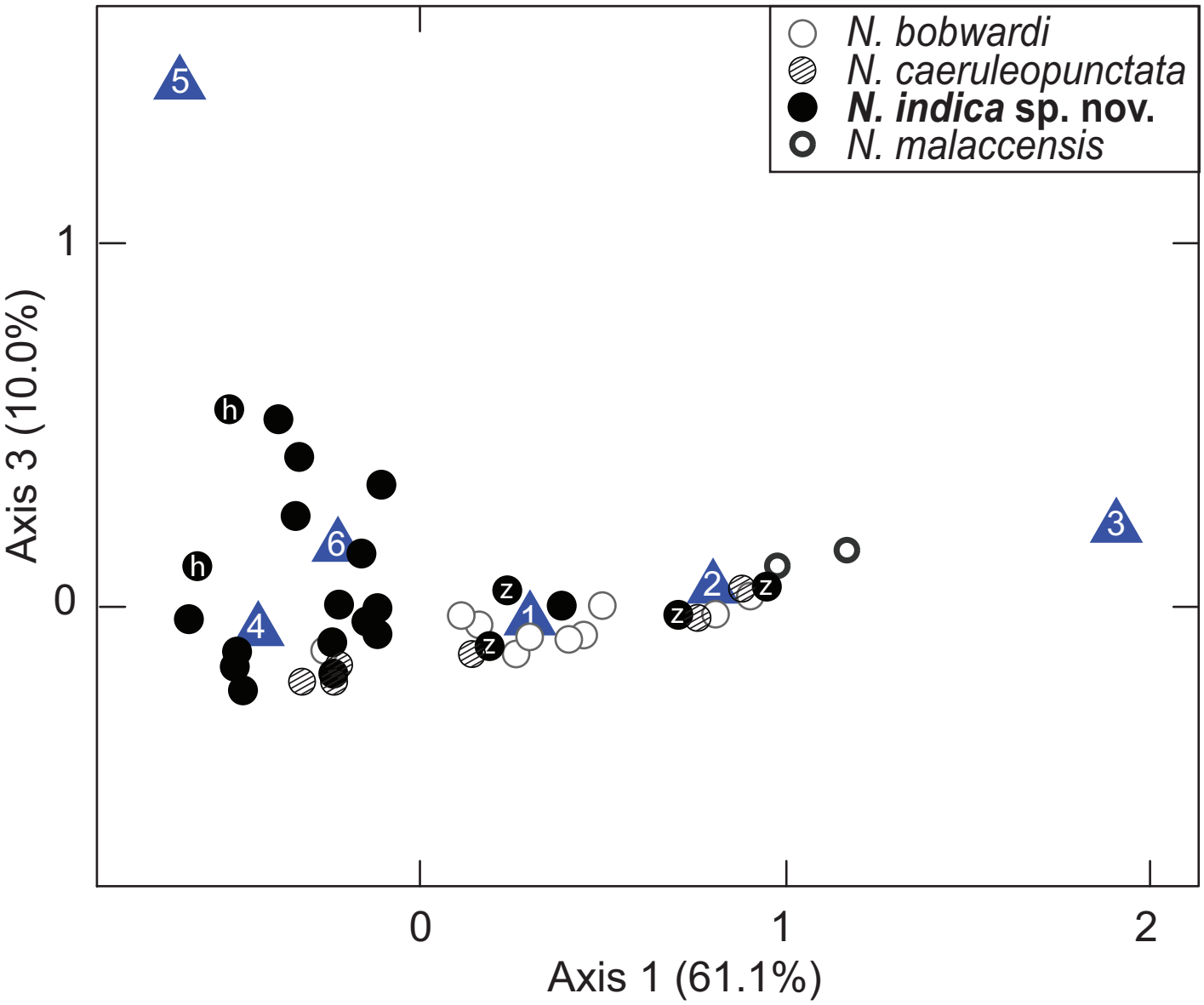

N. bobwardi
N. caeruleopunctata
N. indica sp. nov.
N. malaccensis
5
6
4
1
2
3
h
h
z
z
z
z
1
0
0
1
2
Axis 3 (10.0%)
Axis 1 (61.1%)

Figure 4 - Pavan-Kumar et al.

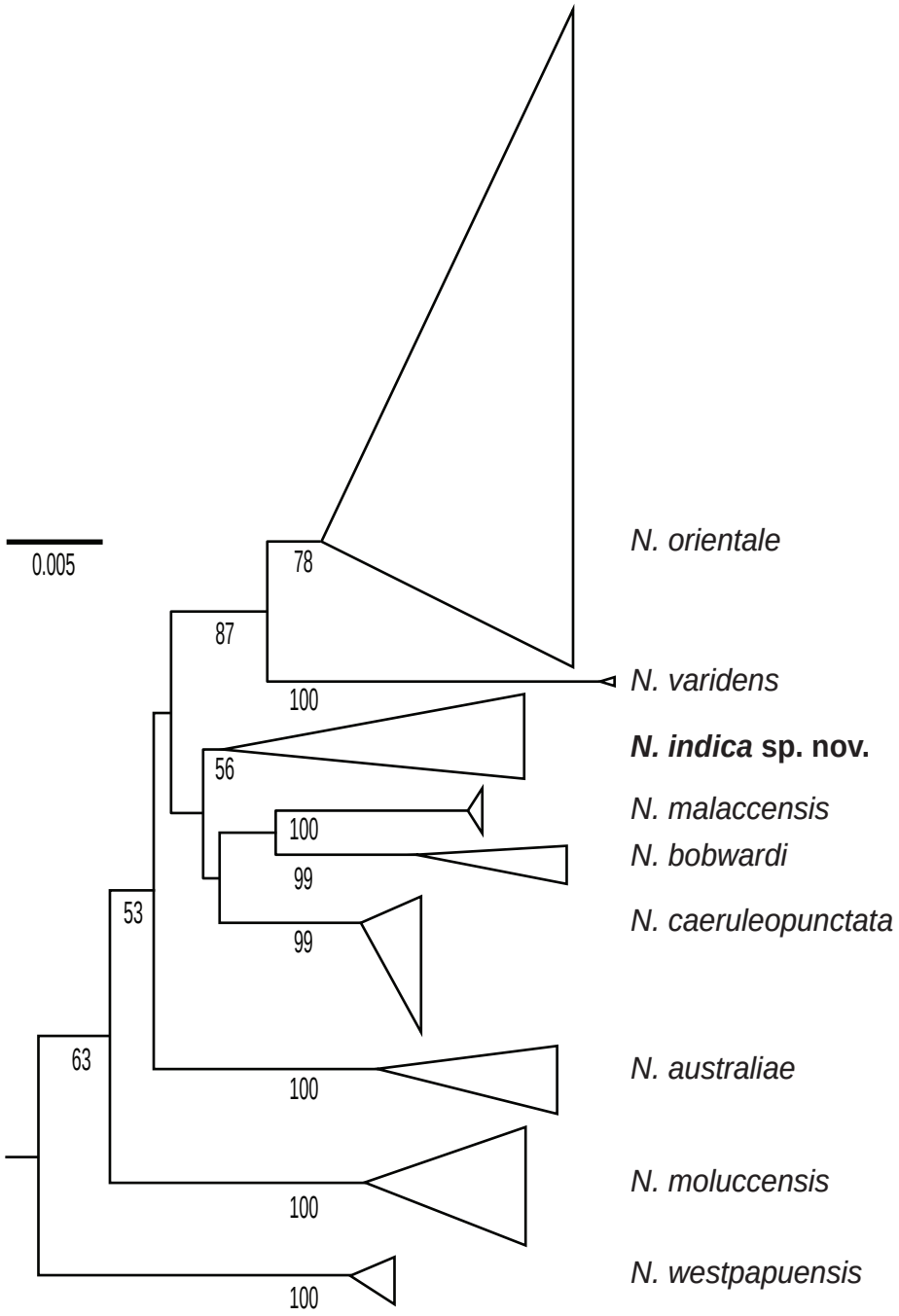
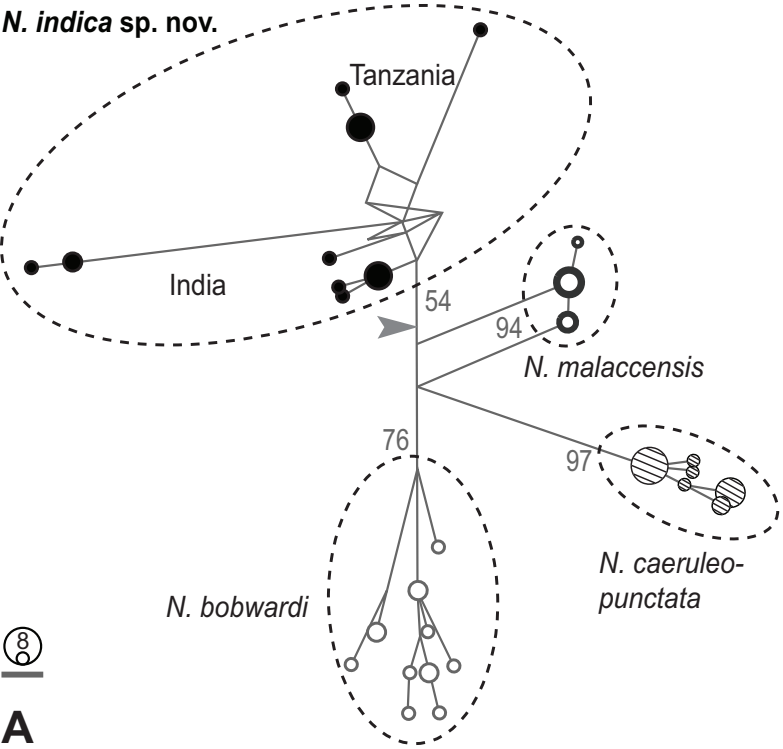
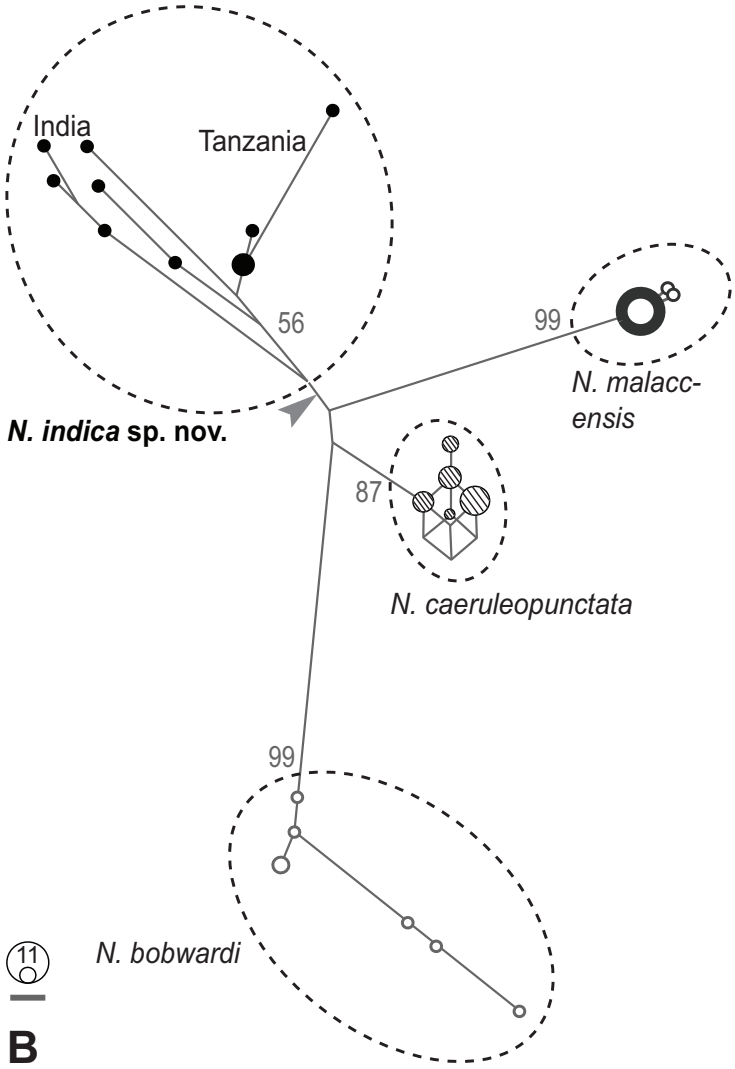


Figure 5


N. indica sp. nov.
Tanzania
India
54
94
N. malaccensis
76
97
N. caeruleo-
punctata
N. bobwardi
8
A

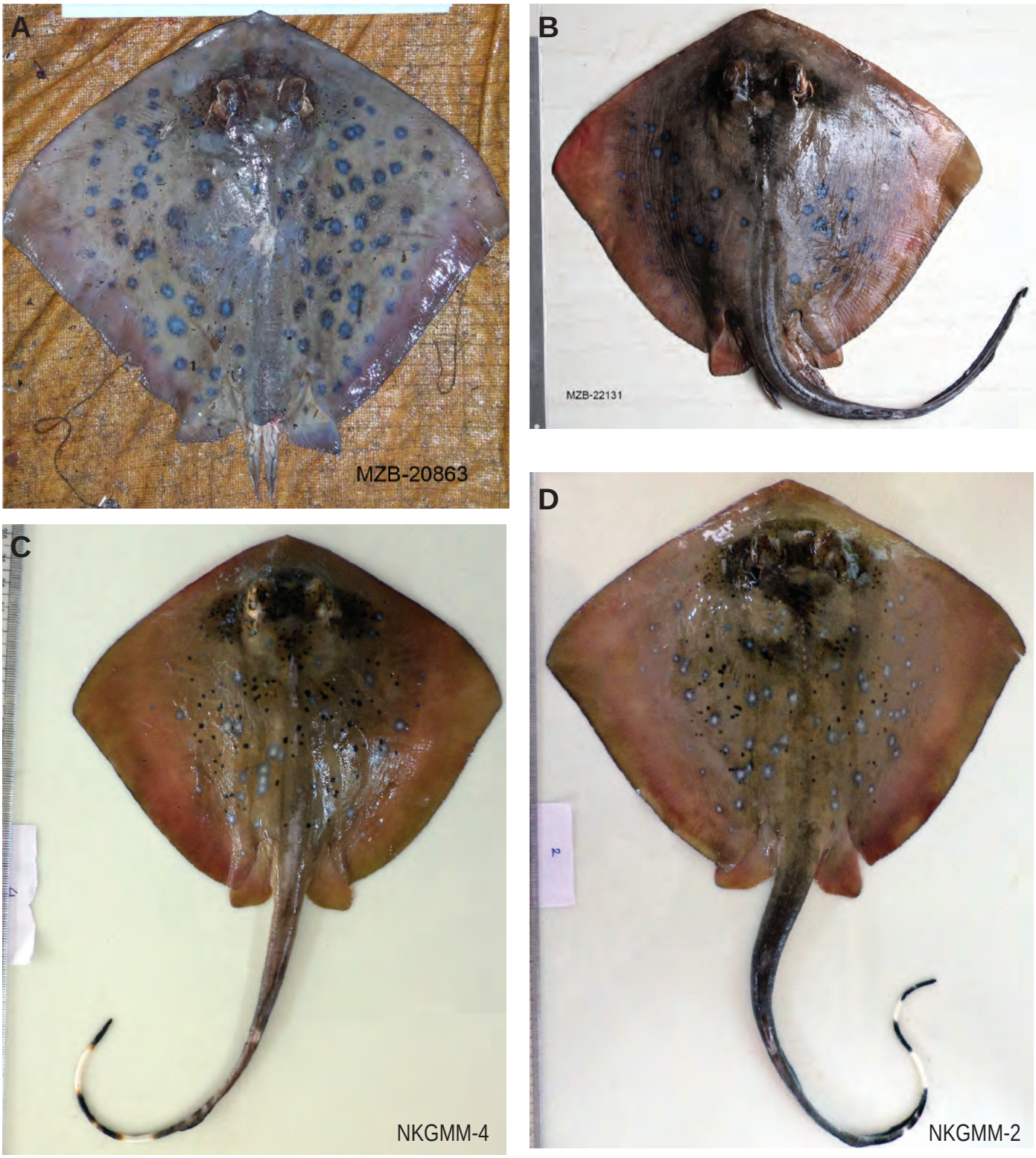

India
Tanzania
56
99
N. malacc-
ensis
N. indica sp. nov.
87
N. caeruleopunctata
99
N. bobwardi
11
B

Supplementary material to:

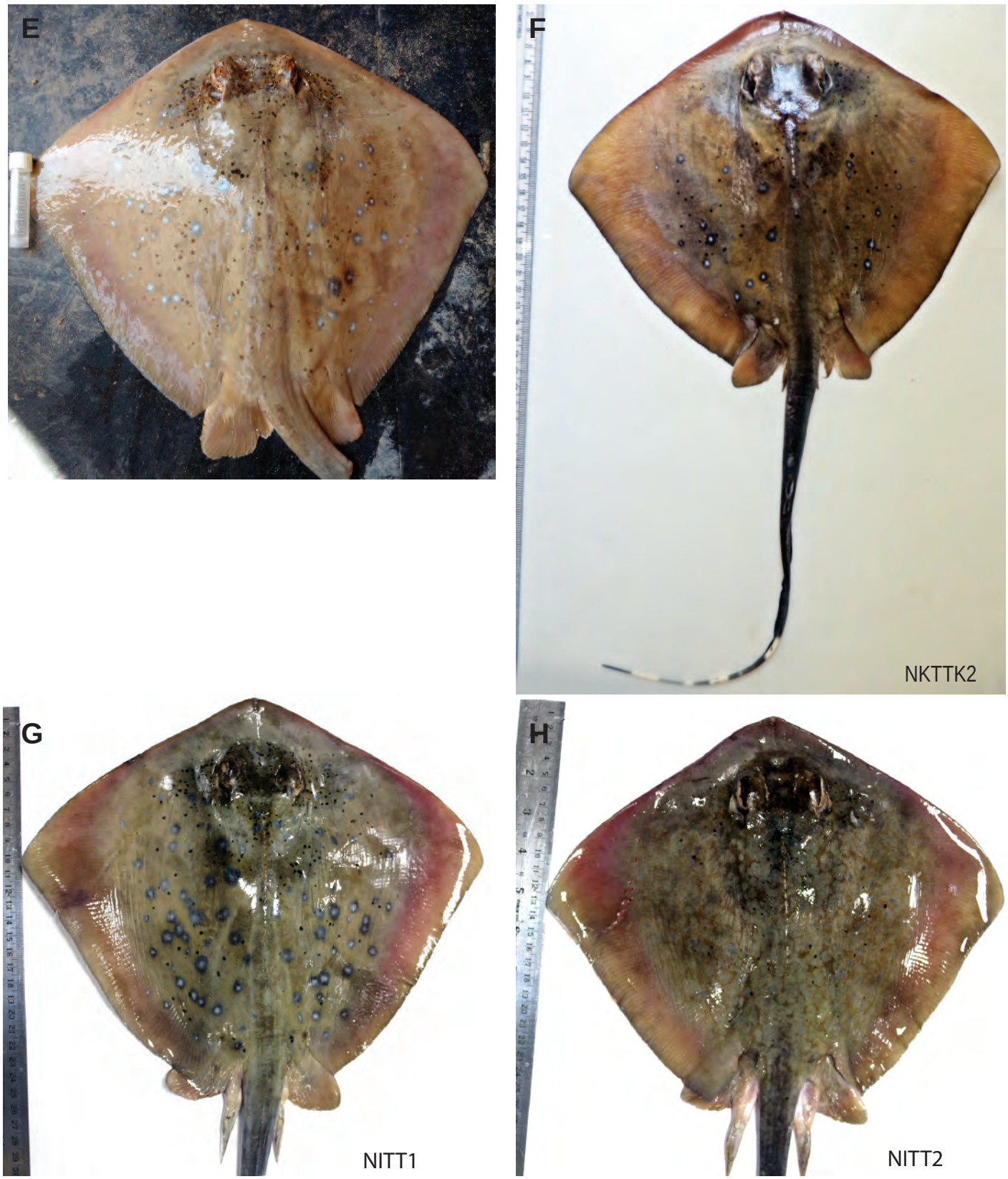
***Neotrygon indica* sp. nov., the Indian-Ocean blue spotted maskray (Myliobatoidei, Dasyatidae)**

By: Annam Pavan-Kumar, Rajan Kumar, Pranali Pitale, Kang-Ning Shen, and Philippe Borsa

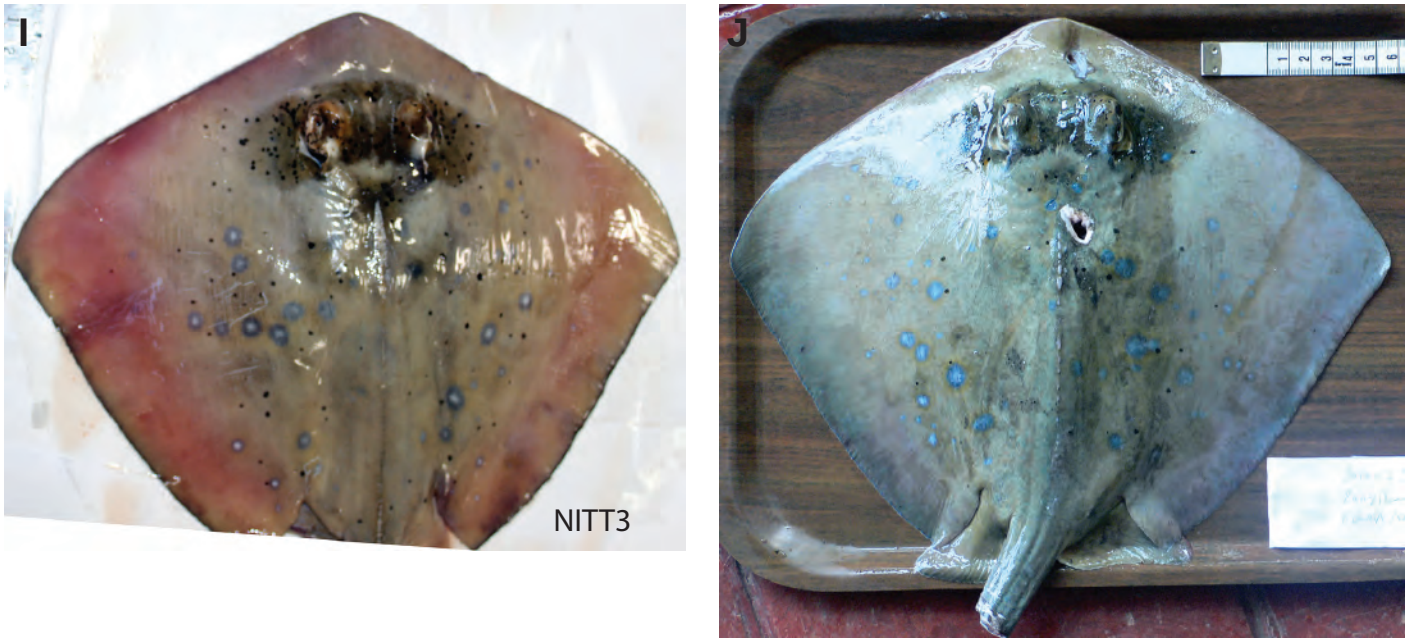
Supplementary Figs. S1-S8 here appended.



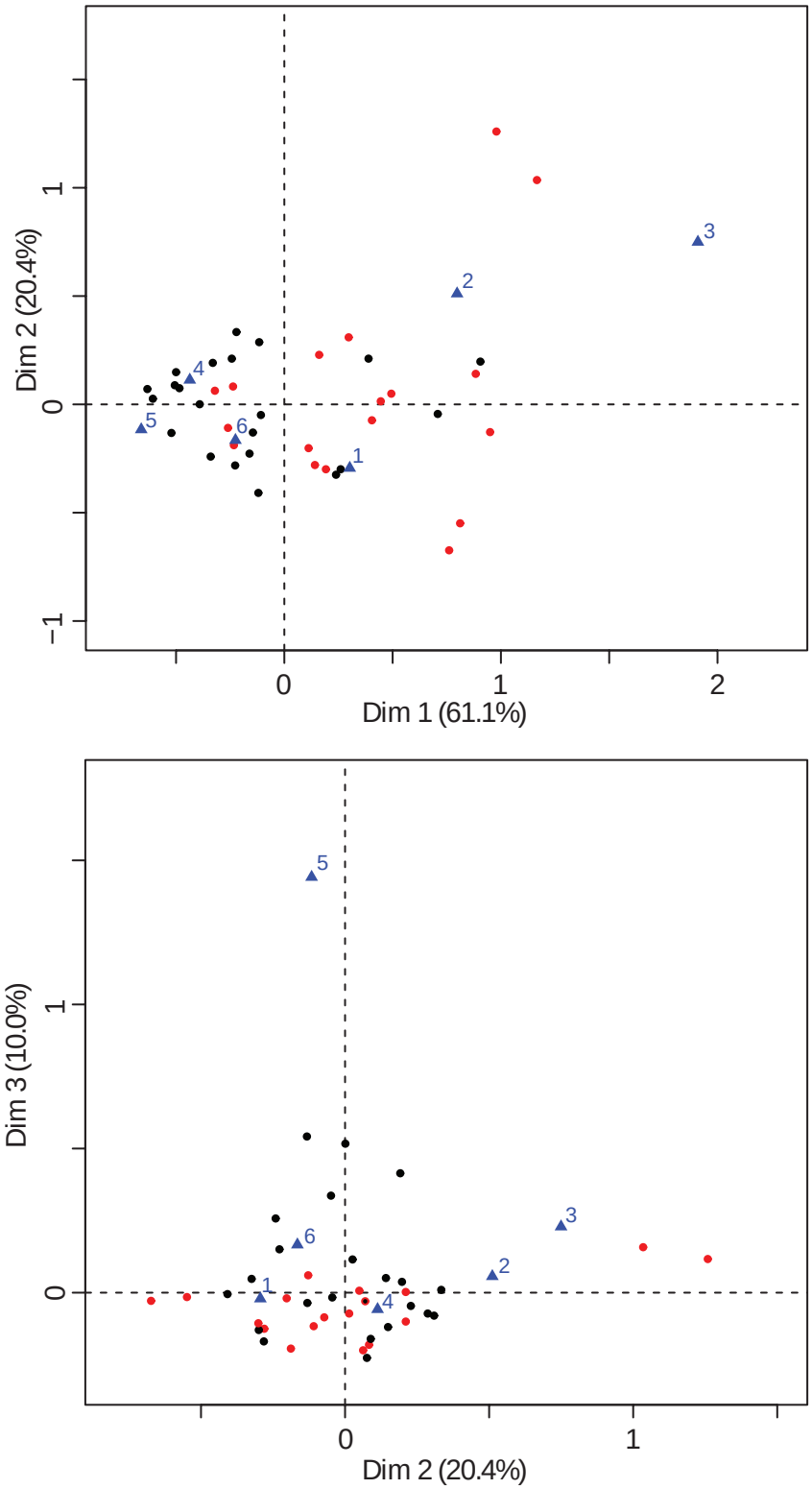
Supplementary Fig. S1. *Neotrygon* spp. Photographs of new specimens analyzed for dorsal spot patterns. **A.** *N. australiae* specimen MZB-20863 from Tanjung Sulamo, western Timor island, Aug. 2010 (courtesy of I.S. Arlyza, LIPI and T. Arifin, Indonesian ministry of Fisheries). **B.** *N. caeruleopunctata* specimen no. MZB-22131 from Kedonganan, Bali island, Sep. 2013 (courtesy of Rumini, Proyek BioKar). **C.** *N. indica* sp. nov. specimen no. NKGMM-4 from the gulf of Mannar, India (RK). **D.** *N. indica* sp. nov. specimen no. NKGMM-2 from the gulf of Mannar, India (RK).



Supplementary Fig. S1 (continued). E. *N. indica* sp. nov. specimen no. NKTT1 from Tuticorin, India (RK). **F.** *N. indica* sp. nov. specimen no. NKTT2 from Tuticorin, India (RK). **G.** *N. indica* sp. nov. specimen no. NITT1 from Tuticorin, India, Dec. 2017 (APK). **H.** *N. indica* sp. nov. specimen no. NITT2 from Tuticorin, India, Dec. 2017 (APK); the spot patterns of this specimen were not scored, because of a dense cover of pale blotches masking the blue pigmentation.

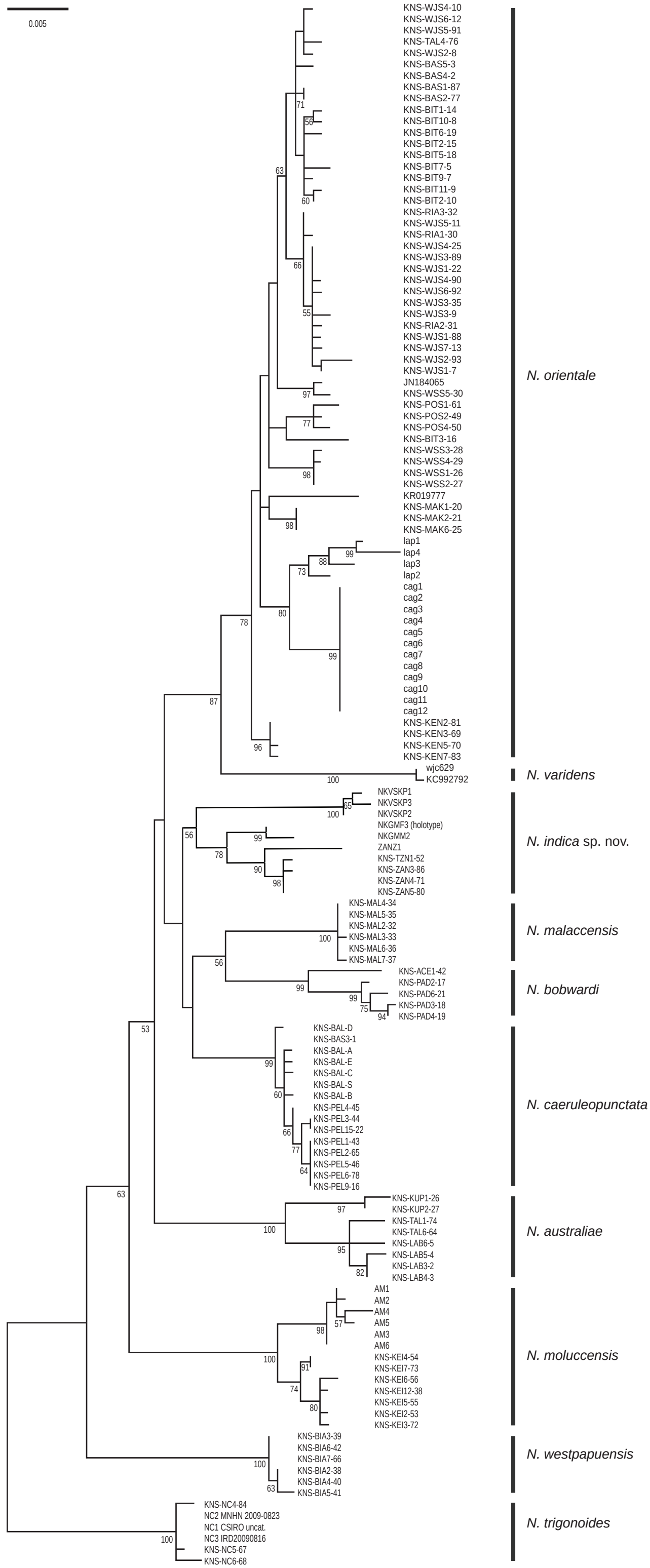


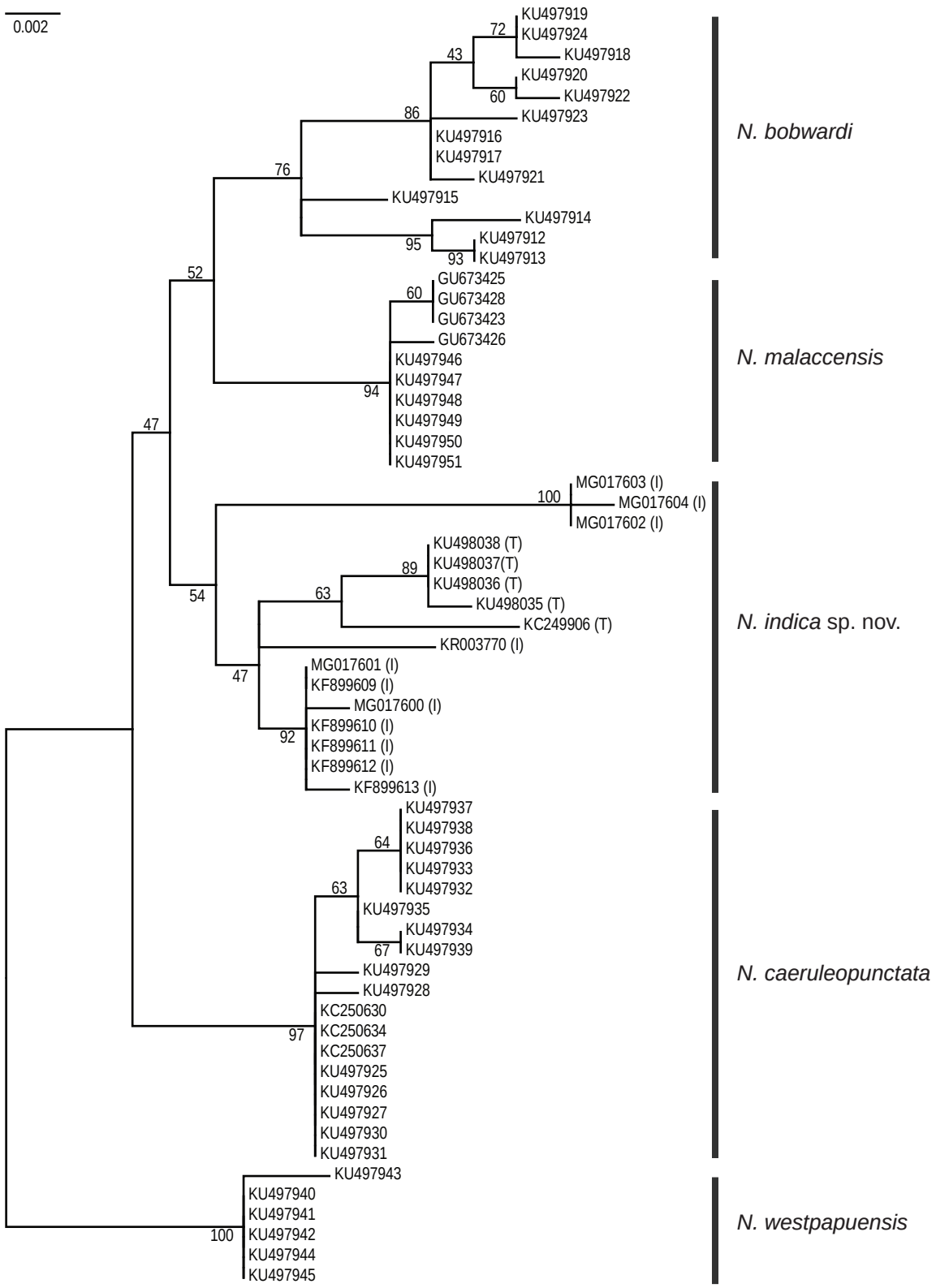
Supplementary Fig. S1 (continued). I. *N. indica* sp. nov. specimen no. NITT3 from Tuticorin, India, Dec. 2017 (APK). **J.** *N. indica* sp. nov. specimen no. IRD-20170720-A from Zanzibar, Tanzania, July 2017 (PB).



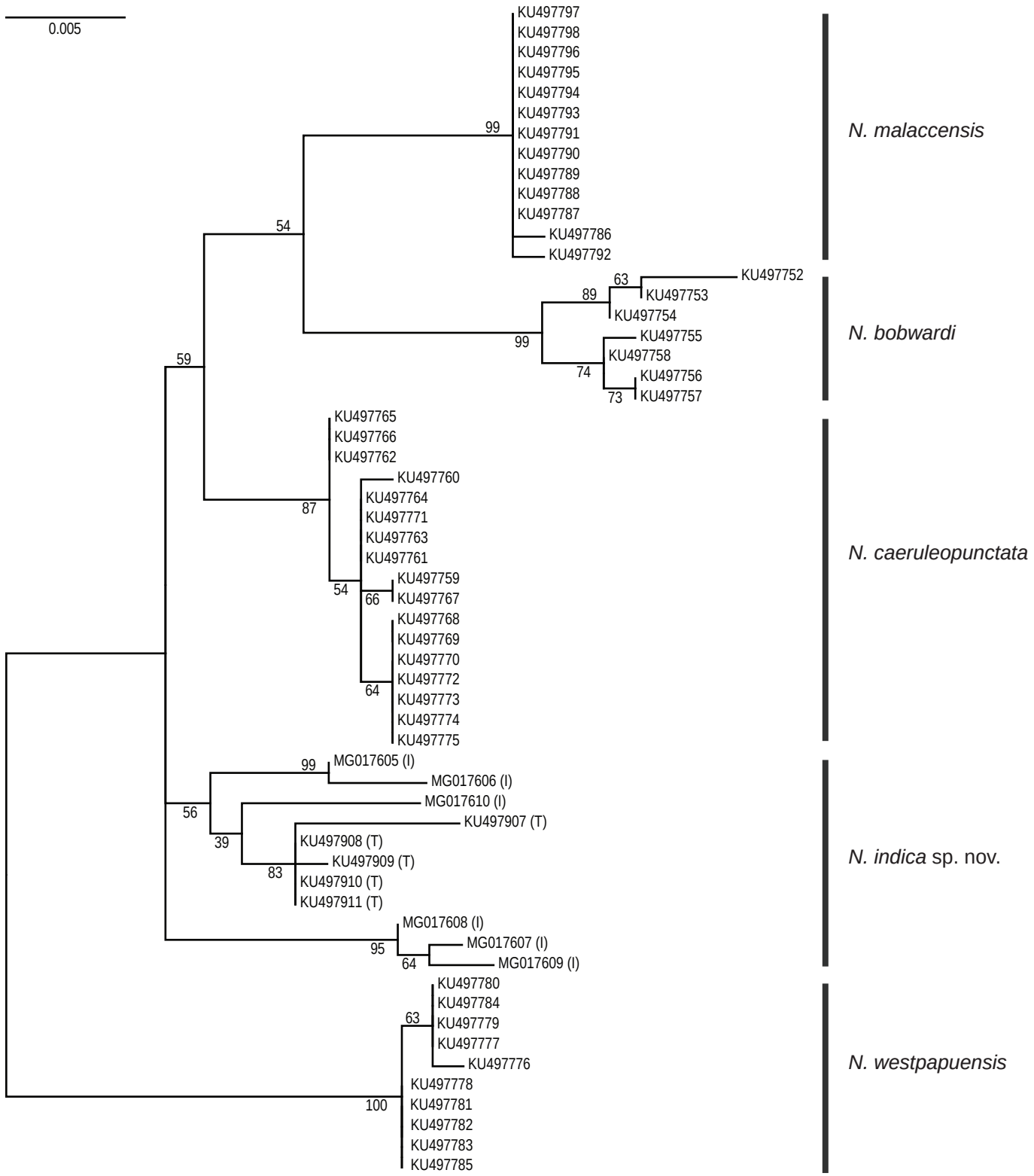
Supplementary Fig. S2. Correspondence analysis (CA): output of FactoMineR [Lê et al. 2008] representing the projection of individuals of the blue-spotted maskray species complex (*black circles*: *N. indica* sp. nov.; *red circles N. bobwardi*, *N. caeruleopunctata*, *N. malaccensis*) along CA axes 1 and 2 (above) and CA axes 2 and 3 (below). *Blue triangles* indicate the position of the variables used to characterize spot patterns: *1*. Small ocellated blue spots; *2*. Medium-sized ocellated blue spots; *3*. Large ocellated blue spots; *4*. Dark speckles; *5*. Dark spots; *6*. Occipital mark

Supplementary Fig. S3. Maximum-likelihood tree of concatenated *CO1* + *cytochrome b* haplotypes in the blue-spotted maskray (*Neotrygon* spp.), rooted by *N. trigonoides*. The substitution model was TN93+G. Numbers at a node are bootstrap scores, from 600 bootstrap resampling runs under MEGA6.

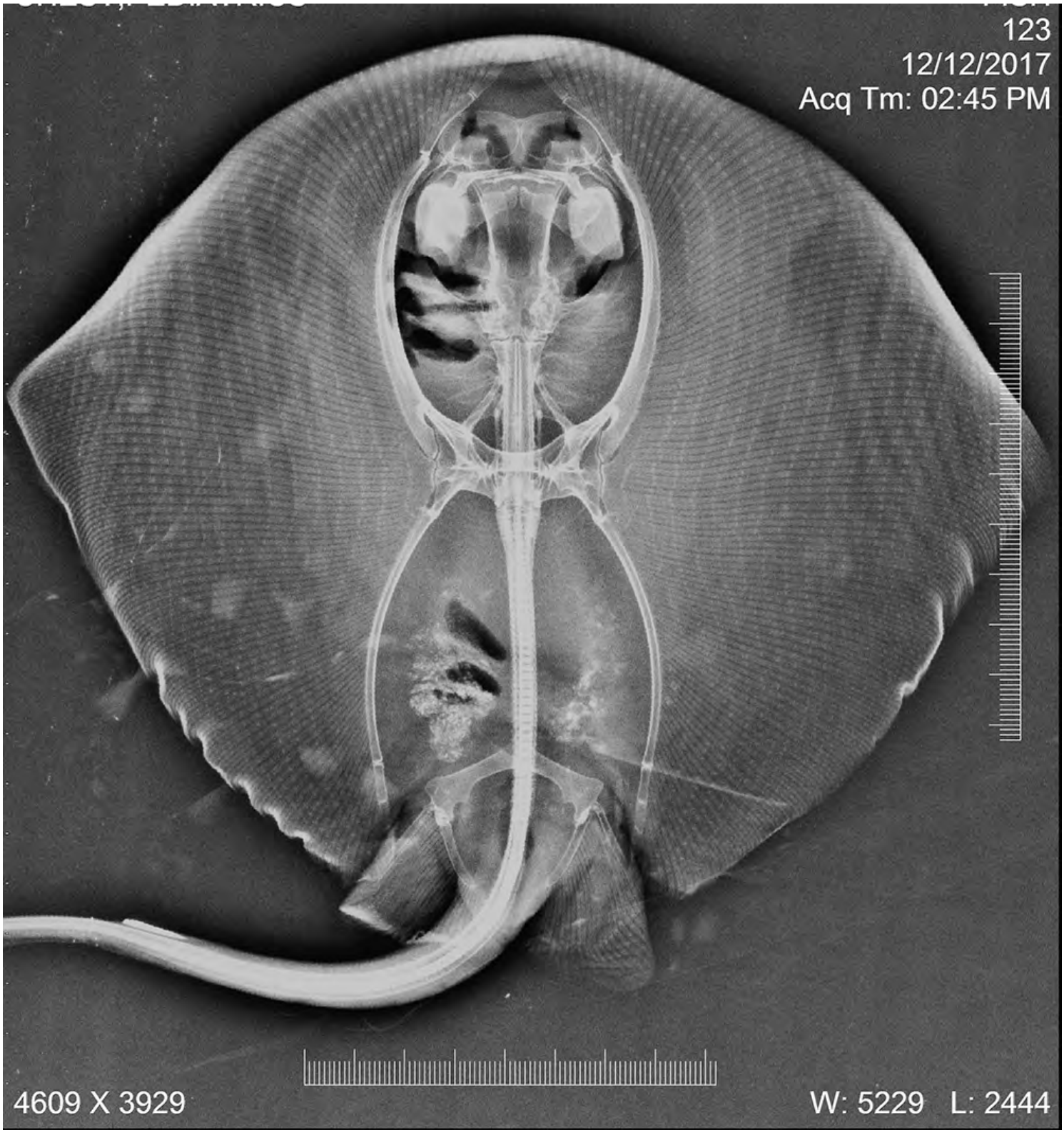




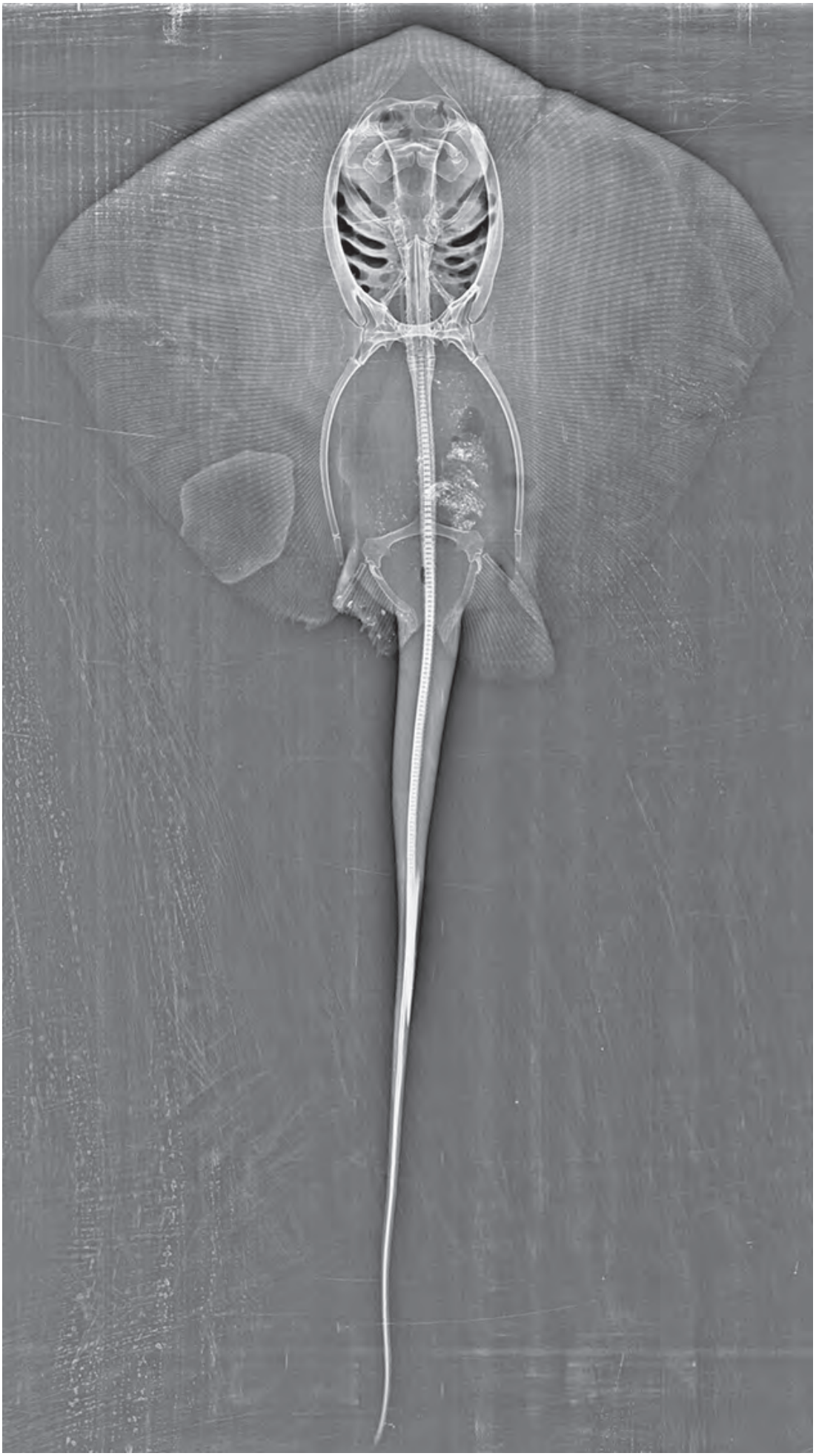
Supplementary Fig. S4. Maximum-likelihood tree of *CO1* haplotypes in the Indian-Ocean blue-spotted maskray sub-group (*Neotrygon* spp.), rooted by *N. westpapuensis*. Nucleotide sequences were trimmed to a single core length of 638 bp and the dataset (63 sequences) was analyzed under MEGA6. The substitution model was K2+G. Numbers at a node are bootstrap scores, from 600 bootstrap resampling runs under MEGA6. *Neotrygon indica* sp. nov. specimens from India are labelled "(I)"; specimens from Tanzania are labelled "(T)".



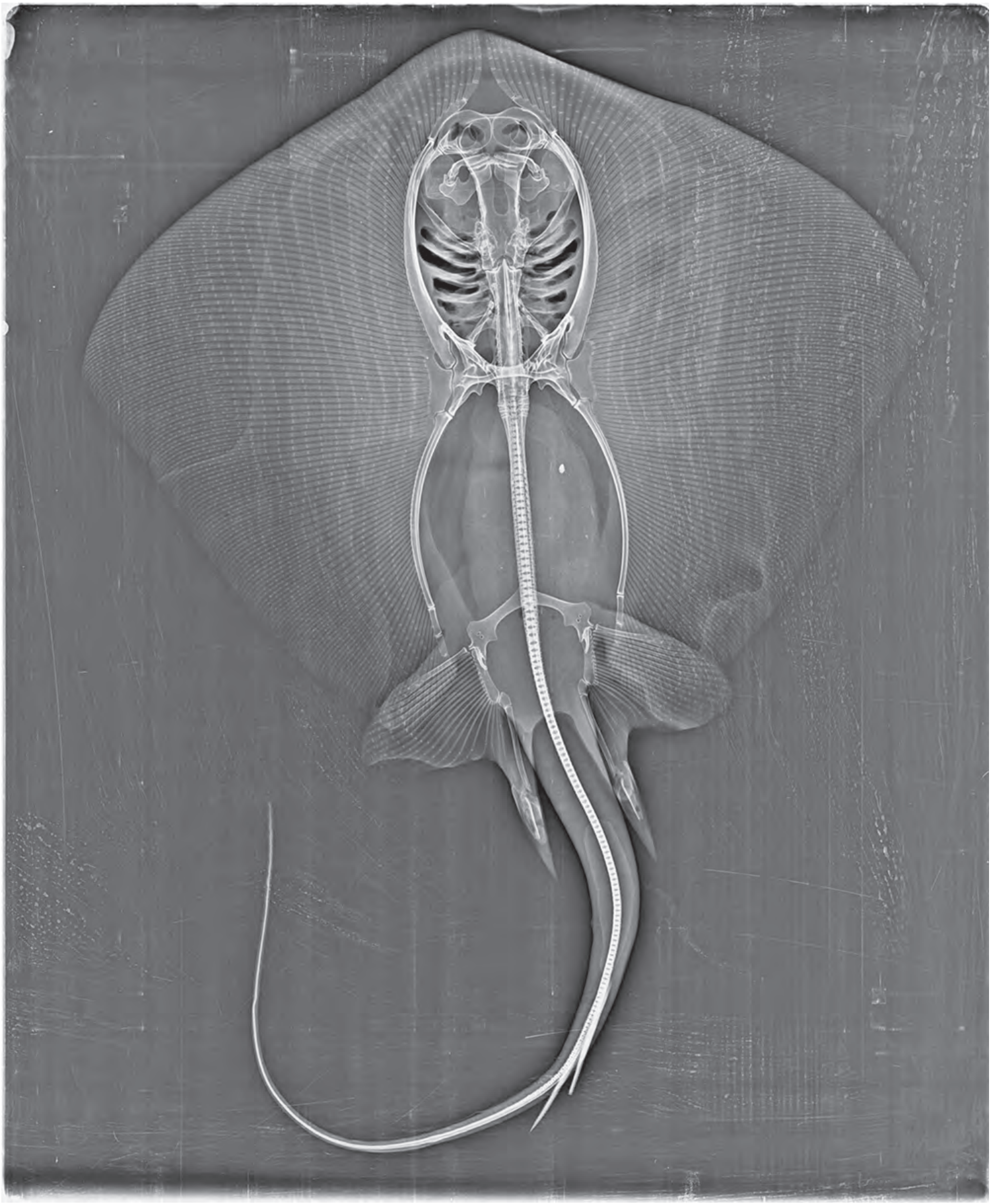
Supplementary Fig. S5. Maximum-likelihood tree of *cytochrome-b* haplotypes in the Indian-Ocean blue-spotted maskray sub-group (*Neotrygon* spp.), rooted by *N. westpapuensis*. Nucleotide sequences were trimmed to a single core length of 795 bp and the dataset (58 sequences) was analyzed under MEGA6. The substitution model was TN93+G. Numbers at a node are bootstrap scores, from 600 bootstrap resampling runs under MEGA6. *Neotrygon indica* sp. nov. specimens from India are labelled "(I)"; specimens from Tanzania are labelled "(T)".



Supplementary Fig. S6. *Neotrygon indica* sp. nov. X-ray photograph of holotype, used for the count of pectoral-fin radials.



Supplementary Fig. S7. *Neotrygon indica* sp. nov. X-ray photograph of paratype, used for the count of pectoral-fin radials.



Supplementary Fig. S8. *Neotrygon indica* sp. nov. X-ray photograph of specimen NITT1 from Tuticorin, India, used for the count of pectoral-fin radials. The number of pectoral-fin radials was 108-109 including propterygion 45-46, mesopterygion 14-15 and metapterygion 48-49.