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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

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Abstract Nine morphologically similar but genetically distinct lineages in the blue-spotted maskray species complex, previously *Neotrygon kuhlii* (Müller and Henle) qualify as cryptic species. Four of these lineages have been previously described as *Neotrygon australiae* Last, White and Séret, *Neotrygon caeruleopunctata* Last, White and Séret, *Neotrygon orientale* Last, White and Séret, and *Neotrygon varidens* (Garman), but the morphological characters used in the descriptions offered poor diagnoses and their geographic distributions were not delineated precisely. The objective of the present work is to complete the description of the cryptic species in the complex. Here, an additional four lineages are described as new species on the basis of their mitochondrial DNA sequences: *Neotrygon bobwardi*, whose distribution extends from the northern tip of Aceh to the western coast of Sumatera; *Neotrygon malaccensis*, sampled from the eastern part of the Andaman Sea and from the Malacca Strait; *Neotrygon moluccensis*, from the eastern half of the Banda Sea; and *Neotrygon westpapuensis* from the central portion of northern West Papua. The geographic distributions of *N. australiae*, *N. caeruleopunctata*, *N. orientale*, and *N. varidens* are updated. For each species, a diagnosis is provided in the form of a combination of private or partly-private nucleotides at 2-4 nucleotide sites along a 519-base pair fragment of the *CO1* gene. We believe that the present taxonomic revision will provide information relevant to the sound management and conservation of cryptic species of the blue-spotted maskray in the Coral Triangle region.

Keywords: molecular taxonomy; diagnosis; distribution

1 INTRODUCTION

Species usually are the fundamental units in biogeography, community ecology and conservation ecology. Since pioneer allozyme studies about five decades ago (Manwell and Baker, 1963), molecular genetic markers have been essential in uncovering cryptic species within nominal species once believed to be widely distributed (Bickford et al., 2007). Cryptic species are a common occurrence in marine ecosystems (Knowlton, 1993; Zemplak et al., 2009; Nygren, 2014; Durand and Borsa, 2015). Ignoring them may have adverse consequences in marine fisheries management, biodiversity management, and conservation (Krishnamurthy, 2012; Van Campenhout et al., 2014; Borsa et al., 2015; Pante et al., 2015). It is therefore important to accurately delineate cryptic species. The step beyond, naming species, is essential in biodiversity studies because formally designating a species by its binominal name allows to link and compare the results of scientists working separately on conspecific samples (Pante et al., 2015). DNA sequences potentially provide a profusion of diagnostic characters, hence are useful tools in modern taxonomy and essential ones to delineate and describe cryptic species (Tautz et al., 2003; Vogler and Monaghan, 2006; Cook et al., 2010; Jörger and Schrödl, 2013). In contrast, morphometrics as presented in some recent taxonomic work in Elasmobranchs (e.g., Last et al., 2016) did not offer clear diagnoses. In our view, morphometric descriptions in this case should be undertaken a posteriori, i.e. once species of a complex of cryptic species have been delineated and described based on molecular genetics.

Cryptic species have been reported in morphologically intractable species complexes of Indo-West Pacific fishes (e.g., Zemplak et al., 2009; Durand and Borsa, 2015; Randall and Victor, 2015) including stingrays (Naylor et al., 2012; Arlyza et al., 2013b; Borsa et al., 2013c). In a recent paper (Borsa et al., 2016b), the maximum-likelihood phylogeny of *CO1*- and *cytochrome b* gene haplotypes determined nine main lineages within the blue-spotted maskray, previously *Neotrygon kuhlii* (Müller and Henle, 1841). Analysis of coalescence patterns (Pons et al., 2006) generally confirmed that these lineages represent separate species. Also, the lineages are geographically distributed in a parapatric fashion, indicating reproductive isolation (Bull, 1991). Eight of these lineages occur in the Coral-Triangle region and the ninth one occurs in the Indian Ocean, from India to Tanzania (Borsa et al., 2016b). A specimen from Vanikoro in the Santa Cruz archipelago, northeastern Coral Sea has been designated as lectotype of *N. kuhlii* (Last et al., 2016) and three of the lineages, all from the Coral Triangle, have been described as new species, namely *N. australiae* Last, White and Séret, 2016, *N. caeruleopunctata* Last, White and Séret, 2016 and *N. orientale* Last, White and Séret, 2016. A fourth one, *N. varidens* (Garman, 1885) has been resurrected from synonymy with *N. kuhlii* (Last et al., 2016). None of the nine lineages uncovered by Borsa et al. (2016b) was *N. kuhlii* according to its recent re-description (Borsa and Béarez, 2016). Five of these nine lineages remain undescribed, illustrating the limitations of morphological characters to distinguish them. Two additional lineages up to now represented each by a single haplotype also require attention (Borsa et al., 2016b).

The present paper is a follow-up of Borsa et al. (2016b), whose purpose was to delineate the cryptic species in the blue-spotted maskray species complex. We emphasize the distinction between species delineation, species description, and species identification, although DNA markers may be used in all three cases (Vogler and Monaghan, 2006; Cook et al., 2010). In the present paper, the nucleotide sequence of a portion of the *CO1* gene is used to diagnose species in a formal taxonomic description. The same genetic marker is the standard in DNA-barcoding, where it is used as a means of identifying individuals to species by sequence similarity (Vogler and Monaghan, 2006; Ratnasingham and Hebert, 2007). The aims of the present paper are (1) to describe as new species, on the basis of their DNA sequences, the still-anonymous lineages of

the blue-spotted maskray previously under *N. kublii*; (2) to provide a molecular diagnosis for each of the nine lineages previously under *N. kublii*; (3) to provide or update their distributions, based on the available nucleotide-sequence data at the *CO1*- and *cytochrome b* gene loci.

2 METHODS

To evaluate the diagnosticity of the morphological characters used in the description or re-description of *N. australiae*, *N. caeruleopunctata*, *N. kublii* and *N. orientale* (Last et al., 2016), we tabulated their ranges of values in each of the four species. We then assessed whether a given character enabled the distinction of at least one of the species from the other species by looking at possible disjunctions in the ranges of values.

All individuals included in the present survey along with sampling details, accession numbers in the GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov/>) and registration numbers in the Barcoding of Life Datasystem (BOLD; <http://www.barcodinglife.com/>; Ratnasingham and Hebert, 2007) are listed in Borsa et al. (2016b). Voucher specimens of blue-spotted maskray were deposited at the Museum Zoologicum Bogoriense (MZB) in Cibinong, Indonesia. These include the holotypes of the four new species, which were photographed before their preservation in 96% alcohol / 4% formalin solution. Sampling details, sex, and disk width (DW) were recorded (Borsa et al., 2016b). Tissue samples used for DNA extraction were deposited at LIPI-P2O in Ancol, Jakarta; a partial list of tissue samples is available from Arlyza et al. (2013a).

The phylogenetic tree of the blue-spotted maskray species complex (previously *N. kublii*), based on the concatenated partial nucleotide sequences of the *CO1* and *cytochrome b* genes showed nine main clades which characterized *N. australiae*, *N. caeruleopunctata*, *N. orientale*, *N. varidens* and 5 other lineages that remained undescribed. The New Caledonian maskray *N. trigonoides* was considered as outgroup to the species complex (Borsa et al., 2013a). Voucher specimens deposited in museum collections are available for four of these lineages, i.e., clades II, III, VII and VIII of Borsa et al. (2016b) (Fig. 1b), but not yet for the fifth (clade I), hereafter referred to as the Indian-Ocean maskray. Two additional lineages, each represented by a single *CO1* haplotype (Supplementary Table S1) were not included in our phylogeny. In the following, they will be referred to as the Guadalcanal maskray and the Ryukyu maskray.

The partial *CO1* gene sequences here used to describe the holotypes of new species were 611 bp long. Variable nucleotide sites at the *CO1* locus were identified automatically using MEGA6 (Tamura et al., 2013) on the matrix of 314 partial *CO1* gene sequences extracted from Borsa et al. (2016b). Diagnostic nucleotide sites were identified along a core 519-bp sub-fragment common to all 314 sequences by visual examination of the ExCel file (Microsoft Corporation, Redmond WA) that showed all the variable sites (Supplementary Table S1). The ExCel file was generated by MEGA6. *Cytochrome b* gene sequences were not available for the type specimens of the new species except one: MZB-20864 from Ambon, to be chosen as paratype of *N. moluccensis* sp. nov. Variable nucleotide sites at the *cytochrome b* locus were similarly identified automatically using MEGA6 (Tamura et al., 2013) on the matrix of 159 partial *cytochrome b* gene sequences extracted from Borsa et al. (2016b). Diagnostic nucleotide sites were similarly identified along a core 1127-bp sub-fragment sequenced in at least one individual of each species, by visual examination of the ExCel file with variable sites generated by MEGA6 (Supplementary Table S2).

The BOLD datasystems distinguishes clusters of sequences that qualify as operational taxonomic units (OTUs) i.e., putative species using the Refined Single Linkage (RESL) algorithm. The latter “clusters sequences with high similarity and connectivity and separates those with lower similarity and sparse connectivity” (Ratnasingham and Hebert, 2013). Each OTU thus identified is allocated a unique barcode

index number (BIN) in BOLD. We established the homology of BIN numbers with the blue-spotted maskray lineages examined in the present study by visual inspection of the placement of the corresponding *CO1* gene sequences retrieved from BOLD in a maximum-likelihood tree of 314 *CO1* gene sequences extracted from Borsa et al. (2016b), rooted by *N. trigonooides*. The tree was constructed with MEGA6 (Tamura et al., 2013) using the Tamura 3-parameter model (Tamura, 1992) with gamma-distributed rate differences among sites, a choice based on the Bayesian information scores provided by MEGA6.

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3 RESULTS AND DISCUSSION

The way four species previously under *N. kublii*, namely *N. australiae*, *N. caeruleopunctata*, *N. orientale*, *N. varidens* have been diagnosed is not satisfactory (Table 1). Of all 19 characters used in the diagnoses of these four species, only one, the ratio of preoral length to mouth width, enabled the distinction of *N. kublii* from the other three species considered, but this was based on very low sample sizes (Table 1). Also, preoral length concerns a fleshy part of the body whose shape is susceptible to be influenced by environmental conditions during development and growth. Snout angle apparently enabled the distinction of *N. caeruleopunctata* and *N. orientale* from *N. australiae* and *N. kublii* (Table 1), but the repeatability and precision of this measurement are likely to be poor, because of the subjectivity in defining linear sides to snout at its apex. The morphological key proposed by Last et al. (2016) was mostly based on the morphological traits of a few (1-10) individuals per species, with no attempt to assess presumed interspecific differences against infra-specific variation. The values of most morphological characters claimed to be diagnostic of a species overlapped across species (Table 1), suggesting that they are not sufficient to fully distinguish closely related, though genetically distinct species in the blue-spotted maskray species complex. Moreover, the apportion of environmental vs. genetic determination in the traits measured has not been evaluated. Therefore, the lack of diagnostic morphometric characters used for the “in-depth morphometric analyses” performed by Last et al. (2016) on the blue-spotted maskray species complex raises the question of their taxonomic utility. This question is a crucial one, not restricted to the blue-spotted maskray species complex, because it has been claimed that these analyses follow “standard taxonomic practices used for the family group (stingrays)” (Last et al., 2016). We are here questioning the relevance of the morphometric diagnoses provided thus far in the blue-spotted maskray (Last et al., 2016), as in another species complexes in stingrays (Borsa, 2017). In contrast, we observe that mitochondrial-DNA sequences provide clear species-specific clusters, offering much better diagnoses than so-called standard morphological measurements (Naylor et al., 2012; Puckridge et al., 2013; Borsa et al., 2016b; Last et al., 2016; Borsa, 2017). To eventually design a useful morphological key, multivariate analysis of morphometric characters should be undertaken posterior to molecular clustering, and the most discriminant of these characters, if any, should be selected. The discriminant characters can then be used for identification purposes if no DNA barcoding is possible. This is beyond the scope of the present paper.

Thus, the need arises for a molecular diagnosis of the species previously under *N. kublii*. Here, a 519-bp fragment of the nucleotide sequence of the *CO1* gene provided at least one, up to four diagnostic or quasi-diagnostic nucleotides for eight (i.e. *N. australiae*, *N. caeruleopunctata*, *N. varidens*, clades I, II, III, VII, VIII) out of the nine main lineages of blue-spotted maskray previously under *N. kublii* (Supplementary Table S1). Although no diagnostic nucleotide site was scored along this fragment for the remaining lineage (*N. orientale*), the latter was easily diagnosed by a combination of nucleotides at four nucleotide sites (see Taxonomy section). Two more lineages (Guadalcanal maskray, Ryukyu maskray), each represented by a single sequence were also taken into account for selecting diagnostic sites in the other lineages. Six of the nine blue-spotted maskray lineages treated in the present revision are currently represented in BOLD (see details in Taxonomy section). A distinct BIN number has been allocated to three of them (*N. australiae*, *N. orientale*, *N. varidens*) illustrating the taxonomic value of DNA barcodes in maskrays. However, *N. caeruleopunctata*, clade I and clade III which are reciprocally monophyletic (Borsa et al., 2016b) shared the same single BIN, exposing the limitations of the RESL analysis on *CO1* barcodes when addressing shallow divergences between clades, especially when sample sizes are low. It should be emphasized that BIN assignment on BOLD is a dynamic process, where individual BINs might split or merge each time new sequences are added (Ratnasingham and Hebert 2013; Steinke et al., 2017). Similarly, a 1127-bp fragment of the nucleotide sequence of the *cytochrome b* gene provided at least one, up to nine diagnostic or quasi-diagnostic nucleotides for eight (i.e. *N. australiae*, *N. caeruleopunctata*, *N. varidens*, clades I, II, III, VII, VIII) out of the nine main lineages of blue-spotted maskray previously under *N. kublii* (Supplementary Table S2). Last, partial sequences of the nuclear recombination activating protein 1 (*RAG-1*) gene distinguish two main clades among species in the genus *Neotrygon* (Puckridge et al., 2013). One clade includes all the assayed sequences of *N. caeruleopunctata*, *N. trigonoides*, clade I and clade III; the other clade includes all the assayed sequences of *N. australiae*, *N. leylandi*, *N. picta*, *N. orientale* and *N. varidens* (see figure 3B of Borsa et al., 2016b).

We recognize several cryptic species within *N. kublii* under its previous definition (Borsa et al., 2016b) and these species have parapatric distribution (Fig. 1a). Given this, a specimen can also be identified to species from the locality where it has been collected, provided the locality of collection does not lie on the line of contact between geographically adjacent species. The samples examined by Borsa et al. (2016b) spanned the whole Indo-Malay-Papua archipelago, allowing us to map the geographic distribution of each of the eight lineages present in this region (Fig. 1a) with higher accuracy than previous research (Arlyza et al., 2013a; Borsa et al., 2013a; Puckridge et al., 2013; Last et al., 2016). Geographic distributions are updated in the following Taxonomy section and previous speculative statements concerning the distribution of *N. australiae* (Last et al., 2016) are also corrected.

The blue-spotted maskray is currently categorized as “data deficient” by the International Union for the Conservation of Nature (Fahmi et al., 2015). There are currently no species-specific conservation measures in place for this species complex (Fahmi et al., 2015). It is therefore urgent to have these species recognized and named, so appropriate species-specific conservation measures can start to be implemented. The present descriptions are exclusively based on genetic diagnoses, unlike “standard” taxonomic descriptions in sharks and rays (White and Last, 2012; Last et al., 2016). The authors of recent “standard” descriptions in stingrays have claimed the new species were diagnosed based on morphology, but in some cases it can be inferred that the species were actually delineated from DNA sequences and not from morphology (see our present reassessment of Last et al., 2016; Table 1). The rationale for basing a new species description on genetic data has been repeatedly written in black ink (Tautz et al., 2003; Cook et al., 2010). In short, there is no objective reason to dismiss DNA-based descriptions when DNA sequences prove effective and sufficient as the only

description of a species. New animal species have to be named and described according to the rules of zoological nomenclature (International Commission of Zoological Nomenclature, 1999), which at no moment specify which particular approaches, whether morphological, or genetical, or else, should be adopted or discarded to delineate and diagnose species. Indeed, the essence of the taxonomic description of a species is the science, and not the type of data on which it is based. Unless one arbitrarily imposed as the sole acceptable definition of a species the narrow morphological species concept, morphology thus should be considered as a mere possible source of taxonomic characters among many other potential sources (Cook et al., 2010). In invertebrates, species of the blue mussel have been re-described based on allozyme diagnoses (McDonald and Koehn, 1988), and the field of entomology offers an emblematic example of DNA-only descriptions (Brower, 2010). In Actinopterygian fishes, new genera have been proposed based on DNA phylogenies exclusively (Craig and Hastings, 2007; Durand et al., 2012), and countless species have been described or resurrected based primarily on genetic markers (e.g. Baldwin et al., 2011; Borsa et al., 2013c, 2014; Woodland and Anderson, 2014; Randall and Victor, 2015; Shen et al., 2017). We believe that the present taxonomic revision will provide part of the information that is urgently needed for a sound management and conservation of cryptic species of the blue-spotted maskray in the Coral Triangle region.

4 TAXONOMY

Maskrays, genus *Neotrygon* Castelnau, 1873 belong to family Dasyatidae (Jordan, 1888). The type species of the genus is *N. trigonooides* (Castelnau, 1873), which has been recently resurrected from synonymy with *N. kuhlii* (Borsa et al., 2013a).

4.1 Description of four new maskray, *Neotrygon* spp. species previously under *N. kuhlii*

***Neotrygon bobwardi* sp. nov.** <http://zoobank.org/58F189B8-3504-46FD-B4DB-95CAE1712612>. Clade II (Borsa et al., 2016b); *Neotrygon kuhlii* clade II (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kuhlii* (Borsa et al., 2013a).

Type material: the holotype (Fig. 2a) is a male specimen, 40 cm disk DW, collected by ISA on 28 April 2009 from local fishermen at the Meulaboh, Aceh fish landing place (04°07'N 96°08'E). It is registered at MZB under no. MZB-20843; it is also registered at LIPI-P2O in Ancol, Jakarta under no. LIPI-4406. The specimen is preserved in 96% alcohol and 4% formalin mixture. A subsample of tissue, numbered NK-ME3 is currently preserved in alcohol at -20°C in the freezers of the genetics laboratory at LIPI-P2O. Paratypes are MZB-20842 (LIPI-4407), male, 32 cm DW from Pulau Breueh, Aceh, collected on 23 April 2009 by ISA; MZB-20844 (LIPI-4404), male, 20 cm DW from Sibolga, northwestern Sumatra, collected on 24 March 2009 by ISA; and MZB-20845 (LIPI-4411) from Padang, West Sumatra, collected in August 2009 by ISA.

Description: the partial *CO1* gene sequence of the holotype, which has GenBank accession no. JX304805, is 5'-CTGGCCTCAGTTTACTTATCCGAACAGAACTAAGCCAACCAGGCGCTTTACTGGGTGATGATCAAATTTATAATGTAATCGTCACTGCCACGCCTTCGTAATAATCTTCTTTATGGTAATGCCAATTATAATTGGTGGGTTTGGCAACTGACTGGTGCCCCTGATAATTGGGGCTCCGGACATAGCCTTTCCACGAATAAACAAACATAAGTTTTTACTTCTACCTCCCTCATTCCCTATTACTGCTGGCCTCAGCAGGAGTAGAAGCCGGAGCCGGAACAGGTTGAACAGTTTATCCCCCATTAGCTGGTAATCTAGCAC

ATGCCGGAGCTTCTGTAGACCTTACAATCTTCTCTCTTTCACCTAGCA
GGTGTTCCTCTATTCTGGCATCCATCAACTTTATCACACAATTAT
TAATATAAAACCACCTGCAATCTCCCAGTATCAAACCCCAATTATTCG
TCTGATCTATTCTTGTTACA ACTGTGCTTCTCCTGCTATCTCTACCA
GTCCTAGCAGCTGGCATTACCATACTCCTCACAGACCGAAATCTTA
ATACA ACTTTCTTTGACCCAGCTGGGGGAGGAGATCCCAATTCTTTA
CCAACACCTC-3'.

Diagnosis: based on Supplementary Table S1, *N. bobwardi* sp. nov. is distinguished from all other species currently under *N. kublii* by nucleotide G at nucleotide site 210 of the *CO1* gene; it also has C at nucleotide site 240, a character that it shares with no species other than *N. malaccensis* sp. nov. Based on Supplementary Table S2, *N. bobwardi* sp. nov. is distinguished from other species previously under *N. kublii* by C at nucleotide site 546 of the *cytochrome b* gene; it also has C at nucleotide site 399, a character that it shares with no species other than *N. malaccensis* sp. nov., G at nucleotide 57 and T at nucleotide site 111, characters that it shares with no species other than *N. caeruleopunctata* sp. nov., and T at nucleotide site 327, a character that it shares with no species other than *N. westpapuensis* sp. nov.

Distribution: the type locality of *N. bobwardi* sp. nov. is Meulaboh, northwestern coast of Sumatra Island. Based on present study, its distribution includes the northern tip of the Aceh region and all the western coast of Sumatra south to Padang.

Etymology: the species is named after Robert D. (Bob) Ward, one of the leaders of the fish barcoding initiative (Ward et al., 2009). One of his noted contributions in this field was the DNA-barcoding survey of Australian chondrichthyans, which included bluespotted maskray samples from the Coral Triangle region (Ward et al., 2008). R.D. Ward and co-authors suspected the occurrence of cryptic species within the nominal species *N. kublii*, based on an unusually high level of genetic divergence among geographic populations, at the *CO1* locus. We chose to name after him the bluespotted maskray species that occurs on the Indian-Ocean coast of Sumatra, as an acknowledgement of his contribution to the systematics of chondrichthyans.

Proposed vernacular names: Bob Ward's bluespotted maskray (English); pari masker total biru Pak Bob (Indonesian); raie pastenague à points bleus de Bob Ward (French).

***Neotrygon malaccensis* sp. nov.** <http://zoobank.org/69B1F017-99AD-4E5E-81BE-2684E2192D1A>.

Clade III (Borsa et al., 2016b); *Neotrygon kublii* clade III (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kublii* clade 7 (Puckridge et al., 2013); *Neotrygon kublii* (Borsa et al., 2013a). Also BIN number BOLD:AAA5611 in BOLD.

Type material: the holotype (Fig. 2b) is a female specimen, 40 cm DW, collected by ISA on 06 December 2008 at the fish landing site of Kuala Lama, northeastern Sumatra Island (03°26'N 99°16'E). It is registered at the Museum Zoologicum Bogoriense under no. MZB-20847 (one of two specimens); it is also registered at LIPI-P2O under no. LIPI-4401 (one of two specimens). Whole specimen preserved in 96% alcohol and 4% formalin. A subsample of tissue, designated NK-MSKL3 is currently preserved in alcohol at -20°C in the freezers of the genetics laboratory at LIPI-P2O. The single paratype is the larger specimen under registration no. MZB-20847 (LIPI-4401; tissue sample no. NK-MSKL4); this is a female, 42 cm DW, from Kuala Lama, collected in March 2009 by ISA.

Description: the partial *CO1* gene sequence of the holotype, which has GenBank accession no. JX304818, is 5'-CTGGCCTCAGTTTACTTATCCGAACAGAACTGAGCCAACCAGGCGCTTTACTGGGTGATGATCAAATTTATAATGTAATCGTCACTGCCCA

CGCCTTCGTAATAATCTTCTTTATAGTAATGCCAATTATAATTGGTG
GGTTTGGCAACTGACTAGTGCCCCTGATAATTGGGGCTCCGGACAT
AGCCTTTCACGAATAAACAAACATAAGTTTTTGACTTCTACCTCCCT
CATTCTATTACTGCTAGCCTCAGCAGGAGTAGAAGCCGGAGCCGG
AACAGGTTGAACAGTTTATCCTCCATTAGCTGGTAATCTAGCACAT
GCCGGAGCTTCTGTAGACCTTACAATCTTCTCTCTTACCTAGCAG
GTGTTTCCCTCTATTTTGGCATCCATCAACTTTATCACAAACAATTATT
AATATAAAACCACCTGCAATCTCCAATATCAAACCCCATTTATTCGT
CTGATCTATTCTTGTTACAACCTGTACTTCTCCTGCTATCCCTACCAG
TCCTAGCAGCTGGCATTACTATACTCCTCACAGACCGAAATCTTAAT
ACAACCTTCTTTGACCCAGCTGGGGGAGGAGATCCCATTCTTTACC
AACACCTC -3'.

Diagnosis: based on Supplementary Table S1, *N. malaccensis* sp. nov. is distinguished from other species previously under *N. kublii* by G at nucleotide site 126 of the *CO1* gene. Most *N. malaccensis* sp. nov. individuals have T at nucleotide site 478, a character that was otherwise present in a single *N. orientale* individual; *N. malaccensis* sp. nov. also has C at nucleotide site 240, a character that it shares exclusively with *N. bobwardi* sp. nov. and T at nucleotide site 393, a character that it shares exclusively with the Guadalcanal maskray. Based on Supplementary Table S2, *N. malaccensis* sp. nov. is distinguished from other species previously under *N. kublii* by C at nucleotide sites 129 and 441, G at nucleotide site 405, and T at nucleotide site 264 of the *cytochrome b* gene; T at nucleotide site 225 is quasi-diagnostic; it also has C at nucleotide 399, a character that it shares with no species other than *N. bobwardi* sp. nov. Based on Puckridge et al. (2013), partial haplotypes at the *RAG-1* locus enable the distinction of *N. malaccensis* sp. nov. from *N. australiae*, *N. orientale* and *N. varidens*, but not from either *N. caeruleopunctata*, *N. trigonoides* or the Indian-Ocean maskray.

Distribution: the type locality of *N. malaccensis* sp. nov. is Kuala Lama, Malacca Strait (03°39'N 98°59'E). Specimens of the same species were sampled from Perbaungan, a neighbouring locality in the Malacca Strait and from the western coast of the Kra isthmus, Thailand. Known distribution, based on present study: northern part of Malacca Strait and eastern Andaman Sea.

Etymology: epithet *malaccensis* refers to the geographic origin of the type material, the Malacca Strait. It is the latinized geographical adjectival form of noun “Malacca”.

Proposed vernacular names: the Malacca Strait bluespotted maskray (English); pari masker totol biru Selat Melaka (Indonesian); raie pastenague masquée à points bleus du détroit de Malacca (French).

***Neotrygon moluccensis* sp. nov.** <http://zoobank.org/626567B0-3F01-4233-B7CA-2F617DE9186B>.

Clade VII (Borsa et al., 2016b); *Neotrygon kublii* Clade VII (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kublii* (Borsa et al., 2013a).

Type material: the holotype (Fig. 2c) is a female specimen, 30.5 cm DW, collected by A. Kusnadi on 08 April 2009 in front of the LIPI laboratories in Tual, Kei Islands (05°38'S 132°44'E). It is registered at the Museum Zoologicum Bogoriense under no. MZB-20866; also registered at LIPI-P2O under no. LIPI-4405. Preserved in 96% alcohol and 4% formalin. A subsample of tissue, numbered ARA1 is currently preserved in alcohol at -20°C in the freezers of the genetics laboratory at LIPI-P2O. The single paratype is MZB-20864 (LIPI-4400), female, 36 cm DW from Ambon Bay, collected on 23 October 2008 by ISA.

Description: the partial *CO1* gene sequence of the holotype, which has GenBank accession no. JX304898, is 5'-CTGTTCTTAGTTTACTTATCCGAAACAGAACTAACCCACCAGGCG

CTTTACTGGGTGATGATCAAATTTATAATGTAATCGTCACTGCCCA
CGCCTTCGTAATAATCTTCTTTATAGTAATGCCAATTATAATTGGTG
GGTTTGGTAATTGACTAGTGCCCCTGATGATTGGGGCTCCGGACA
TAGCCTTTCCACGAATAAACAAACATAAGTTTCTGACTTCTACCTCCC
TCATTCCTATTACTGCTAGCCTCAGCAGGAGTAGAAGCCGGACCTG
GGACAGGTTGAACAGTTTATCCCCCATTAGCTGGTAATCTACCACA
TGCCGGAGCTTCTGTAGACCTTACAATCTTTTCTCTTCACCTAGCAG
GTGTTTCTCTATTCTAGCATCCATCAACTTTATCACAAACAATTATT
AATATAAAACCACCTGCAATCTCCAGTATCAAACCCCATTAATTCGT
CTGATCTATTCTTGTTACAACCTGTACTTCTCCTGCTATCCCTACCAG
TCCTAGCAGCTGGCATTACTATACTCCTCACAGACCGAAATCTTAAT
ACAACCTTTCTTTGACCCAGCTGGAGGAGGAGATCCCATTCTTTACC
AACACCTC-3'.

Diagnosis: based on Supplementary Table S1, *N. moluccensis* sp. nov. is distinguished from other species previously under *N. kublii* by T at nucleotide site 447 of the *CO1* gene; *N. moluccensis* sp. nov. also has G at nucleotide site 261, a character that it shares with the Guadalcanal maskray and with a single *N. australiae* individual, and C at nucleotide site 309, a character that it shares with the Guadalcanal maskray and with a few individuals from the Indian Ocean. Based on Supplementary Table S2, *N. moluccensis* sp. nov. is distinguished from other species previously under *N. kublii* by C at nucleotide site 519, G at nucleotide site 453, and T at nucleotide sites 291, 906 and 1125 of the *cytochrome b* gene; it also has C at nucleotide site 246, a character that it shares with no species other than *N. caeruleopunctata*, and G at nucleotide site 1116, a character that it shares with no lineage other than the Indian-Ocean maskray.

Distribution: the type locality of *N. moluccensis* sp. nov. is Tual, Kei Islands, Moluccas, Indonesia. Specimens of the same species were sampled from Ambon, Moluccas. Known distribution, based on present study: eastern half of Banda Sea.

Etymology: epithet *moluccensis* refers to the geographic origin of the type material, the Molucca islands. It is the latinized geographical adjectival form of noun “Molucca”.

Proposed vernacular names: Moluccan bluespotted maskray (English); pari masker total biru Maluku (Indonesian); raie pastenague masquée à points bleus des Moluques (French).

***Neotrygon westpauensis* sp. nov.** [http://zoobank.org/565C798C-249E-4BA9-9B02-](http://zoobank.org/565C798C-249E-4BA9-9B02-ED2189C33FA2)

[ED2189C33FA2](http://zoobank.org/565C798C-249E-4BA9-9B02-ED2189C33FA2). Clade VIII (Borsa et al., 2016b); *Neotrygon kublii* clade VIII (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kublii* (Borsa et al., 2013a).

Type material: the holotype (Fig. 2d) is a female specimen, 36 cm DW, collected by Alvi Sitepu on 06 June 2009 in Biak, West Papua. It is registered at the Museum Zoologicum Bogoriense under no. MZB-20867; it is also registered at LIPI-P2O under no. LIPI 4408. The specimen is preserved in 96% alcohol and 4% formalin mixture. A subsample of tissue, numbered NK-BK5 is currently preserved in alcohol at -20°C in the freezers of the genetics laboratory at LIPI-P2O.

Description: the partial *CO1* gene sequence of the holotype, which has GenBank accession no. JX304909 is 5'-CTGGCCTCAGTTTACTTATCCGAACAGAACTAAGCCAACCAGGCGCTTTACTGGGTGATGATCAAATTTATAATGTAATCGTCACTGCCCA
CGCCTTCGTAATAATCTTCTTTATAGTAATGCCAATTATAATTGGTG
GGTTTGGTAATTGACTAGTACCCCTAATAATTGGGGCTCCGGACAT

AGCCTTTCCACGAATGAACAACATAAGTTTTGACTTCTGCCTCCCT
 CATTCTACTACTGCTAGCCTCAGCAGGGGTAGAAGCCGGAGCCG
 GAACAGGTTGAACAGTTTATCCCCATTAGCTGGTAATCTAGCACA
 TGCCGGAGCTTCTGTAGACCTTACAATCTTCTCTCTTTCACCTAGCAG
 GTGTTTCCTCTATTCTGGCATCCATCAACTTTATCACACAATTATT
 AATATAAAACCACCTGCAATCTCCCAGTATCAAACCCCATTTATTCGT
 CTGATCTATTCTTGTTACAACCTGTACTTCTCCTGCTATCCCTACCAG
 TCCTAGCAGCTGGCATTACTATACTCCTCACAGACCGAAATCTTAAT
 ACAACTTTCTTTGACCCAGCTGGAGGGGGAGATCCCATTTCTTTACC
 AACACCTC-3'.

Diagnosis: based on Supplementary Table S1, *N. westpapuensis* sp. nov. is distinguished from all other species previously under *N. kublii* by G at nucleotide 354 of the *CO1* gene; it also possesses G at nucleotide site 294, a character that it shares with no other species than *N. caeruleopunctata* and C at nucleotide site 334, a character that is otherwise present in the Ryukyu maskray only. Based on Supplementary Table S2, *N. westpapuensis* sp. nov. is distinguished from other species previously under *N. kublii* by C at nucleotide site 733, G at nucleotide sites 76, 372, 528 and 753, and T at nucleotide sites 48, 57, 228 and 792 of the *cytochrome b* gene; it also has T at nucleotide site 327, a character that it shares with no species other than *N. bobwardi* sp. nov.

Distribution: the type locality of *N. westpapuensis* sp. nov. is Biak Island north of Cenderawasih Bay, West Papua. As all samples we have of this species originate from Biak and from nearby Numfor Island only, amid a wide unsampled region (Fig. 1a), no distribution map can be inferred at present. The geological features of the northern coastline of the Vogelkop peninsula, where the shallow-water habitat favourable to blue-spotted maskrays is absent, isolate the reef fauna of Cenderawasih Bay from that of western West Papua further west. Further research is needed to delineate the precise geographic distribution of *N. westpapuensis* sp. nov.

Etymology: Named for the country of type locality, West Papua as it is spelled since 1961 (Saltford, 2003). Epithet *westpapuensis* is the latinized geographical adjectival form of noun “West Papua”, with no emendation.

Proposed vernacular names: West Papuan blue-spotted maskray (English); pari masker total biru West Papua (Indonesian); raie pastenague masquée à points bleus de Papouasie Occidentale (French).

4.2 Molecular diagnoses and updated distributions of *N. australiae*, *N. caeruleopunctata*, *N. orientale* and *N. varidens*

***Neotrygon australiae* Last, White and Séret 2016.** *Neotrygon kublii* clade V (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kublii* clade 5 (Puckridge et al., 2013); clade *Neotrygon kublii* 4 of Naylor et al. (2012); *Neotrygon kublii* (Borsa et al., 2013a; Cerutti-Pereyra et al., 2012). Also BIN number BOLD:AAA5609 in BOLD.

Diagnosis: based on Supplementary Table S1, *N. australiae* is distinguished from other species previously under *N. kublii* by T at nucleotide sites 124, 171 and 435 of the *CO1* gene; C at nucleotide site 321 is quasi-diagnostic. Based on Supplementary Table S2, *N. australiae* is distinguished from other species previously under *N. kublii* by A at nucleotide site 750, C at nucleotide sites 556 and 831, and T at nucleotide site 705 of the *cytochrome b* gene; T at nucleotide site 642 is partly diagnostic. Based on Puckridge et al. (2013), partial haplotypes at the *RAG-1* locus enable the distinction of *N. australiae* from *N. caeruleopunctata*, *N. malaccensis* sp. nov., *N. trigonoides* and the Indian-Ocean maskray, but not from either *N. orientale* or *N. varidens*.

Distribution: the type locality of *N. australiae* is southwest of Weipa, Gulf of Carpentaria (Last et al., 2016). Known distribution, based on Borsa et al. (2016b) (Fig. 1a): Timor Island, Rote Island, Lesser Sunda islands including Lombok and Flores, Sahul shelf, from Ningaloo Reef to Torres Strait and the northern extremity of the Great Barrier Reef. Last and co-authors wrote that the distribution of *N. australiae* also encompassed eastern Indonesia, West Papua and New Guinea, but this is totally unwarranted. Not only did these authors examine no blue-spotted maskray sample from these regions, but previous research had shown that the blue-spotted maskray samples from Ambon and Kei Islands in the Moluccas and from Biak Island in West Papua corresponded to distinct mitochondrial clades, themselves distinct from the clade that characterizes *N. australiae* (Arlyza et al., 2013a; Borsa et al., 2016a).

Remark: Last et al. (2016) assigned Müller and Henle's syntype from New Guinea (MNHN-IC-A-7931; collected by J.R.C. Quoy and J.P. Gaimard during the hydrographic expedition of the *Astrolabe*, 1826-1829) to *N. australiae*, without justification. They placed the sampling locality of that specimen in "West Papua, New Guinea, 7°30'S 132°30'E". These coordinates actually designate a location in the deep ocean 64 km east-southeast of the eastern tip of Larat Island in the Tanimbar archipelago, Moluccas. Not only the habitat at this location is unsuitable to *N. kublii*, which is a demersal, shallow-water chondrichthyan, but there is no indication from the collectors' notes (Quoy and Gaimard, 1835; Bauchot, 1994) or from the expedition commander's logbook (Dumont d'Urville, 1833) that the Tanimbar archipelago was ever visited by the *Astrolabe*. It has been instead determined that the New Guinea syntype originates from Manokwari in northern West Papua (00°52'S 134°04'E) (Borsa and Béarez, 2016). Based on sampling location, specimen MNHN-IC-A-7931 more likely belongs to *N. westpapuensis* sp. nov. than *N. australiae*. Sequencing fresh specimens from Manokwari would have helped verify this but so far we only accessed specimens from Pulau Numfoor (Borsa et al., 2016b), ca. 50 km east of Manokwari Bay.

***Neotrygon caeruleopunctata* Last, White and Séret 2016.** *Neotrygon kublii* clade VI (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kublii* clade 6 (Puckridge et al., 2013); *Neotrygon kublii* (Borsa et al., 2013a; Ward et al., 2008). Also BIN number BOLD:AAA5611 in BOLD.

Diagnosis: based on Supplementary Table S1, *N. caeruleopunctata* is distinguished from other species currently under *N. kublii* by the simultaneous possession of T at nucleotide site 457 of the *CO1* gene; *N. caeruleopunctata* also has G at nucleotide site 294, a character that it shares exclusively with *N. westpapuensis* sp. nov., C at nucleotide site 270, a character that it shares exclusively with a proportion of *N. orientale* individuals, and C at nucleotide site 567, a character that it shares exclusively with the Guadalcanal maskray. Based on Supplementary Table S2, *N. caeruleopunctata* is distinguished from other species previously under *N. kublii* by C at nucleotide sites 513 of the *cytochrome b* gene; the quartet (G, T, C, C) at nucleotide sites (57, 111, 327, 399) is also diagnostic. Based on Puckridge et al. (2013), partial haplotypes at the *RAG-1* locus enable the distinction of *N. caeruleopunctata* from *N. australiae*, *N. orientale* and *N. varidens*, but not from either *N. malaccensis* sp. nov., *N. trigonoides* or the Indian-Ocean maskray.

Distribution: the type locality of *N. caeruleopunctata* is Kedonganan Bay, Bali, Indonesia. All *N. caeruleopunctata* specimens sampled thus far originated from the Indian-Ocean coasts of Java and Bali (Fig. 1a). Four *N. caeruleopunctata* specimens included in Puckridge et al. (2013), whose partial *CO1* gene sequence was identical to the holotype of the species were reportedly sampled in Sadang, an inland locality of Central Java. However, figure 3 of Puckridge et al. (2013) suggests that the sample originated from the southern coast of Java Island.

***Neotrygon orientale* Last, White and Séret, 2016.** *Neotrygon kublii* clade IV (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kublii* clades 2 and 3 (Puckridge et al., 2013); clade *Neotrygon kublii* 1 of Naylor et al. (2012); *Neotrygon kublii* (Ward et al., 2008; Last et al., 2010; Aschliman et al., 2012; Borsa et al., 2013a; Shen et al., 2016). Also BIN number BOLD:ABZ6131 in BOLD.

Diagnosis: based on Supplementary Table S1, *N. orientale* is distinguished from other species previously under *N. kublii* by having a combination of C at nucleotide site 228 of the *CO1* gene that it shares exclusively with *N. varidens* and the Guadalcanal maskray, T at nucleotide site 405, which excludes *N. varidens* and the pair (C, A) at nucleotide sites (420, 522), which excludes the Guadalcanal maskray. Based on Supplementary Table S2, *N. orientale* has C at nucleotide site 255 of the *cytochrome b* gene, a character that it shares with no species other than *N. varidens*. Based on Puckridge et al. (2013), partial haplotypes at the *RAG-1* locus enable the distinction of *N. orientale* from *N. caeruleopunctata*, *N. malaccensis* sp. nov., *N. trigonoides* and the Indian-Ocean maskray, but not from either *N. australiae* or *N. varidens*.

Distribution: the type locality of *N. orientale* is the eastern Java Sea off the southeastern tip of Borneo (Last et al., 2016). Based on the present study, its wide distribution includes the South China Sea, the Java Sea, the Sulu Sea, the Sulawesi Sea, the Philippines and Sulawesi Island (Fig. 1a). Also sampled from Tanjung Luar, but exact geographic origin unknown (see Borsa et al., 2013b: 237).

***Neotrygon varidens* (Garman, 1885).** *Neotrygon varidens* (Last et al., 2016); *Neotrygon kublii* clade IV (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kublii* clade 1 (Puckridge et al., 2013); clade *Neotrygon kublii* 2 of Naylor et al. (2012); *Neotrygon kublii* (Ward et al., 2008; Cerutti-Pereyra et al., 2012; Wang et al., 2012; Arlyza et al., 2013b, Borsa et al., 2013a; Chen et al., 2014); *Dasybatus varidens*, original combination (Garman, 1885). Also BIN number BOLD:ABZ6130 in BOLD.

Diagnosis: based on Supplementary Table S1, *N. varidens* is diagnosed relative to other species previously under *N. kublii* by (C, T) at nucleotide sites (405, 456) of the *CO1* gene; *N. varidens* also has C at nucleotide site 507, which it shares exclusively with a proportion of *N. bobwardi* sp. nov. individuals, and C at nucleotide site 228, which it shares exclusively with *N. orientale* and the Guadalcanal maskray. Based on Supplementary Table S2, *N. varidens* is unique by having A at nucleotide site 480, C at nucleotide site 120, and T at nucleotide site 402 of the *cytochrome b* gene; it also has C at nucleotide site 255, a character that it shares with no species other than *N. orientale*, and T at nucleotide site 973, a character that it shares with no species other than *N. bobwardi* sp. nov. Based on Puckridge et al. (2013), partial haplotypes at the nuclear recombination activating protein 1 (*RAG-1*) locus enable the distinction of *N. varidens* from *N. caeruleopunctata*, *N. malaccensis* sp. nov., *N. trigonoides* and the Indian-Ocean maskray, but not from either *N. australiae* or *N. orientale*.

Distribution: the type locality of *N. varidens* is Hong Kong (Garman, 1885). Its known distribution, based on Borsa et al. (2016b) is the South China Sea.

4.3 Other *Neotrygon* spp. lineages requiring further attention

Guadalcanal maskray. Guadalcanal maskray (Borsa et al., 2016b); *Neotrygon kublii* (Last et al., 2016).

The single vouchered specimen available is CSIRO H7723-01 from Honiara, Guadalcanal, Solomon Islands. Its partial *CO1* gene sequence, which is unique among blue-spotted maskrays has been published (Last et al., 2016). The limited morphological data available show that the Guadalcanal maskray is a species different from *N. kublii* (Borsa and Béarez, 2016).

Indian-Ocean maskray. 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.

Voucher material for this clade includes a specimen collected by A. Pavan Kumar and colleagues on 15 August 2011 in Visakhapatnam, Andhra Pradesh state of India, Bay of Bengal. The specimen has been discarded (A. Pavan Kumar, pers. comm.) 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 BOLD under accession no. BOLD:ACB9305. Most (12/14) Indian Ocean maskray individuals in our dataset possessed T at nucleotide site 607 of the *CO1* gene, a character that was otherwise present in only 2/130 *N. orientale* individuals (Supplementary Table S1). From the material genetically examined thus far, the Indian bluespotted maskray's distribution includes the Indian coast of the Bay of Bengal (Visakhapatnam, Chennai), the Indian coast of the Laccadives Sea (Kerala), and eastern Africa (Borsa et al., 2016b).

Ryukyu maskray. Ryukyu maskray (Borsa et al., 2016b); *Neotrygon kublii* clade IV (Arlyza et al., 2013a; Borsa et al., 2016a); *Neotrygon kublii* clade 4 (Puckridge et al., 2013); *Neotrygon kublii* (Borsa et al., 2013a; Yagishita et al., 2009). Also BIN number BOLD:ACH3785 in BOLD.

If more material confirms that the Ryukyu maskray is genetically distinct from other blue-spotted maskrays, then this should be erected as a new species. The Ryukyu maskray has been reported from Ishigaki-shima; it was represented by a single nucleotide sequence (Yagishita et al., 2009) in our dataset.

5 DATA AVAILABILITY

All nucleotide sequences referred to in the present work have been deposited in GenBank (<https://www.nlm.nih.gov/>). New species names have been registered in ZooBank (<http://zoobank.org/>). The holotypes of the four new species described in this paper have been deposited at MZB, Cibinong, Indonesia.

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analysis tools: ISA, PB, TBH, KNS. Wrote the paper: PB. The authors declare no financial conflict of interest.

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Table 1 Ranges of values for 19 morphological characters claimed to be diagnostic of four species in the blue-spotted maskray species complex (data compiled from Last et al., 2016). *Diagnosticity*: utility of character to diagnose at least one of the four species. *N*, sample size

Character	Species (<i>N</i>)				Diagnosticity of character
	<i>N. australiae</i> (9)	<i>N. caeruleo-punctata</i> (10)	<i>N. kublii</i> (3) ^a	<i>N. orientale</i> (6)	
Disc width (DW) (cm)	>45	>47	>30	>38	no
DW / disc length (DL)	1.2–1.3	1.2–1.3	~1.2	1.2–1.3	no
Angle of snout (°)	101–103	125–130	~107	124–129	?
Snout length / interorbital width	1.6–2.1	1.8–2.4	1.7–2.1	1.9–2.5	no
Length from snout tip to pectoral-fin insertion / distance from snout tip to DW	1.7–1.8	1.9–2.0	1.8–1.9	1.8–2.0	no
Disc width / distance from snout tip to DW	2.3–2.5	2.6–2.9	2.5–2.6	2.7–2.9	no
Preoral length / mouth width	1.8–2.3	1.6–2.3	2.4–2.8	1.8–2.2	? ^c
Prenasal length / internasal distance	1.3–1.6	1.4–1.8	1.5–1.8	1.3–1.6	no
Interspiracular distance / DW (%)	14–16	13–14	13–15	13–16	no
Nostril length / DW (%)	3.4–4.0	2.5–3.5	2.8–4.1	3.1–3.8	no
Nasal curtain width / DW (%)	8.4–9.1	8.0–9.4	8.0–8.3	8.9–10.0	no
Mouth width / DW (%)	6.5–7.3	6.6–7.9	6.4–6.8	7.0–7.5	no
Horizontal distance from cloaca to caudal sting base / DL (%)	42–52	51–56	~55	48–56	no
Pectoral-fin radials	105–113	105–110	113 ^b	105–110	no
Total vertebral centra (including synarcual)	129–134	133–142	133 ^b	125–141	no
Trunk centra (including synarcual)	37–41	38–43	39 ^b	36–42	no
Largest spot on disc / eye width	0.7–1.2	0.5–0.8	0.3–0.5	0.5–0.9	no
Size of largest blue spot on medial belt / DW (%)	3.3–5.6	1.7–2.7	~2.1	2.8–3.3	no
Number of blue spots on medial belt	2–17	0–3	0–6	0–6	no

^a including one specimen of the Guadalcanal maskray, which is a distinct species as indicated by pigmentation patterns (Borsa and Béarez, 2016)

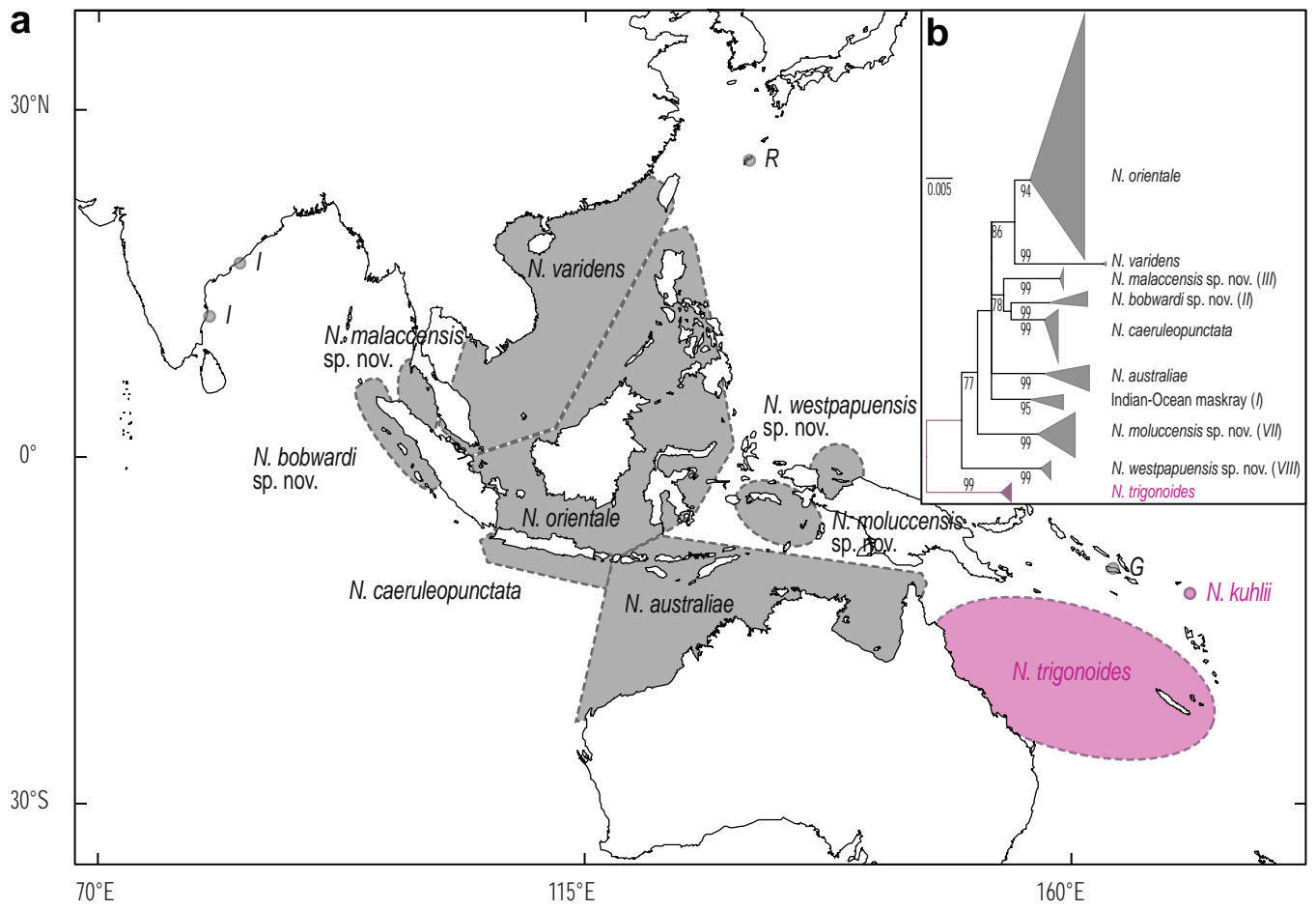
^b from a single specimen of the Guadalcanal maskray

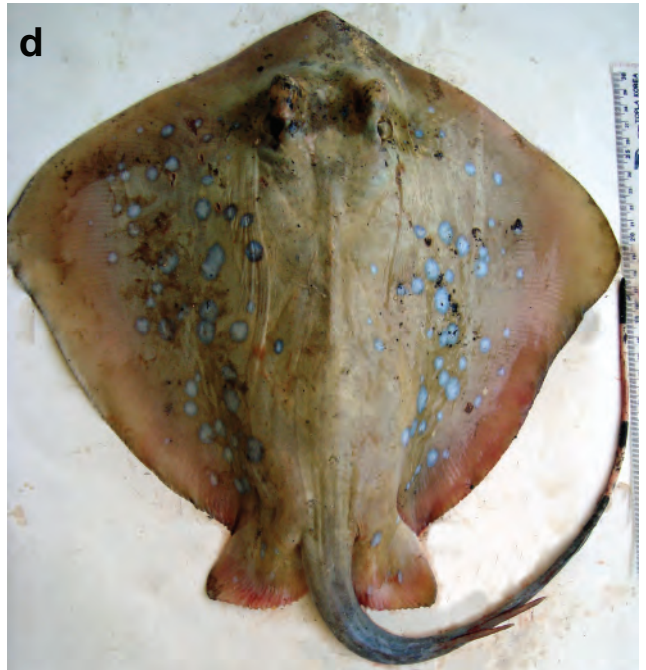
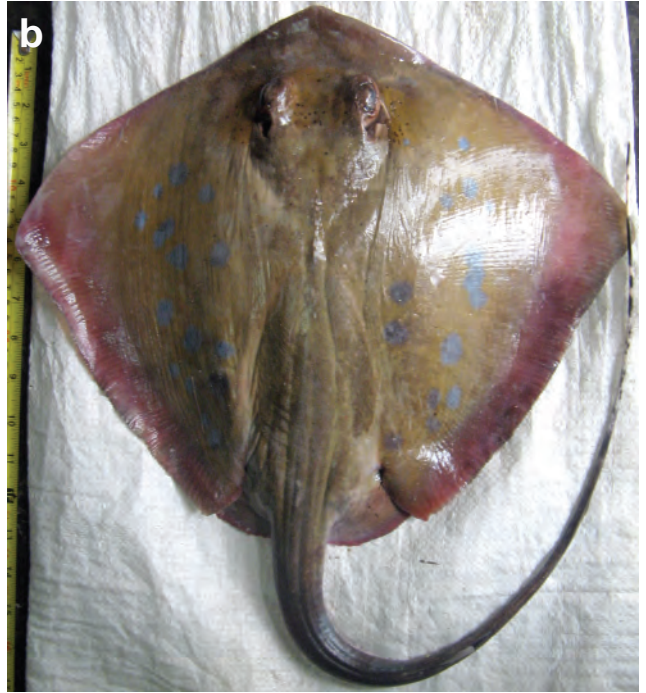
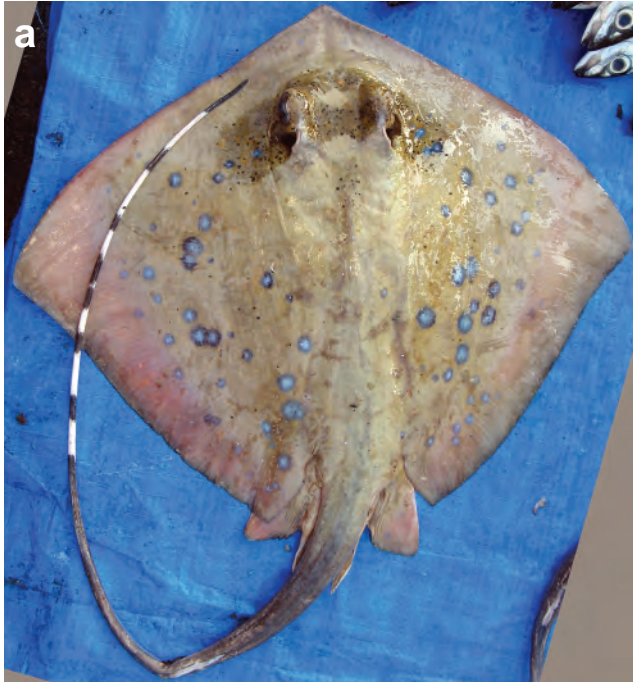
^c ratio of preoral length / mouth width possibly singles out *N. kublii* compared to the other species, but the sample size is too small (*N*=2 when excluding the Guadalcanal maskray) for the comparison to be meaningful

Captions to figures

Fig. 1 Geographic structure of the blue-spotted maskray species complex. a. Geographic ranges of eight blue-spotted maskray species previously under *Neotrygon kuhlii*, including four new species [from the point map of Borsa et al. (2016b)] (*dotted grey envelopes*). *Dotted purple ellipse*. *N. trigonooides*. Circles indicate the sampling sites of three additional lineages [Guadalcanal maskray (*G*); Indian-Ocean maskray (*I*) and Ryukyu maskray (*R*)] and the type-locality of *N. kuhlii*. b. Simplified maximum-likelihood phylogenetic tree of blue-spotted maskray species previously under *N. kuhlii*, including four new species, based on nucleotide sequences of the concatenated CO1+ cytochrome *b* gene fragments (127 sequences, trimmed to a core length of 1415 bp; Borsa et al., 2016b). New Caledonian maskray *N. trigonooides* was used as outgroup (Borsa et al., 2013a). Tree constructed under MEGA6 (Tamura et al., 2013) (Tamura-Nei model with gamma-distributed rate differences among sites + invariant sites; partial deletion). Numbers at a node are bootstrap scores (from 600 bootstrap resampling runs). Roman numbers in brackets follow clade nomenclature of Arlyza et al. (2013a). No nucleotide sequence was available for *N. kuhlii*, which based on colour patterns and geographic proximity is likely a close relative of *N. trigonooides* if not synonymous with it (Borsa and Béarez, 2016).

Fig. 2 Holotypes of four new maskray species previously under *Neotrygon kuhlii* (Müller and Henle, 1841). a. *Neotrygon bobwardi* sp. nov., no. MZB-20843 from Meulaboh, Aceh; male, 40 cm DW (photograph by ISA). b. *Neotrygon malaccensis* sp. nov., no. MZB-20847 from Kuala Lama, Malacca Strait; female, 40 cm DW (photograph by ISA). c. *Neotrygon moluccensis* sp. nov., no. MZB-20866 from Tual, Kei Islands; female, 30.5 cm DW. d. *Neotrygon westpapuensis* sp. nov., no. MZB-20867 from Biak, West Papua; female, 36 cm DW.





Supplementary Table S1 Variable nucleotide sites from the *CO1* sequence dataset used to diagnose blue-spotted maskray species formerly under *Neotrygon kuhlii* (Müller and Henle 1841). The sequence dataset comprises 314 individual sequences including 182 retrieved from GenBank, 128 from Borsa et al. (2016b), three from the Academia Sinica Institute of Zoology, Taipei, and one from Last et al. (2016). *Highlighted blue*: nucleotides diagnostic or quasi-diagnostic of one of the 8 blue-spotted maskray lineages assigned to a nominal species following the present revision, namely, *N. australiae*, *N. bobwardi* sp. nov., *N. caeruleopunctata*, *N. malaccensis* sp. nov., *N. moluccensis* sp. nov., *N. orientale*, *N. varidens*, and *N. westpapuensis* sp. nov.; *highlighted grey*: nucleotides that are diagnostic or quasi-diagnostic to two of the species. Nucleotide sites numerotated starting from the origin of the *CO1* gene in *N. orientale*, GenBank accession no. JN184065. The fragment used in this alignment is 519 bp long, spanning nucleotide sites 106-624. *Dot*: nucleotide identical to first sequence of table (*N. australiae*: DQ108184); *dash*: no data. Indian-Ocean maskray is clade *I* of Borsa et al. (2016b)

GenBank / specimen no.	Nucleotide site no.
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 3 3 3 3 3 3 3 3 3 3 3 3 3
	1 2 2 2 2 2 3 3 3 3 4 5 5 6 6 7 7 7 7 9 9 9 0 1 1 2 2 2 3 3 3 3 4 4 4 5 5 5 6 6 7 8 8 9 9 9 0 0 1 1 2 2 3 3 3 4 4 4 4
	4 1 4 6 8 9 1 2 5 8 4 3 9 0 5 1 2 4 7 2 3 8 1 0 6 2 6 8 1 4 7 9 0 3 9 0 2 8 1 7 0 0 5 0 4 7 5 9 6 8 1 7 1 4 9 0 2 8 9
<i>N. australiae</i>	
DQ108184	C G T A G C A A A T A T A A T T A C T C G A C A G T A T T G T G T C A G G G A G T G T G A C G T C G C A C T G C A A G
GU673441 C C
HM902468
JQ765536
JQ765537
KC250626 T . . . C C
KC250627
KC250632 T . . . C C
KC250635
KC250642
KC250645
JX304874 T . . . C C
JX304875 T . . . C C
LIP1 4428 T
KNS-LAB2-1 T . . . C C T
KNS-LAB3-2 T . . . C C
KNS-LAB4-3 T . . . C C
KNS-LAB5-4 T . . . C C
KNS-LAB6-5 T . . . C . . . C
KNS-LAB7-6 T . . . C C
KNS-LAB9-2 T . . . C C
KNS-LAB18-11 T . . . C C
KNS-TAL1-74 T . . . C C
KNS-TAL2-75 T . . . C C
KNS-TAL6-64 T . . . C C
KNS-KUP1-26 G
KNS-KUP2-27 T
KNS-KUP4-29 T
<i>N. bobwardi</i> sp. nov. (formerly clade <i>II</i> ; Borsa et al. 2016b)	
JX304798	. . C C A . . C . . . G . C . . . C . . . A A T G . .

JX304799	. . C C . . G . . A . . C G C . G . . A A T G . .
JX304800	. . C C . . G . . A . . C . A . . G C . G . . A . . C A T G . .
JX304801	. . C C . . T . . A . . C G C . G . . A . . C A T G . .
JX304802	. . C G A . . C G C . G A T G . .
JX304803	. . C C A . . C G . C C A A T G . .
JX304804	. . C C A . . C G C . G A T G . .
JX304805	. . C C A . . C G C . G A T G . .
JX304806	. . C C . . G . . A . . C T G C A T T G . .
JX304807	. . C C . . G . . A . . C G C A T T G . .
JX304808	. . C C A . . C G C A T T G . .
JX304809	. . C C . . G . . A . . C G C A T T G . .
JX304810	. . C C . . G . . A . . C G C A T T G . .
JX304811	. . C C . . G . . A . . C G C A T T G . .
JX304812	. . C C . . G . . A . . C G C A T T G . .
JX304813	. . C C . . G . . A . . C G C A T T G . .
JX304814	. . C C . . G . . A . . C G C A T T G . .
JX304815	. . C C . . G . . A . . C G C A T T G . .
JX304828	. . C C . . G . . A . . C G C A T T G . .
KNS-ACE1-12	. . C C A . . C G C . G A T G . .
KNS-ACE2-13	. . C C A . . C G C . G A T G . .
KNS-ACE3-14	. . C C A . . C G C . G A T G . .
KNS-ACE12-60	. . C C A . . C G . C C A A T G . .
KNS-PAD1-16	. . C C . . G . . A . . C G C A T T G . .
KNS-PAD2-17	. . C C . . G . . A . . C G C A T T G . .
KNS-PAD3-18	. . C C . . G . . A . . C G C . C A A T T G . .
KNS-PAD4-19	. . C C . . G . . A . . C G C . C A A T T G . .
KNS-PAD6-21	. . C C . . G . . A . . C G C . C A T T G . .
KNS-PAD9-48	. . C C . . G . . A . . C G C A T T G . .
KNS-PAD10-49	. . C C . . G . . A . . C G C . C A T T G . .
KNS-PAD12-50	. . C C . . G . . A . . C G C A T T G . .
KNS-PAD14-52	. . C C . . G . . A . . C G C . C A A T T G . .

N. caeruleopunctata

EU398736	. . C C A . . C C C G A T
EU398742	. . C C A . . C C C G A T
EU398743	. . C C A . . C C C G A T
EU398744	. . C C A . . C C C G A T
EU398745	. . C C A . . C C C G A T
EF609342	. . C C A . . C C C G A T
JX304860	. . C C A . . C C C G A T
KC250629	. . C C A . . C C C G A T
KC250630	. . C C A . . C C C G A T
KC250634	. . C C A . . C C C G A T
KC250637	. . C C A . . C C C G A T

KC250639	C	C	A	C	C	C	G	A	T
KNS-BAL-A	C	C	A	C	C	C	G	A	T
KNS-BAL-B	C	C	A	C	C	C	G	A	T
KNS-BAL-C	C	C	A	C	C	C	G	A	T
KNS-BAL-D	C	C	A	C	C	C	G	A	T
KNS-BAL-E	C	C	A	C	C	C	G	A	T
KNS-BAL-S	C	C	A	C	C	C	G	A	T
KNS-BAS3-1	C	C	A	C	C	C	G	A	T
KNS-PEL1-43	C	C	A	C	C	C	G	A	T
KNS-PEL2-65	C	C	A	C	C	C	G	A	T
KNS-PEL3-44	C	C	A	C	C	C	G	A	T
KNS-PEL4-45	C	C	A	C	C	C	G	A	T
KNS-PEL5-46	C	C	A	C	C	C	G	A	T
KNS-PEL6-78	C	C	A	C	C	C	G	A	T
KNS-PEL9-16	C	C	A	C	C	C	G	A	T
KNS-PEL15-22	C	C	A	C	C	C	G	A	T

N. malaccensis sp. nov. (formerly clade III; Borsa et al. 2016b)

GU673423	C	G	C	A	C	A	C	A	T
GU673425	C	G	C	A	C	A	C	A	T
GU673426	C	G	C	A	C	C	C	A	T
GU673427	C	G	C	A	C	C	C	A	T
GU673428	C	G	C	A	C	A	C	A	T
JX304816	C	G	C	A	C	C	C	A	T
JX304817	C	G	C	A	C	C	C	A	T
JX304818	C	G	C	A	C	C	C	A	T
JX304819	C	G	C	A	C	C	C	A	T
JX304820	C	G	C	A	C	C	C	A	T
JX304821	C	G	C	A	C	C	C	A	T
JX304822	C	G	C	A	C	C	A	A	T
JX304823	A	C	G	C	A	C	C	T	A
JX304824	C	G	C	A	C	C	C	A	T
JX304825	C	G	C	A	C	C	C	T	A
JX304826	C	G	C	A	C	C	C	A	T
JX304827	A	C	G	C	A	C	C	A	T
KNS-MAL2-32	C	G	C	A	C	C	C	A	T
KNS-MAL3-33	C	G	C	A	C	C	C	A	T
KNS-MAL4-34	C	G	C	A	C	C	C	A	T
KNS-MAL5-35	C	G	C	A	C	C	C	A	T
KNS-MAL6-36	C	G	C	A	C	C	C	A	T
KNS-MAL7-37	C	G	C	A	C	C	C	A	T

N. moluccensis sp. nov. (formerly clade VII; Borsa et al. 2016b)

JX304892	C	C	A	C	A	T	A	G	C	A	T
JX304893	C	C	A	C	A	T	A	G	C	A	T

JX304894	. . C C A . . C A . . T A G C . A T
JX304895	. . C . C . C . . C A . . C A . . T A G C . A T
JX304896	. . C . C C A . . C A . . T A G C . A T
JX304897	. . C C A . . C A . . T A G C . A T
JX304898	. . C . C . C . . C A . . C T G C . A T
JX304899	. . C C A . . C T G C . A T
JX304900	. . C C A . . C T G C . A T
JX304901	. . C C A . . C T G C . A T
JX304902	. . C C A . . C T G C . A T
JX304903	. . C C A . . C T G C . A T
JX304904	. . C C A . . C T G C . A T
JX304905	. . C C A . . C T G C . A T
KNS-KEI2-53	. . C C A . . C T G C . A T
KNS-KEI3-72	. . C C A . . C T G C . A T
KNS-KEI4-54	. . C C A . . C T G C . A T
KNS-KEI5-55	. . C C A . . C T G C . A T
KNS-KEI6-56	. . C C A . . C T G C . A T
KNS-KEI7-73	. . C C A . . C T G C . A T
KNS-KEI12-38	. . C C A . . C T G C . A T
KNS-KEI13-39	. . C C A . . C T G C . A T
KNS-KEI15-41	. . C C A . . C T G C . A T

N. orientale

EU398737	T . C C A . . C . . G C T A T . T G .
EU398738	T . C C A . . C . . G C T A T . T G .
EU398739	. . C C C A . . C C T A T . T
EU398740	T . C C A . . C . . G C T A T . T G .
EU398741	T . C C A . . C . . G C C T A T . T G .
GU673709	. . C C C A . . C C T A T . T
JN184065	. . C C A . . C C . A . . T T A T . T
JX304829	. . C C C A . . C C T A T . T
JX304830	. . C C A . . C . . G C T A T . T G .
JX304831	T . C C A . . C . . G C T A T . T G .
JX304832	. . C C C A . . C C T A T . T
JX304833	. . C C C A . . C C T A T . T
JX304834	T . C C A . . C . . G C T A T . T G .
JX304835	T . C C A . . C . . G C T A T . T G .
JX304836	T . C C A . . C . . G C T A T . T G .
JX304837	. . C C C A . . C C T A T . T
JX304838	. . C C C A . . C C T A T . T
JX304839	T . C C A . . C . . G C T A T . T G .
JX304840	T . C C A . . C . . G C T A T . T G .
JX304841	T . C C A . . C . . G C T A T . T G .
JX304842	T . C C A . . C . . G C T A T . T G .

JX304843	T	C		C		A	C	G		C						T	A	T	T		G	
JX304844	T	C		C		A	C	G		C						T	A	T	T		G	
JX304845	T	C		C		A	C	G		C						T	A	T	T		G	
JX304847		C		C		A	C	G		C						T	A	T	T		G	
JX304848		C		C		A	C	G		C						T	A	T	T		G	
JX304849		C		C		A		G		C						T	A	T	T		G	
JX304850		C		C		A	C	G		C						T	A	T	T		G	
JX304851	T	C		C		A	C	G		C						T	A	T	T		G	
JX304852	T	C		C		A	C	G		C						T	A	T	T		G	
JX304853		C		C C G		A	C			C						A	T T	A	T	T		T
JX304854		C		C		A	C	G		C						T	A	T	T		G	
JX304855		C		C		A	C	G		C						T	A	T	T		G	
JX304856		C		C C		A	C			C						C	T	A	T	T		
JX304857	T	C		C		A	C	G		C						T	A	T	T		G	
JX304858		C		C C		A	C			C						T	A	T	T			
JX304859	T	C		C		A	C	G		C						T	A	T	T		G	
JX304861		C		C		A	C			C						C	T	A	T	T		
JX304862		C		C		A	C			C						T	A	T	T			
JX304863		C		C		A	C			C						T	A	T	T			
JX304864		C		C		A	C			C						T	A	T	T			
JX304865		C		C		A	C			C						T	A	T	T			
JX304866		C		C		A	C			C						T	A	T	T			
JX304867		C		C C		A	C			C						T	A	T	T			
JX304869		C		C		A	C T			C						C	T	A	T	T		
JX304870		C		C		A	C T			C						C	T	A	T	T		
JX304871		C		C		A	C T			C						C	T	A	T	T		
JX304872		C		C		A	C T			C						C	T	A	T	T		
JX304873		C		C		A	C T			C						C	T	A	T	T		
JX304876		C		C		A	C			C						A	T	A	T	T		
JX304877		C		C		A	C			C						A	T	A	T	T		
JX304878		C		C		A	C			C						A	T	A	T	T		
JX304879		C		C		A	C			C						A	T	A	T	T		
JX304880		C		C		A	C			C						A	T	A	T	T		
JX304881		C		C		A	C			C						A	T	A	T	T		
JX304882		C		C		A	C			C						A	T	A	T	T		
JX304883		C		C		A	C			C						A	T	A	T	T		
JX304884		C		C		A	C			C						A	T	A	T	T		
JX304885		C		C		A	C			C						A	T	A	T	T		
JX304886		C		C		A	C			C						A	T	A	T	T		
JX304887		C		C		A	C			C						A	T	A	T	T		
JX304888		C		C G		A	C			C						T	A	T	T			
JX304889		C		C		A	C			C						T	A	T	T			
JX304890		C		C		A	C			C						T	A	T	T			

JX304891	. . C C A . . C C C T A T . T
KC249903	. . C C A . . C C A T A T . T
KC249904	. . C C A . . C C A T T A T . T
KC249905	. . C C A . . C C A T T A T . T
KM073024	. . C C A . . C C T T A T . T
KM073025	. . C C A . . C C T T A T . T
KP856772	T . C C A . . C . . G C T A T . T T G .
KP856773	. . C C A . . C . . G C T A T . T G .
KR019777	. . C C A . . C C A . . C . . T A T . T
KNS-BAS1-87	. . C C A . . C C T A T . T
KNS-BAS2-77	. . C C A . . C C T A T . T
KNS-BAS4-2	. . C C A . . C C T A T . T
KNS-BAS5-3	. . C C A . . C T G C T A T . T
KNS-BAS6-4	. . C C A . . C T G C T A T . T
KNS-BIT1-14	. . C C A . . C T C T A T . T
KNS-BIT2-15	. . C C A . . C C T A T . T
KNS-BIT3-16	. . C C A . . C C T A T . T G .
KNS-BIT4-17	. . C C A . . C C T A T . T
KNS-BIT5-18	. . C C A . . C C T A T . T
KNS-BIT6-19	. . C C A . . C C T A T . T
KNS-BIT7-5	. . C C A . . C C T A T . T
KNS-BIT8-6	. . C C A . . C C T A T . T G .
KNS-BIT9-7	. . C C A . . C C T A T . T
KNS-BIT10-8	. . C C A . . C C T A T . T
KNS-BIT11-9	. . C C A . . T C C T A T . T
KNS-BIT12-10	. . C C A . . C C T A T . T
KNS-KEN2-81	. . C C A . . C C T A T . T
KNS-KEN3-69	. . C C A . . C C T A T . T
KNS-KEN4-51	. . C C A . . C C T A T . T
KNS-KEN5-70	. . C C A . . C C T A T . T
KNS-KEN7-82	. . C C A . . C C T A T . T
KNS-MAK1-20	. . C C A . . C T C . . C C T A T . T
KNS-MAK2-21	. . C C A . . C T C . . C C T A T . T
KNS-MAK4-23	. . C C A . . C T C . . C C T A T . T
KNS-MAK5-24	. . C C A . . C C T A T . T
KNS-MAK6-25	. . C C A . . C T C . . C C T A T . T
KNS-POS1-61	. . C C A . . T C C T T A T . T G .
KNS-POS2-49	. . C C A . . C C T T A T . T G .
KNS-POS04-50	. . C C A . . C C T T A T . T G .
KNS-RIA1-30	. . C C A . . C . . G C T A T . T G .
KNS-RIA2-31	T . C C A . . C . . G C T A T . T G .
KNS-RIA3-32	. . C C A . . C . . G C T A T . T G .
KNS-RIA4-34	. . C C A . . C . . G C T A T . T G .

KNS-TAL4-76	. . C C C A . . C C T A T . T
KNS-WJS1-22	T . C C A . . C . . G C T A T . T G .
KNS-WJS2-23	. . C C C A . . C C T A T . T
KNS-WJS4-25	T . C C A . . C . . G C T A T . T G .
KNS-WJS1-88	T . C C A . . C . . G C T A T . T G .
KNS-WJS2-93	T . C C A . . C . . G C T A T . T G .
KNS-WJS3-89	T . C C A . . C . . G C T A T . T G .
KNS-WJS4-90	T . C C A . . C . . G C T A T . T G .
KNS-WJS5-91	. . C C C A . . C C T A T . T
KNS-WJS6-92	T . C C A . . C . . G C T A T . T G .
KNS-WJS1-7	T . C C A . . C . . G C T A T . T G .
KNS-WJS2-8	. . C C C A . . C C T A T . T
KNS-WJS3-9	T . C C A . . C . . G C T A T . T T G .
KNS-WJS4-10	. . C C C A . . C C T A T . T
KNS-WJS5-11	. . C C A . . C . . G C T A T . T G .
KNS-WJS6-12	. . C C C A . . C C T A T . T
KNS-WJS7-13	T . C C A . . C . . G C T A T . T G .
KNS-WJS3-35	T . C C A . . C . . G C T A T . T G .
KNS-WSS1-26	. . C C A . . C C T A T . T
KNS-WSS2-27	. . C C A . . C C T A T . T
KNS-WSS3-28	. . C C A . . C C T A T . T
KNS-WSS4-29	. . C C A . . C C T A T . T
KNS-WSS5-30	. . C C A . . C C T A T . T
<i>N. varidens</i>	
EU398733	. . C C A . . T C T A T . T
EU398734	. . C C A . . T C T A T . T
EU398735	. . C C A . . T C T A T . T
JQ681494	. . C C G . . T C T A T . T
JQ765561	. . C C A . . T C T A T . T
JQ765562	. . C C A . . T C T A T . T
JX263422	. . C C A . . T C T A T . T
KC249902	. . C C A . . T C T A T . T
JX304846	. . C C A . . T C T A T . T
JX304868	. . C C A . . T C T A T . T
KC250640	. . C C A . . T C T A T . T
KC992792	. . C C A . . T C T A T . T
KM073023	. . C C A . . T C T A T . T
ASIZP0806084	. . C C A . . T C T A T . T
ASIZP0806153	. . C C A . . T C T A T . T
ASIZP0806154	. . C C A . . T C T A T . T
<i>N. westpauensis</i> sp. nov. (formerly clade VIII; Borsa et al. 2016b)	
JX304906	. . C C A . . C T A A G T C
JX304907	. . C . C C T . A C T T A A G T C

JX304908	. . C C A . . C T . . A A G T . . C
JX304909	. . C C A . . C T . . A A G T . . C
JX304910	. . C C A . . C T . . A A G T . . C
JX304911	. . C C A . . C T . . A A G T . . C
JX304912	. . C C A . . C T . . A A G T . . C
JX304913	. . C C A . . C T . . A A G T . . C
JX304914	. . C C A . . C T . . A A G T . . C
JX304915	. . C C A G . C T . . A A G T . . C
ISA-bia13	. . C C A G . C T . . A A G T . . C
ISA-bia25	. . C C A G . C T . . A A G T . . C
ISA-bia30	. . C C A . . C T . . A A G T . . C
KNS-BIA2-38	. . C C A . . C T . . A A G T . . C
KNS-BIA3-39	. . C C A . . C T . . A A G T . . C
KNS-BIA4-40	. . C C A . . C T . . A A G T . . C
KNS-BIA5-41	. . C C A G . C T . . A A G T . . C
KNS-BIA6-42	. . C C A . . C T . . A A G T . . C
KNS-BIA7-66	. . C C A . . C T . . A A G T . . C
Guadalcanal maskray	
CSIRO H 7723-01	. . C C . . G . . A A . . C G A C . . T C
Indian-Ocean maskray	
HM467799	. . C C A . . C C A A T
JX263421	. . C C A . . C A A C . . T
JX978329	. . C C . . C . . A . . C A A T A
KC249906	. . C C A . . C A A C . . T A
KF899609	. . C C A . . C A A A T
KF899610	. . C C A . . C A A A T
KF899611	. . C C A . . C A A A T
KF899612	. . C C A . . C A A A T
KF899613	. . C C A . . C A A A T
KR003770	. . C C A . . C A A T T
KNS-TZN1-52	. . C C A . . C A A T G
KNS-ZAN3-86	. . C C A . . C A A T
KNS-ZAN4-71	. . C C A . . C A A T
KNS-ZAN5-80	. . C C A . . C A A T
Ryukyu maskray	
AB485685	. . C C . . G . . A . . C A A T A T . . T C . . G . .

JX304799	. T C T C C G A C
JX304800 C T C G C
JX304801 C T A C A G C
JX304802 C T C C G C
JX304803 C T C T
JX304804 C T C G . T C
JX304805 C T C G . T C
JX304806 C T C C .
JX304807 A C T C C
JX304808 C T A C C .
JX304809 C T C C .
JX304810 C T C C .
JX304811 C T C C .
JX304812 C T C C .
JX304813 C T C C .
JX304814 C T C C .
JX304815 C T C C .
JX304828 C T A C C .
KNS-ACE1-12 C T C C G C
KNS-ACE2-13 C T C C G C
KNS-ACE3-14 C T C T G C
KNS-ACE12-60 C T C .
KNS-PAD1-16 C T C C .
KNS-PAD2-17 C T C C .
KNS-PAD3-18 C T C T C
KNS-PAD4-19 C T C C .
KNS-PAD6-21 C T C C .
KNS-PAD9-48 A C T C C
KNS-PAD10-49 C T C C T
KNS-PAD12-50 C T C C T T
KNS-PAD14-52 C T C C .
<i>N. caeruleopunctata</i>	
EU398736 C T C T C
EU398742 C T C T C
EU398743 C T C T C
EU398744 C T C T C
EU398745 C T C T C
EF609342 C T C T C
JX304860 C T C T C
KC250629 C T C T C
KC250630 C T C T C
KC250634 C T C T C
KC250637 C T C T C

KC250639	C	T	C	T	C	C
KNS-BAL-A	C	T	C	T	C	C
KNS-BAL-B	C	T	C	T	C	C
KNS-BAL-C	C	T	C	T	C	C
KNS-BAL-D	C	T	C	T	C	C
KNS-BAL-E	C	T	C	T	T	C
KNS-BAL-S	C	T	C	T	C	C
KNS-BAS3-1	C	T	C	T	C	C
KNS-PEL1-43	C	T	C	T	C	C
KNS-PEL2-65	C	T	C	T	C	C
KNS-PEL3-44	C	T	G	C	T	C
KNS-PEL4-45	C	T	C	T	C	C
KNS-PEL5-46	C	T	C	T	C	C
KNS-PEL6-78	C	T	C	T	C	C
KNS-PEL9-16	C	T	C	T	C	C
KNS-PEL15-22	C	T	G	C	T	C

N. malaccensis sp. nov. (formerly clade III; Borsa et al. 2016b)

GU673423	C	T	T	C	T	A
GU673425	C	T	T	C	T	A
GU673426	C	T	T	C	T	A
GU673427	C	T	T	C	T	A
GU673428	C	T	T	C	T	A
JX304816	C	T	T	C	T	A
JX304817	C	T	T	C	T	A
JX304818	C	T	T	C	T	A
JX304819	C	T	T	C	T	A
JX304820	C	T	T	C	T	A
JX304821	C	T	T	C	T	A
JX304822	C	T	T	C	T	A
JX304823	C	T	T	C	T	A
JX304824	C	T	T	C	T	A
JX304825	C	T	G	C	T	A
JX304826	C	T	T	C	T	A
JX304827	A	C	T	C	T	A
KNS-MAL2-32	C	T	T	C	T	A
KNS-MAL3-33	C	T	T	C	T	A
KNS-MAL4-34	C	T	T	C	T	A
KNS-MAL5-35	C	T	T	C	T	A
KNS-MAL6-36	C	T	T	C	T	A
KNS-MAL7-37	C	T	T	C	T	A

N. moluccensis sp. nov. (formerly clade VII; Borsa et al. 2016b)

JX304892	C	T	C	T	A	T
JX304893	C	T	A	C	T	T

JX304894 C T C T A
 JX304895 . A C T C T A
 JX304896 C C T C T A T
 JX304897 C T C T A
 JX304898 C G T C T A
 JX304899 G T C T A
 JX304900 G T C T A
 JX304901 G T C T A
 JX304902 T T C T A
 JX304903 G T C T A
 JX304904 G T C T A
 JX304905 G T C T A
 KNS-KEI2-53 G T C T A
 KNS-KEI3-72 G T C T A
 KNS-KEI4-54 T T C T A
 KNS-KEI5-55 G T C T A
 KNS-KEI6-56 G T C T A
 KNS-KEI7-73 T T C T A
 KNS-KEI12-38 G T C T A
 KNS-KEI13-39 G T C T A
 KNS-KEI15-41 G T C T A

N. orientale

EU398737 T C C T C A T
 EU398738 T C T C A T
 EU398739 T C T C A T
 EU398740 T C T C A T
 EU398741 T C T C A T
 GU673709 T C T C A T
 JN184065 T C T C A T
 JX304829 T C T C A T
 JX304830 T C T C A T
 JX304831 T C T C A T
 JX304832 A T C T C A T
 JX304833 T C T C A T
 JX304834 T C T C A T
 JX304835 T C T C A T
 JX304836 A T C T C A T
 JX304837 T C T C A T
 JX304838 T C T C A T
 JX304839 T C T C A T T
 JX304840 T C T C A T
 JX304841 T C T C A T
 JX304842 T C T C A T

JX304843	T	C	T	.	.	C	A	T	T	.
JX304844	T	C	T	.	.	C	A	T	.	.
JX304845	T	C	T	.	.	C	A	T	.	.
JX304847	T	C	T	.	.	C	A	T	.	.
JX304848	T	C	T	.	.	C	A	C	.	.	.	T	.	.
JX304849	T	C	T	.	.	C	A	T	.	.
JX304850	T	C	T	.	.	C	A	T	.	.
JX304851	T	C	T	.	.	C	A	T	.	.
JX304852	T	C	T	.	.	C	A	T	.	.
JX304853	T	T	.	.	.	C	T	.	.	C	A	T	.	.
JX304854	T	C	T	.	.	C	A	T	.	.
JX304855	T	C	T	.	.	C	A	T	.	.
JX304856	.	C	.	.	T	C	T	.	.	C	A	T	.	.
JX304857	T	C	T	.	.	C	A	T	.	.
JX304858	T	C	T	.	.	C	A	T	.	.
JX304859	T	C	T	.	.	C	A	T	.	.
JX304861	C	T	.	.	A
JX304862	T	C	T	.	.	C	A	T	.	.
JX304863	T	C	.	.	.	A	C	T	.	.	C	A
JX304864	T	C	.	.	.	C	T	.	.	C	A
JX304865	C	T	.	.	C	A	T	.	.
JX304866	C	T	.	.	C	A	T	.	.
JX304867	T	C	.	.	.	C	T	.	.	C	A
JX304869	T	C	T	.	.	A
JX304870	T	C	T	.	.	A
JX304871	T	C	T	.	.	A
JX304872	T	C	T	.	.	A
JX304873	T	C	T	.	.	A
JX304876	C	A	T	.	A
JX304877	C	A	T	.	A
JX304878	C	A	T	.	A
JX304879	C	A	T	.	A
JX304880	C	A	T	.	A
JX304881	C	A	T	.	A
JX304882	C	A	T	.	A
JX304883	C	A	T	.	A
JX304884	C	A	T	.	A
JX304885	C	A	T	.	A
JX304886	C	A	T	.	A
JX304887	C	A	T	.	A
JX304888	.	G	.	.	T	C	A	.	.	C	T	T	.	.	A
JX304889	T	.	A	.	.	C	.	T	.	.	.	T	.	.	A
JX304890	T	C	A	.	.	C	T	.	.	A

JX304891	G	T	C A	C	T	T	A		
KC249903		T		C		A T T	A		
KC249904		T		C		T	C A		T
KC249905		T		C		T	C A		T
KM073024		T		C		T	C A		T
KM073025		T		C		T	C A	G	T
KP856772		T		C		T	C A		T
KP856773		T		C		T	C A		T
KR019777		T		C		A T T	G A		
KNS-BAS1-87		T		C		T	C A		T
KNS-BAS2-77		T		C		T	C A		T
KNS-BAS4-2		T		C		T	C A		T
KNS-BAS5-3		T		C		T	C A		T
KNS-BAS6-4		T		C		T	C A		T
KNS-BIT1-14		T		C		T	C A		T
KNS-BIT2-15		T		C		T	C A		T
KNS-BIT3-16		T	G	C		A T	A		A
KNS-BIT4-17		T		C		T	C A	T	T
KNS-BIT5-18		T		C		T	C A		T
KNS-BIT6-19		T		C		T	C A		T
KNS-BIT7-5		T		C		T	C A		T
KNS-BIT8-6		T	G	C		A T	A		A
KNS-BIT9-7		T		C		T	C A	G	T
KNS-BIT10-8		T		C		T	C A	T	T
KNS-BIT11-9		T		C		T	C A		T
KNS-BIT12-10		T		C		T	C A		T
KNS-KEN2-81		T		C		T	A		
KNS-KEN3-69		T		C		T	A		
KNS-KEN4-51		T		C		T	A		
KNS-KEN5-70		T		C		T	A		
KNS-KEN7-82		T		C		T	A		
KNS-MAK1-20		T		C		T	A		
KNS-MAK2-21		T		C		T	A		
KNS-MAK4-23		T		C		T	A		
KNS-MAK5-24		T		C		T	C A		T
KNS-MAK6-25		T		C		T	A		
KNS-POS1-61		T		C		T	C A		
KNS-POS2-49		T		C		T	C A		
KNS-POS04-50		T		C		T	C A		
KNS-RIA1-30		T		C		T	C A		T
KNS-RIA2-31		T		C		T	C A		T
KNS-RIA3-32		T		C		T	C A		T
KNS-RIA4-34		T		C		T	C A		T

KNS-TAL4-76			T		C			T		C A			T
KNS-WJS1-22			T		C			T		C A			T
KNS-WJS2-23			T		C			T		C A			T
KNS-WJS4-25			T		C			T		C A			T
KNS-WJS1-88			T		C			T		C A			T
KNS-WJS2-93			T		C			T		C A			T
KNS-WJS3-89			T		C			T		C A			T
KNS-WJS4-90			T		C			T		C A			T
KNS-WJS5-91			T		C			T		C A			T
KNS-WJS6-92			T		C			T		C A	T		T
KNS-WJS1-7			T		C			T		C A			T
KNS-WJS2-8			T		C			T		C A			T
KNS-WJS3-9			T		C			T		C A			T
KNS-WJS4-10			T		C			T		C A			T
KNS-WJS5-11			T		C			T		C A			T
KNS-WJS6-12			T		C			T		C A			T
KNS-WJS7-13			T		C			T		C A			T
KNS-WJS3-35			T		C			T		C A			T
KNS-WSS1-26			T	C		C		T		C A			T
KNS-WSS2-27			T	C		C		T		C A			T
KNS-WSS3-28			T	C		C		T		C A	T		T
KNS-WSS4-29			T	C		C		T		C A			T
KNS-WSS5-30			C			C		T		C A			T
<i>N. varidens</i>													
EU398733			T	C		C		T		T	C		T
EU398734			T	C		C		T		T	C		T
EU398735			T	C		C		T		T	C		T
JQ681494			T	C		C		T		T	C		T
JQ765561			T	C		C		T		T	C		T
JQ765562			T	C		C		T		T	C		T
JX263422			T	C		C		T		T	C		T
KC249902			T	C		C		T		T	C		T
JX304846			T	C		C		T		T	C		T
JX304868			T	C		C		T		T	C	T	T
KC250640			T	C		C		T		T	C		T
KC992792			T	C		C		T		T	C		T
KM073023			T	C		C		T		T	C		T
ASIZP0806084			T	C		C		T		T	C		T
ASIZP0806153			T	C		C		T		T	C		T
ASIZP0806154			T	C		C		T		T	C		T
<i>N. westpauensis</i> sp. nov. (formerly clade VIII; Borsa et al. 2016b)													
JX304906	G			C		T		A	C				
JX304907	G			C		T			C				

KNS-PEL3-44	- - -	G	T	T	A	C	C	C
KNS-PEL4-45	T C	G	T	T	A	C	C	C
KNS-PEL5-46	- - -	G	T	T	A	C	C	C
KNS-PEL6-78	T C	G	T	T	A	C	C	C
KNS-PEL9-16	- - -	G	T	T	A	C	C	C
KNS-PEL15-22	- - G	G	T	T	A	C	C	C
<i>N. malaccensis</i> sp. nov. (formerly clade III; Borsa et al. 2016b)								
KNS-MAL1-31	- - -		T		A	T	T	C
KNS-MAL2-32	- - -		T		A	T	T	C
KNS-MAL3-33	T C		T		A	T C	T	C
KNS-MAL4-34	- C		T		A	T	T	C
KNS-MAL5-35	T C		T T		A	T	T	C
KNS-MAL6-36	- - -		T		A	T	T	C
KNS-MAL7-37	- - -		T		A	T	T C	C
KNS-MAL10	- - -		T		A	.	T	C
KNS-MAL11	- - - G		T		A	T	T	C
KNS-MAL12	G C		T		A	T	T	C
KNS-MAL14	T C		T		A	T	T	C
KNS-MAL16	T C		T		A	T	T	C
KNS-MAL17	T C		T		C A	T	T	C
<i>N. moluccensis</i> sp. nov. (formerly clade VII; Borsa et al. 2016b)								
AM1	- - - C		T		A T	C	C	T
AM2	- C	C	T		A T	C	C	T
AM3	- C	C	T		A T	C	C	T
AM4	- - - C		T		A T	C	C	T
AM5	- - - C		T		A T	C	C	T
AM6	G C	C	T		A T	C	C	T
KNS-KEI2-53	- - - C		T C		A T	C		T
KNS-KEI3-72	- C	C	T C		A T	C		T
KNS-KEI4-54	T C	C	T C		A T	C	C	T
KNS-KEI5-55	T C	C	T C		A T	C		T
KNS-KEI6-56	- - - C		T C		A T	C		T A
KNS-KEI7-73	- - - C		T C		A T	C	C	T
KNS-KEI8-34	- - - C	C	T C		A T	C	C	T
KNS-KEI10-36	- - - C		T C		A T	C		T
KNS-KEI12-38	- - - C		T C		A T	C		T
KNS-KEI18-44	- - - -		T C		A T	C		T
<i>N. orientale</i>								
JN184065	T C		T G		A	C	C	
KR019777	T C		T G		A	C	C	
cag1	- - -		T G		A	C	C	
cag2	- C		T G		A	C	C	

KNS-BIA5-41	T C	T T	G	T	A T	T	T
KNS-BIA6-42	- -	T T	G	T	A T	T	T
KNS-BIA7-66	T C	T T	G	T	A T	T	T
KNS-BIA9	- -	T T	G	T	A T	T	T
KNS-BIA10	- C	T T	G	T	A T	T	T
KNS-BIA13	- -		G	T	A T	T	T
Indian-Ocean maskray							
KU497907	T C		A	T	C	A	C
KNS-TZN1-52	- C		A	T T	C	A	C
KNS-ZAN3-86	T C		A	T T	C	A	C
KNS-ZAN4-71	- -		A	T T	C	A	C
KNS-ZAN5-80	T C		A	T T	C	A	C

KNS-PEL3-44						T			C		G	C				T	T		C	A			C							
KNS-PEL4-45						T			C		G	C				T	T		C	A			A	C						
KNS-PEL5-46						T			C		G	C				T	T		C	A			C							
KNS-PEL6-78						T			C		G	C				T	T		C	A			C							
KNS-PEL9-16						T			C		G	C				T	T		C	A			C							
KNS-PEL15-22						T			C		G	C				T	T		C	A			C							
<i>N. malaccensis</i> sp. nov. (formerly clade III; Borsa et al. 2016b)																														
KNS-MAL1-31			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL2-32			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL3-33			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL4-34			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL5-35			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL6-36			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL7-37			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL10			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL11			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL12			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL14			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL16			C	C	G		C	T	A							T	T		G	C	A			C						
KNS-MAL17			C	C	G		C	T	A							T	T		G	C	A			C						
<i>N. moluccensis</i> sp. nov. (formerly clade VII; Borsa et al. 2016b)																														
AM1			G				T	G								C	C		T			C		C						
AM2			G				T	G								C	C		T			C		C						
AM3			G				T	G								C	C		T			C		C						
AM4			G				T	G								C	C		T			C		C						
AM5			G				T	G								C	C		T			C		C						
AM6			G				T	G								C	C		T			C		C						
KNS-KEI2-53			G				T	G								C	C		T	T		C		A	A	C				
KNS-KEI3-72			G				T	G								C	C		T	T		C			A	C				
KNS-KEI4-54			G				T	G								C	C		T	T		C			A	C				
KNS-KEI5-55			G				T	G								C	C		T	T		C			A	C				
KNS-KEI6-56			G				T	G								C	C		T	T		C			A	C				
KNS-KEI7-73			G				T	G								C	C		T	T		C			A	C				
KNS-KEI8-34			G				T	G								C	C		T	T		C			A	C				
KNS-KEI10-36			G				T	G								C	C		T	T		C			A	A	C			
KNS-KEI12-38			G				T	G								C	C		T	T		C	A			A	C			
KNS-KEI18-44			G				T	G								C	C		T	T		C				A	C			
<i>N. orientale</i>																														
JN184065			C				C	T											T	T			C			A	C			
KR019777			G				T												T	T			C				A	C		
cag1			G				T												T	T			C					A	C	
cag2			G				T												T	T			C						A	C

KNS-POS2-49				T								T	T			C		G		C	G								
KNS-POSO4-50				T								T	T			C				C	G								
KNS-RIA1-30				T								T	T			C	A	G		A	C								
KNS-RIA2-31				T								T	T			C				A	C								
KNS-RIA3-32				T								T	T			C		G		A	C								
KNS-TAL4-76				T								T	T			C				A	C								
KNS-TAL5-63				T								T	T			C		G		A	C								
KNS-WJS1-22				T								T	T			C		G		A	C								
KNS-WJS4-25				T								T	T			C		G		A	C								
KNS-WJS1-88				T								T	T			C		G		A	C	G							
KNS-WJS2-93																													
KNS-WJS3-89				T								T	T			C		G		A	C								
KNS-WJS4-90				T								T	T			C		G		A	C								
KNS-WJS5-91				T								T	T			C		G		A	C								
KNS-WJS6-92				T								T	T			C		G		A	C								
KNS-WJS1-7				T								T	T			C		G	A	A	C								
KNS-WJS2-8				T								T	T			C	A	G		A	C								
KNS-WJS3-9				T								T	T			C		G		A	C								
KNS-WJS4-10				T								T	T			C		G		A	C								
KNS-WJS5-11				T								T	T			C		G		A	C								
KNS-WJS6-12				T								T	T			C		G		A	C								
KNS-WJS7-13				T								T	T		C			G		A	C								
KNS-WJS1				T								T	T			C	A	G		A	C								
KNS-WJS3-35				T								T	T			C		G		A	C								
KNS-WJS4-36				T								T	T			C	A	G		A	C								
KNS-WSS1-26	G			T								T	T			C				C									
KNS-WSS2-27	G			T								T	T			C				C									
KNS-WSS3-28	G			T								T	T			C				C									
KNS-WSS4-29	G											T	T			C				C									
KNS-WSS5-30		C			C							T	T			C				A	C								
KNS-WSS6-31	G			T								T	T			C				C									
<i>N. varidens</i>																													
EU870496				T				T	A			A								T			C	A			C		
KC992792				T				T	A			A		G						T			C	A			C		
wjc629				T				T	A			A		G						T			C	A			C		
wjc625				T				T	A			A		G						T			C	A			C		
wjc628				T				T	A			A		G						T			C	A			C		
<i>N. westpauensis</i> sp. nov. (formerly clade VIII; Borsa et al. 2016b)																													
KNS-BIA1-37		G	G					T	A			G			G				T	T	T		C			A	C		C
KNS-BIA2-38		G	G					T	A			G			G				T	T	T		C			A	C		C
KNS-BIA3-39		G	G					T	A			G			G				T	T	T		C			A	C		C
KNS-BIA4-40		G	G					T	A			G			G				T	T	T		C			A	C		C

KNS-BIA5-41 G G	T A	G	G	T T T	C	A C	C
KNS-BIA6-42 G G	T A	G	G	T T T	C	A C	C
KNS-BIA7-66 G G	T A	G	G	T T T	C	A C	C
KNS-BIA9 G G	T A	G	G	T T T	C	A C	C
KNS-BIA10 G G	T A	G	G	T T T	C	A C	C
KNS-BIA13 G G	T A	G	G	T T T	C	A C	C
Indian-Ocean maskray								
KU497907 G	C . T			T . T	C A	A C	
KNS-TZN1-52					T . T	C A	A C	
KNS-ZAN3-86					T . T	C A	A C	
KNS-ZAN4-71					T . T	C A	A C	
KNS-ZAN5-80					T . T	C A	A C	

KNS-PEL3-44 C . A T . . T . . C A
 KNS-PEL4-45 C . A T . . T . . C A
 KNS-PEL5-46 C . A T . . T . . C A
 KNS-PEL6-78 C . A T . . T . . C A
 KNS-PEL9-16 C . A T . . T . . C A
 KNS-PEL15-22 C . A T . . T . . C A

N. malaccensis sp. nov. (formerly clade III; Borsa et al. 2016b)

KNS-MAL1-31 C T . A T . . C A T
 KNS-MAL2-32 C T . . T . . C A T C
 KNS-MAL3-33 C T . . T . . C A T C
 KNS-MAL4-34 C T . . T . . C A T C
 KNS-MAL5-35 C T . . T . . C A T C
 KNS-MAL6-36 C T . . T . . C A T C
 KNS-MAL7-37 C T . . T . . C A T C
 KNS-MAL10 C T . . T . . C A T C
 KNS-MAL11 C T . . T . . C A T C
 KNS-MAL12 C T . . T . . C A T C
 KNS-MAL14 C T . . T . . C A T C
 KNS-MAL16 C T . . T . . C A T C
 KNS-MAL17 C T . . T . . C A T C

N. moluccensis sp. nov. (formerly clade VII; Borsa et al. 2016b)

AM1 C . A A . C T . . T . . C . T T G T
 AM2 C . A A . C T . . T . . C . T T G T
 AM3 C . A A . C T . . T . . C . T T G T
 AM4 C . A A . C T . . T . . C . T T G T
 AM5 C . A A . C T . . T . . C . T T G T
 AM6 C . A A . C T . . T . . C . T T G T
 KNS-KEI2-53 T . A C . A . C T . . T . . C T G G T
 KNS-KEI3-72 T . A C . A . C T . . T . . C T C G G T
 KNS-KEI4-54 T . A A . C T . . T . . C T G G T
 KNS-KEI5-55 T . A C . A . C T . . T . . C T T G G T
 KNS-KEI6-56 T . A C . A . C T . . T . . C T G G T
 KNS-KEI7-73 T . A A . C T . . T . . C T G G T
 KNS-KEI8-34 T . A A . C T . . T . . C T G G T
 KNS-KEI10-36 T . A C . A . C T . . T . . C T G G T
 KNS-KEI12-38 T . A C . A . C T . . T . . C T G G T
 KNS-KEI18-44 T . A C . A . C T . . T . . C T G G T

N. orientale

JN184065 C A . . T . . T . . C A G T
 KR019777 C A . . T . . T . T C C . A T
 cag1 C A . . T . . T . T C C . A T
 cag2 C A . . T . . T . T C C . A T

KNS-BIA5-41	C	G	A	T	A	T	T	C	T	G	T	T
KNS-BIA6-42	C	G	A	T	A	T	T	C	G	T	T	
KNS-BIA7-66	C	G	A	T	A	T	T	C	G	T	T	
KNS-BIA9	C	G	A	T	A	T	T	C	G	T	T	
KNS-BIA10	C	G	A	T	A	T	T	C	T	G	T	T
KNS-BIA13	C	G	A	T	A	T	T	C	G	T	T	
Indian-Ocean maskray												
KU497907	C	A	A	T	T	C	A	T	C	T	T	G
KNS-TZN1-52	C	A	A	T	T	C	A	T	T	T	T	G
KNS-ZAN3-86	C	A	A	C	T	T	C	A	T	T	T	
KNS-ZAN4-71	C	A	A	T	T	C	A	T	T	T	T	G
KNS-ZAN5-80	C	A	A	T	T	C	A	T	T	T	T	G