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Multiple cryptic species in the blue-spotted maskray (Myliobatoidei: Dasyatidae: *Neotrygon* spp.): an update

*Espèces cryptiques multiples chez la pastenague masquée à points bleus (Myliobatoidei: Dasyatidae: *Neotrygon* spp.) : actualisation*

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ABSTRACT

Previous investigations have uncovered divergent mitochondrial clades within the blue-spotted maskray, previously *Neotrygon kuhlii* (Müller and Henle). The hypothesis that the blue-spotted maskray may consist of a complex of multiple cryptic species has been proposed, and four species have been recently described or resurrected. To test the multiple-cryptic species hypothesis, we investigated the phylogenetic relationships and coalescence patterns of mitochondrial sequences in a sample of 127 new individuals from the Indian Ocean and the Coral Triangle region, sequenced at both the *CO1* and *cytochrome b* loci. The maximum-likelihood (ML) tree of concatenated *CO1*+*cytochrome b* gene sequences, rooted by the New Caledonian maskray *N. trigonoides* yielded 9 strongly supported, main clades. Puillandre's ABGD algorithm detected gaps in nucleotide distance consistent with the ML phylogeny. The general mixed Yule-coalescent algorithm partitioned the dataset into putative species generally consistent with the ML phylogeny. Nuclear markers generally confirmed that distinct mitochondrial clades correspond to genetically isolated lineages. The 9 main lineages identified by ML analysis were geographically distributed in a parapatric fashion, indicating reproductive isolation. The hypothesis of multiple cryptic species is thus validated.

Keywords: parapatric distribution; *Neotrygon kuhlii*; *Neotrygon trigonoides*; Indo-West Pacific; Coral Triangle

RESUME

Des recherches antérieures ont montré des clades mitochondriaux divergents au sein de la raie masquée à points bleus, précédemment appelée *Neotrygon kuhlii* (Müller et Henle). L'hypothèse d'un complexe d'espèces cryptiques chez la raie masquée à points bleus a été proposée ; trois espèces ont été récemment décrites et une quatrième, ressuscitée. Afin de tester l'hypothèse d'espèces cryptiques multiples, nous étudions les relations phylogénétiques et les patrons de coalescence des séquences mitochondriales d'un échantillon de 127 individus de l'océan Indien et de la région du Triangle de Corail, séquencés simultanément aux locus *CO1* et *cytochrome b*. L'arbre de maximum de vraisemblance (ML) des séquences partielles concaténées des gènes *CO1* et *cytochrome b*, raciné par la raie masquée de Nouvelle-Calédonie *N. trigonoides*, montre 9 clades principaux fortement soutenus. A l'aide de l'algorithme ABGD de Puillandre, des lacunes dans la distribution des distance nucléotidiques sont détectées, qui s'avèrent généralement cohérentes avec la phylogénie de ML. L'algorithme mixte de Yule-coalescent (GYMC) partitionne l'ensemble de données en un certain nombre d'espèces putatives dont la délimitation est généralement cohérente avec la phylogénie de ML. Les marqueurs nucléaires confirment, en général, que les clades mitochondriaux distincts caractérisent des lignées génétiquement isolées. Les 9 lignées principales identifiées par l'analyse de ML montrent une distribution parapatric, ce qui indique qu'elles sont reproductivement isolées. L'hypothèse d'espèces cryptiques multiples est donc validée.

Mots-clés : distribution parapatric ; *Neotrygon kuhlii* ; *Neotrygon trigonoides* ; Indo-Pacifique ouest; Triangle de Corail

1. Introduction

Taxonomic studies of sharks and rays have led to an upsurge in new species descriptions within the last decade ([1, 2]; and references therein). In particular, it appears that the number of species in the Coral Triangle region has been considerably under-estimated until recently [1]. Meanwhile, grave concern has been expressed over the risk of extinction in shallow-water species from a number of shark and ray families including Dasyatidae or stingrays. Overfishing of stingrays is particularly severe in the Coral Triangle region [3-5] and management is urgently needed. Species are the fundamental units in many studies of biogeography, community ecology and conservation ecology. Both conservation and fisheries management require that species be clearly identified and populations be delineated [6].

This paper focuses on the blue-spotted maskray, previously *Neotrygon kuhlii* (Müller and Henle, 1841) [7], a stingray species that inhabits Indo-West Pacific coral reefs, lagoons and slopes [8]. The blue-spotted maskray is heavily exploited in Southeast Asia, but its catch rate and mortality rates are poorly known and its population trends are unknown [9, 10]. Authors have distinguished the “Java” (Java Sea) form of blue-spotted maskray from the “Bali” (Kedonganan) form, based on differences in size at birth and male size at maturity and treated them as different species [9]. Molecular population genetics offer the concepts and the practical tools for delineating populations, diagnosing closely related species, and detecting cryptic species. Cryptic species are defined as evolutionary lineages with a substantial amount of genetic distinctiveness and no detectable morphological differences [11-13]. R.D. Ward and co-authors ([14]: 60-62) have noted that at the *CO1* locus, “the *D. kuhlii* group showed considerable within species diversity ... with four subgroups ... One was the sole specimen from Australia (Queensland), one from the six specimens taken from Kedonganan fish market on Bali (Indonesia), one from the five specimens from Muara Angke fish market at Jakarta, Java (Indonesia) and one from the three specimens from the Penghu Islands (Taiwan). Average distances among and within these four groups were 2.80% and 0.18% respectively”. Subsequent genetic studies in the genus *Neotrygon* have focused on its phylogeny [15-17], and on the population genetic structure and phylogeography of the blue-spotted maskray [18, 19]. Additional mitochondrial clades have been uncovered within the blue-spotted maskray [15, 17, 19]. These clades have a parapatric-like distribution [19]. Meanwhile, molecular markers have advanced the systematics of species in the genus *Neotrygon*: cryptic species have been uncovered within *N. ningalooensis* and *N. picta* [17], and the New Caledonian maskray *N. trigonoides* (Castelnau, 1873) [20] has been resurrected [16, 21]. The hypothesis that the blue-spotted maskray may itself consist of a complex of multiple species has been raised repeatedly [14, 15, 17, 22] and was also discussed by us [19, 23]. We emphasized that the parapatric-like population structure uncovered in the Coral Triangle region “points to incipient speciation, where some degree of reproductive isolation has been achieved but ecological compatibility has not yet been reached”. Recently, P.R. Last and co-authors [24] described three new species (*N. australiae*, *N. caeruleopunctata*, *N. orientale*) previously under *N. kuhlii* and resurrected a fourth one, *N. varidens* (Garman 1885) [25]. Diagnostic morphological differences between the species were proposed but no in-depth assessment of inter-specific against intra-specific differences was included [24].

In the present paper, we compile all *CO1* and *cytochrome b* gene sequences published thus far for the blue-spotted maskray and we add new sequences from samples collected in the western

Indian Ocean and throughout the Indo-Malay archipelago, to construct a robust mitochondrial phylogeny and establish an updated distribution of the clades previously uncovered in the Coral Triangle region [14, 15, 17, 19]. We assess whether the different clades, including those recently resurrected or erected as new species [24] correspond to evolutionary significant units that deserve the status of separate species.

2. Materials and methods

2.1 Ethics in sampling and information sharing

All specimens examined for the present study were independently captured by commercial fishers prior to being sub-sampled for DNA. Some of the sampling localities in our survey (Fig. 1) were in countries that have adopted a principle of state sovereignty over biological resources, including genetic resources with no commercial use [26]. This includes the Philippines, where sampling took place under a collaborative research agreement between the National Taiwan University, Taipei and the University of San Carlos, Cebu; and Indonesia, within the framework of a memorandum of understanding between LIPI, Jakarta and IRD, Marseille. Some of the samples were from West Papua, a former Dutch colony currently administered by Indonesia [27, 28] that has no control over its own natural resources [29]. For equitable sharing of results and knowledge, the data produced in the course of our study will be made accessible from the following open-access repositories: Hal-IRD (<http://www.hal.ird.fr/>; hal@ird.fr), GenBank (<http://www.ncbi.nlm.nih.gov>) [30], and DIPnet (<http://www.indopacificnetwork.wikispaces.com/>).

2.2. Sampling

A total of 362 individuals, including 341 blue-spotted maskray and 21 New Caledonian maskray *N. trigonoides*, was utilized in the present study. This total includes 203 individuals whose *CO1* or *cytochrome b* gene sequences were compiled from either the literature [14, 16, 17, 19, 31–38], the BOLD barcode database, the Cryobank DNA-barcode database (<http://cryobank.sinica.edu.tw/chi/>), or the GenBank nucleotide-sequence database; and 159 new individuals sampled from local fish-landing places throughout the Indo-Malay archipelago, in eastern Africa, and in New Caledonia. All individuals included in the present survey are listed in Supplementary Table S1, along with sampling details, GenBank accession numbers, voucher specimen details, and references. An overview of the density of blue-spotted maskray samples across the Indo-West Pacific, with a focus on the Coral Triangle region is given in Fig. 1.

2.3. Molecular analyses

A piece of skin or flesh ~0.05 to ~1 cm³ was removed, using surgical scissors, from the pelvic fin, or the pectoral fin, or the tail and was preserved in 95% ethanol at ambient temperature. DNA extraction was done using either the Viogene (Taiwan) tissue genomic DNA extraction protocol, or the DNEasy DNA extraction kit (Qiagen GmbH, Hilden, Germany). DNA was stored in 1X, pH 8.0 TE buffer (AppliChem, Darmstadt, Germany). Polymerase chain reaction (PCR) amplification of a

fragment of the *CO1* gene was done according to [19]. For the PCR-amplification of the *cytochrome b* gene, we used primers *L14735* and *CB7* [39, 40]. The reaction volume was 15 μ L and the reaction mixture contained 0.2 mM dNTPs, 1.5 μ L 10 $\times$ PCR buffer (Bioman, Taipei), 0.5 μ M of each forward and reverse primers, 0.2 U Taq DNA polymerase (Bioman), and 1.0 μ L template DNA. The PCR parameters were: initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation (94°C for 45 s), annealing (48°C for 1 min), and extension (72°C for 1 min), and a final extension step at 72°C for 10 min. Intron 5 of the growth hormone gene, *Gh5*, was PCR-amplified using exon-anchored primers *Gh5F* (5'- A G G C C A A T C A G G A C G G A G C -3') and *Gh6R* (5'- T G C C A C T G T C A G A T A A G T C T C C -3') [41] setting the annealing temperature to 64.5°C and the number of cycles to 35.

The PCR products were sequenced directly using the same primers as those used for the PCR. Sequences were analyzed in an automated ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA) at the sequencing facility of the Taiwan Normal University (Taipei).

2.4. Genetic data analysis

Nucleotide sequences, including those obtained in the present study, and homologous sequences retrieved from the BOLD, Cryobank, and GenBank databases (see Supplementary Table S1) were aligned using BIOEDIT [42]. Four datasets were analyzed: (1) the *CO1* gene sequence dataset, consisting of 330 blue-spotted maskray and *N. trigonoides* sequences aligned over a maximum length of 722 base pairs (bp); (2) the *cytochrome b* gene sequence dataset, consisting of 165 blue-spotted maskray and *N. trigonoides* sequences aligned over a maximum length of 1142 bp; (3) the concatenated *CO1+cytochrome b* gene sequence dataset comprising 127 blue-spotted maskray and 6 *N. trigonoides* sequences; (4) the *Gh5* intron sequence dataset, consisting of 18 blue-spotted maskray and *N. trigonoides* sequences aligned over a maximum length of 359 bp.

The phylogeny of concatenated *CO1+cytochrome b* gene haplotypes was inferred by using the maximum-likelihood (ML) method under MEGA6 [43]. The most likely nucleotide-substitution model, which was determined according to the Bayesian information criterion, was the Tamura-Nei model [44] where a discrete Gamma distribution ($\Gamma=0.76$) 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 [16]. The robustness of nodes in the tree was tested by bootstrap resampling.

The *CO1* and *cytochrome b* gene sequence datasets (Supplementary Table S1) were used separately to assign individuals that were sequenced at only one of either locus to a clade uncovered from the phylogenetic reconstruction described above. For this, an ML tree was constructed using each of the two datasets under MEGA6 and the assignment of a haplotype to a clade was determined visually from its relative position on the tree.

2.5. Delineation of cryptic species

The Automatic Barcode Gap Discovery (ABGD) algorithm proposed by N. Puillandre and co-authors [45] was used to identify gaps in the distribution of pairwise nucleotide distances. The ABGD algorithm detects the largest significant gap in the distribution of pairwise nucleotide

distances and uses it for partitioning the dataset. Gap detection is then recursively applied to previously obtained groups to get finer partitions until there is no further partitioning [45]. The ABGD analysis was run on a matrix of 127 *CO1*+*cytochrome b* gene sequences, trimmed to a core length of 1415 bp. Kimura's [46] two-parameter substitution model was selected and the minimum barcoding gap width was set to 1%.

Branch lengths between species are determined by speciation and extinction rates whereas branch lengths within a species reflect coalescent processes at the level of populations [47]. This results in the distinction of species when mitochondrial clades are substantially deeper than the coalescent haplogroups at the extremities of the tree. We ensured that each of the previously-identified deep mitochondrial clades was genetically distant from its nearest neighbour by several times the mean intra-clade distance. For this, pairwise intra-clade and between-clades genetic distances were estimated on the concatenated *CO1*+*cytochrome b* gene sequence matrix using MEGA6.

To delimit potential cryptic species, we applied the general mixed Yule coalescent (GMYC) algorithm [47] as implemented in the program SPLITS under R [48, 49]. This algorithm uses an ultrametric tree to differentiate branching patterns consistent with coalescent, from speciation processes, which provides a threshold time for the transition between populational and species-level processes. We selected the single-threshold option. The ultrametric tree was constructed using BEAST v. 1.7 [50] based on the same matrix (127 individuals x 1415 bp) as that used for the ABGD analysis. Outgroup *N. trigonoides* was excluded from the analysis, which focused on the blue-spotted maskray exclusively. A non-calibrated relaxed lognormal clock, and a constant coalescent tree were assumed. The model of nucleotide substitution was TN93+G+I (see sub-section 2.4). Ten million generations of Markov-Chain Monte Carlo analysis were run, of which we sampled a total of 10,000 genealogies. Length of burn-in was determined by visual inspection of traces in TRACER v. 1.5 [50]. A single analysis was run, for which convergence was reached rapidly. The TREEANNOTATOR v. 1.7. software [50] was used to produce the final tree, based on maximum clade credibility and mean node height.

3. Results

The complete set of partial *CO1*- and *cytochrome b* gene sequences, which comprises both those compiled from the literature and from nucleotide databases and those produced in the present study is presented as Supplementary Table S2. New *CO1* gene sequences have GenBank accession numbers KU497912-KU498038 and KU521523; new *cytochrome b* gene sequences have GenBank accession numbers KU497752-KU497911 (details in Supplementary Table S1).

The ML tree of concatenated (*CO1*+*cytochrome b*) gene nucleotide sequences, rooted by *N. trigonoides* showed 9 main statistically-supported clades (Fig. 2). Here we distinguished the sister-subclades *IVa* (*N. orientale*) vs. *IVb* (*N. varidens*) within previous Clade *IV* [19, 23]. The 7 other clades were Clades *I-III*, *VII*, *VIII* identified previously [19,23], *N. australiae* (our former Clade *V*), and *N. caeruleopunctata* (our former Clade *VI*). Variation in clade nomenclature across studies [15,17,19,23,24] is summarized in Table 1. Pairwise nucleotide distances between clades ranged from 0.014 to 0.029 (average 0.022), that is, two to 29 times higher than within-clade average distances (0.001-0.007; average 0.004) (Table 2).

Through automatic gap determination using Puillandre's ABGD algorithm, 12 distinct mitochondrial lineages were found, separated from each other by a gap in genetic distance $\geq 1\%$ (Fig. 2). The ABGD partitioning was consistent with the topology of the ML tree. In particular, Clades *II* and *III* were recognized as distinct. The distinction between Clades *IVa* (*N. orientale*) and *IVb* (*N. varidens*) was confirmed. Two subclades were identified within each Clade *I* (Indian-Ocean maskray), and *N. australiae*. All 9 major clades of the ML tree were confirmed by the ABGD partitioning (Fig. 2).

Based on the ultrametric tree of blue-spotted maskray mitochondrial haplotypes (not shown), the likelihood of the null model (i.e., where all mitochondrial sequences belong to a single species) was 1141.9, significantly lower than the maximum likelihood of the GMYC model (1145.1); the likelihood ratio was 6.477 ($P = 0.039$). The estimated number of clusters within the blue-spotted maskray was 13 (95% confidence interval: 10–46), whose boundaries often coincided with the above 9 clades (Fig. 2). The distinction between clades *IVa* (*N. orientale*) and *IVb* (*N. varidens*) was thus confirmed, as was that of *N. caeruleopunctata*. The main exception was the lack of recognition of clades *II* and *III* as separate entities. The individuals collected in the Indian Ocean (our clade *I* [19]) formed a distinct series of three clusters, as did *N. australiae*, and a subcluster was found within clade *VIII* (Fig. 2).

The partition into species that was finally retained considered the tree topology, the nucleotide divergence between lineages, and the results of the ABGD and GMYC analyses. This partition was consistent with the current taxonomy of species, leaving unchallenged the four nominal species *N. australiae*, *N. caeruleopunctata*, *N. orientale* and *N. varidens* (Fig. 2). The inability of the GMYC algorithm to separate clade *II* from clade *III* may be caused by a lack of power, as the two clades were approximately equally distant from each other as they were each from *N. caeruleopunctata* (Table 2).

Once cryptic species of the blue-spotted maskray were identified, it was possible to tentatively assign each individual in the total sample (Supplementary Table S1) to either of them, using the partial *CO1*- or *cytochrome b* gene sequence. The ML tree of *CO1* gene sequences, rooted by *N. trigonoides* (Supplementary Fig. S1) showed the same 9 haplogroups as those highlighted in the phylogenetic tree of Fig. 2, plus a tenth lineage represented by the single *CO1* gene haplotype from the Ryukyu Islands (GenBank AB485685; [31]). All haplogroups had acceptable to strong statistical support, except the haplogroup representing individuals from the Indian Ocean. All 330 individuals characterized by their *CO1* gene sequence (except a single individual from India; GenBank HM467799) unambiguously clustered with either one of the 9 lineages of Fig. 2, or with *N. trigonoides*. The ML tree of *cytochrome b* gene sequences, rooted by *N. trigonoides* (Supplementary Fig. S2) similarly showed the same 9 haplogroups as those highlighted in Fig. 2. Eight out of the 9 haplogroups had strong statistical support, with bootstrap scores between 93% and 100%. All 165 individuals characterized by their *cytochrome b* gene sequence unambiguously clustered with either one of these 9 blue-spotted maskray lineages, or with *N. trigonoides*.

Using the sampling locations of the individuals (Supplementary Table S1), it was possible to delineate the geographic distribution of each lineage (Fig. 1). The 9 lineages of the blue-spotted maskray and the closely-related *N. trigonoides* had parapatric distributions (Fig. 1). Zones of contact were identified between *N. orientale* and *N. varidens* in the southeastern part of the South China Sea, between *N. caeruleopunctata* and *N. orientale* in Bali Strait, and between *N. australiae*

and *N. orientale* in the Lombok area (Fig. 1B). Similar zones of contact are expected between Clade-II and Clade-III lineages east of the northern tip of Aceh, between Clade III and *N. varidens* in the Malacca strait, between *N. caeruleopunctata*, *N. orientale*, and Clade II west of the southwestern tip of Java, and between *N. australiae* and *N. caeruleopunctata* east of southern Bali (Fig. 1B). Other contact zones may be possible between *N. orientale* and Clade VII, between *N. australiae* and Clade VII, and between clade VII and Clade VIII (Fig. 1B).

Eighteen *Neotrygon* spp. individuals (out of 37 tested) were successfully sequenced at the *Gh5* intron locus. The sequences have GenBank accession numbers KU497734-KU497751. The ML tree derived from the alignment of sequences, rooted by *N. trigonoides*, is presented in Fig. 3A.

4. Discussion

The present results enforce Ward et al.'s [14] hypothesis that the blue-spotted maskray is a complex of cryptic species, for the reasons developed in the following.

4.1. Coalescence patterns

Nucleotide distances between major blue-spotted maskray clades were several times higher than distances within, confirming previous observations based on preliminary geographic sampling [14, 15, 17, 19]. Denser geographic sampling (present study) did not lead to a dramatically altered ratio of genetic distance between clades to that within, compared to Ward et al.'s results [14]. The gaps in nucleotide distance remained consistent with the existence of 9 separate main lineages within the blue-spotted maskray. The GMYC algorithm determined that the coalescence patterns within a lineage vs. patterns between lineages generally conformed to the expectations of intra-specific vs. inter-specific processes.

4.2. General concordance of mitochondrial and nuclear differences

In sharks and rays, short mitochondrial DNA sequences at the *CO1* locus (or *CO1* DNA barcodes) have proven their suitability to identify up to 99% species [14]. This high barcoding success might be related to the apparent absence of mitochondrial introgression in this vertebrate group [14], itself pointing to a likely low incidence of inter-specific hybridization. This may be an indirect consequence of complex pre-mating behaviour, which prevents hetero-specific pairs, or of mismatch in the morphologies of male and female genitalia, which hampers copulation. Complex pre-mating behaviour has been reported in sandtiger shark [52] and New Caledonian maskray [53]. Mismatch of male – female copulatory apparatuses has been hypothesized for whiptails of the *Himantura uarnak* (Forsskal, 1775) [54] species complex [35]. Other hypotheses for pre-mating isolation involve chemical or visual cues [17]. Because cases of mitochondrial introgression are virtually unknown in sharks and rays [6], one expects that patterns of inter-specific genetic differentiation at nuclear loci parallel those at the mitochondrial locus.

Nuclear markers have also been used to characterize blue-spotted maskray populations [17, 18]. Significant allele-frequency differences at 2/4 intron loci were observed between adjacent populations from southern Java and Bali Strait [18], corroborating the distinction between previous

Clades *IVb* and *VI* ([19, 23]; present study), hence the recognition of, respectively, *N. orientale* and *N. caeruleopunctata* as separate species. Two major clades were observed at the nuclear locus *RAG-1* [17] (Fig. 3B), that distinguished the lineage from the eastern Andaman Sea (our Clade *III*) and those from the adjacent South China Sea (*N. varidens*) and Java Sea (*N. orientale*). Similarly, the *RAG-1* marker allowed the distinction between the lineage from southern Bali (*N. caeruleopunctata*; our previous Clade *VI*) and that of the adjacent Lombok Island (*N. australiae*; our previous Clade *V*) [17]. Additional information from nuclear marker *Gh5* was produced in the present study. Nucleotide sequences at intron locus *Gh5* confirmed the distinction between *N. orientale* on the one side, and *N. australiae* and *N. caeruleopunctata* on the other side, although some other *Gh5* sequences of individuals determined as *N. orientale* according to their mitochondrial haplotypes appeared to cluster with *N. australiae* and with Clade *I*. This may either signal mitochondrial introgression, or reflect incomplete lineage sorting at the *Gh5* locus. In addition, at this locus, samples from the Moluccas and West Papua (respectively, our Clade-*VII* and Clade-*VIII* lineages) were distinct from all the other samples, and also from each other.

4.3. Parapatric distribution

Repeated lowering of the sea level caused by global temperature oscillation [55] has led to the repeated fragmentation of the marine habitats of the Indo-Malay archipelago in the Pleistocene [56]. In shallow-water species like the blue-spotted maskray, this has caused repeated depletion and fragmentation of the populations, followed by repeated recolonisation of shallow-water habitats once the sea level rose again, and secondary contact between long-isolated populations. Population collapse reduces genetic diversity by the random fixation of a subset of alleles and subsequent population expansion favours new, nascent diversification. This succession of isolation episodes with cyclic population collapses followed by rapid population expansions is thought to have favoured speciation in the blue-spotted maskray [17, 19].

Secondary contact after recolonisation of inundated shallow-water habitat may have been a factor reinforcing genetic isolation instead of promoting re-homogenisation. At the scale of an individual's lifetime, the blue-spotted maskray is a sedentary species. Long-term site fidelity has been inferred from tagging experiments in the closely-related *N. trigonoides*, with similar results for females and males [53]. Relatively poorly dispersing species often form well-delineated parapatric boundaries [57]. One of the proposed mechanisms is that of a narrow and stable hybrid zone, which acts as a geographic barrier to cross-dispersal [57]. While this hypothesis remains to be tested in the blue-spotted maskray species complex, the present study identified several of these parapatric boundaries, on which future genetic studies should focus.

5. Conclusion

Sound conservation and fisheries management requires knowledge on population genetic structure, so as to delineate demes, which are the basic units on which to conduct meaningful demographic analyses. Previous attempts at investigating the population genetic structure of blue-spotted maskray have found substantially higher levels of population differentiation than usual, leading to suspecting cryptic species. Here, molecular markers distinguished nine main separate

lineages within the blue-spotted maskray previously under *N. kuhlii*. These lineages qualify as distinct species, based on levels of genetic divergence, coalescence patterns, concurrent differentiation at nuclear loci, and parapatric distribution, which points to reproductive isolation. We propose that repeated dramatic demographic lows and highs in the Pleistocene, combined with individual sedentarity and possibly homogamy, have driven speciation in the blue-spotted maskray.

Disclosure of interest

We have no conflicts of interest concerning this article.

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.crv.2016.07.004>.

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Table 1

Blue-spotted maskray, *Neotrygon* spp. (formerly *N. kuhlii*) and New Caledonian maskray, *N. trigonoides*. Lineage nomenclature utilized in present study, and its relationship to recent genetic-based studies. Homology between lineages was assessed by the phylogenetic placement of individuals sequenced at different loci and/or by the geographic origin of a sample. Genetic markers indicated in brackets.

Present study	Naylor et al. 2012 [15]	Arlyza et al. 2013 [19, 23]	Puckridge et al. 2013 [17]	Last et al. 2016 [24]
(<i>CO1</i> , <i>cytb</i>)	(<i>ND2</i>)	(<i>CO1</i>)	(<i>CO1</i> +16S, <i>RAG-1</i>)	(<i>CO1</i>)
<i>N. australiae</i>	<i>N. kuhlii</i> 4	<i>N. kuhlii</i> Clade V	Clade 5	<i>N. australiae</i>
<i>N. caeruleopunctata</i>	-	<i>N. kuhlii</i> Clade VI	Clade 6	<i>N. caeruleopunctata</i>
<i>N. orientale</i>	<i>N. kuhlii</i> 1	<i>N. kuhlii</i> Clade IV	Clades 2, 3	<i>N. orientale</i>
<i>N. varidens</i>	<i>N. kuhlii</i> 2	<i>N. kuhlii</i> Clade IV	Clade 1	<i>N. varidens</i>
Clade I	<i>N. kuhlii</i> 3	<i>N. kuhlii</i> Haplogroup I	Clade 8	-
Clade II	-	<i>N. kuhlii</i> Clade II	-	-
Clade III	-	<i>N. kuhlii</i> Clade III	Clade 7	-
Clade VII	-	<i>N. kuhlii</i> Clade VII	-	-
Clade VIII	-	<i>N. kuhlii</i> Clade VIII	-	-
Guadalcanal maskray	-	-	-	<i>N. kuhlii</i>
Ryukyu maskray	-	<i>N. kuhlii</i> Clade IV	Clade 4	-
<i>N. trigonoides</i>	-	<i>N. trigonoides</i>	Clade 9	<i>N. trigonoides</i>

Table 2

Blue-spotted maskray, *Neotrygon* spp. Mean $\pm$ SD pairwise genetic distances (number of nucleotide substitutions per site), estimated from concatenated *CO1*+*cytochrome b* gene fragment sequences (total $N=133$) (MEGA6 [43]; Tamura-Nei model [44]). N sample size.

No.	Clade	N	Mean pairwise genetic distance									
			Within clade	Between clades (net)								
				1	2	3	4	5	6	7	8	9
1	<i>N. australiae</i> .	8	0.007 ± 0.001									
2	<i>N. caeruleopunctata</i>	15	0.002 ± 0.001	0.020 ± 0.003								
3	<i>N. orientale</i>	67	0.007 ± 0.001	0.017 ± 0.003	0.014 ± 0.003							
4	<i>N. trigonoides</i>	6	0.001 ± 0.001	0.032 ± 0.005	0.032 ± 0.005	0.028 ± 0.004						
5	<i>N. varidens</i>	2	0.001 ± 0.001	0.026 ± 0.004	0.025 ± 0.004	0.016 ± 0.003	0.037 ± 0.005					
6	Clade I	5	0.003 ± 0.001	0.018 ± 0.003	0.016 ± 0.003	0.014 ± 0.003	0.028 ± 0.004	0.024 ± 0.004				
7	Clade II	5	0.006 ± 0.001	0.023 ± 0.004	0.015 ± 0.003	0.020 ± 0.003	0.033 ± 0.005	0.027 ± 0.004	0.020 ± 0.003			
8	Clade III	6	0.001 ± 0.000	0.022 ± 0.004	0.017 ± 0.003	0.017 ± 0.003	0.031 ± 0.005	0.026 ± 0.004	0.019 ± 0.003	0.018 ± 0.003		
9	Clade VII	13	0.005 ± 0.001	0.022 ± 0.004	0.021 ± 0.003	0.019 ± 0.003	0.030 ± 0.005	0.027 ± 0.004	0.018 ± 0.003	0.027 ± 0.004	0.026 ± 0.004	
10	Clade VIII	6	0.001 ± 0.000	0.026 ± 0.004	0.023 ± 0.004	0.024 ± 0.004	0.030 ± 0.005	0.030 ± 0.005	0.024 ± 0.004	0.029 ± 0.004	0.028 ± 0.004	0.027 ± 0.004

Fig. 1. Sampling sites of blue-spotted maskrays, *Neotrygon* spp. previously under *Neotrygon kuhlii*, including new samples from present study, samples from the literature, and additional haplotypes from BOLD, Cryobank and GenBank (details in Supplementary Table S1). Individuals were identified to species according to present revision, from either their *COI* or cytochrome *b* gene sequence. Roman numbers *I-VIII* designate the haplogroups or clades reported previously [19, 23], four of which have since been described or redescribed [24] (Table 1). Surface of symbol (circle or triangle) proportional to sample size. A. *I*₁, *I*₂ and *I*₃: *clade I* from, respectively, Tanzania (*N*=7), Tamil Nadu (*N*=7) and Vizakhapatnam (*N*=1). *V*₁ and *V*₇: *N. australiae* from, respectively, Ningaloo Reef (*N*=2) and Gulf of Carpentaria (*N*=5). *G*: Guadalcanal maskray (*N. kuhlii* according to [24]), Honiara, Solomon archipelago (*N*=1); *Open triangles*: *N. trigonoides*; *solid triangle*: Vanikoro, type locality of *N. kuhlii*; *inset*: see Fig. 1B. B. Enlarged map of the Indo-Malay archipelago. *II*₁, *II*₂, *II*₃ and *II*₄: *clade II* from, respectively, Banda Aceh region (*N*=11), Meulaboh (*N*=3), Sibolga (*N*=10) and Padang (*N*=10). *III*₁ and *III*₂: *clade III* from, respectively, Andaman-Sea coast of Thailand (*N*=5) and Kuala Lama region (*N*=25). *N. varidens*: *IV*₁ from Kuala Selangor (*N*=1), *IV*₃ from Haiphong (*N*=2), *IV*₅ from Karimata Strait (*N*=1/8), *IV*₇ from Beibu Gulf (*N*=1), *IV*₈ from Sarawak (*N*=1/4), *IV*₁₃ from Penghu (*N*=4), *IV*₁₄ from Taiwan (*N*=8). *N. orientalis*: *IV*₂ from Riau archipelago (*N*=4), *IV*₄ from the western Java Sea (*N*=40), *IV*₅ from Karimata Strait (*N*=7/8), *IV*₆ from Batang (*N*=7), *IV*₈ from Sarawak (*N*=3/4), *IV*₉ from Bali Strait (*N*=7), *IV*₁₀ from the western Sulawesi Sea (*N*=11), *IV*₁₁ from Sandakan (*N*=2), *IV*₁₂ from Makassar (*N*=7), *IV*₁₅ from Bone Bay (*N*=2), *IV*₁₆ from Tomini Bay (*N*=3), *IV*₁₇ from Cagayan (*N*=12), *IV*₁₈ from Kendari (*N*=7), *IV*₁₉ from Lapu-Lapu (*N*=7), *IV*₂₀ from Bitung (*N*=12), *IV*₂₁ from Tanjung Luar (*N*=2). *VI*₁, *VI*₂, *VI*₃ and *VI*₄: *N. caeruleopunctata* from, respectively, Pelabuhan Ratu (*N*=8), Sadang (*N*=4), Bali Strait (*N*=3) and southern Bali Island (*N*=14). *N. australiae*: *V*₂ from Tanjung Luar (*N*=5), *V*₃ from Labuan Bajo (*N*=8), *V*₄ from off Rote Island (*N*=3), *V*₅ from Tanjung Sulamo (*N*=1), *V*₆ from Kupang (*N*=3), *V*₈ from Torres Strait (*N*=1), *V*₉ from the northern Great Barrier Reef (*N*=1). *VII*₁, *VII*₂: *clade VII* from, respectively, Ambon (*N*=6) and Kei Islands (*N*=20). *VIII*₁, *VIII*₂: *clade VIII* from, respectively, Pulau Numfor (*N*=2) and Biak (*N*=18). *R*: Ryukyu maskray, Ishigaki-shima, Ryukyu archipelago (*N*=1).

Fig. 2. Maximum-likelihood phylogeny (MEGA6 [43]; Tamura-Nei model with gamma-distributed rate differences among sites + invariant sites [44]; partial deletion) of blue-spotted maskrays *Neotrygon* spp. previously under *N. kuhlii*, based on nucleotide sequences of the concatenated *COI*+ *cytochrome b* gene fragments. A total of 133 individual sequences (Supplementary Table S1), aligned over 1640 bp, was retained in the final dataset, after all positions with less than 95% site coverage had been eliminated. *N. trigonoides* was used as outgroup [16]. *Roman numbers I-VIII* designate the haplogroups or clades reported previously [19]. *Numbers at a node* are bootstrap scores (from 600 bootstrap resampling runs). *Vertical black bars* summarize the partition obtained according to Puillandre's gap detection analysis, ABGD. *Vertical grey bars* summarize the partition into species obtained from GMYC analysis (SPLITS; single-threshold option). *Open bars*: partition into species finally retained considering tree topology, nucleotide divergence between lineages, and results of ABGD and GMYC analyses.

Fig. 3. Nuclear phylogenies of the blue-spotted maskray, *Neotrygon* spp. A. Maximum-likelihood phylogeny (MEGA6 [43]; Jukes-Cantor model [51]) with gamma-distributed rate differences among sites; partial deletion] of *Gh5* intron sequences. A total of 18 individual sequences aligned over 356 bp was retained in the final dataset, after all positions with less than 95% site coverage had been eliminated. *N. trigonoides* was used as outgroup [16]. Numbers at a node are bootstrap scores (from 1000 bootstrap resampling runs). B. Summary of the Bayesian phylogeny based on partial *RAG-1* sequences (redrawn from figure 2a of [17]; lineage names edited according to Table 1). Numbers at a node are posterior probabilities.

Figure 1 - Borsa et al.

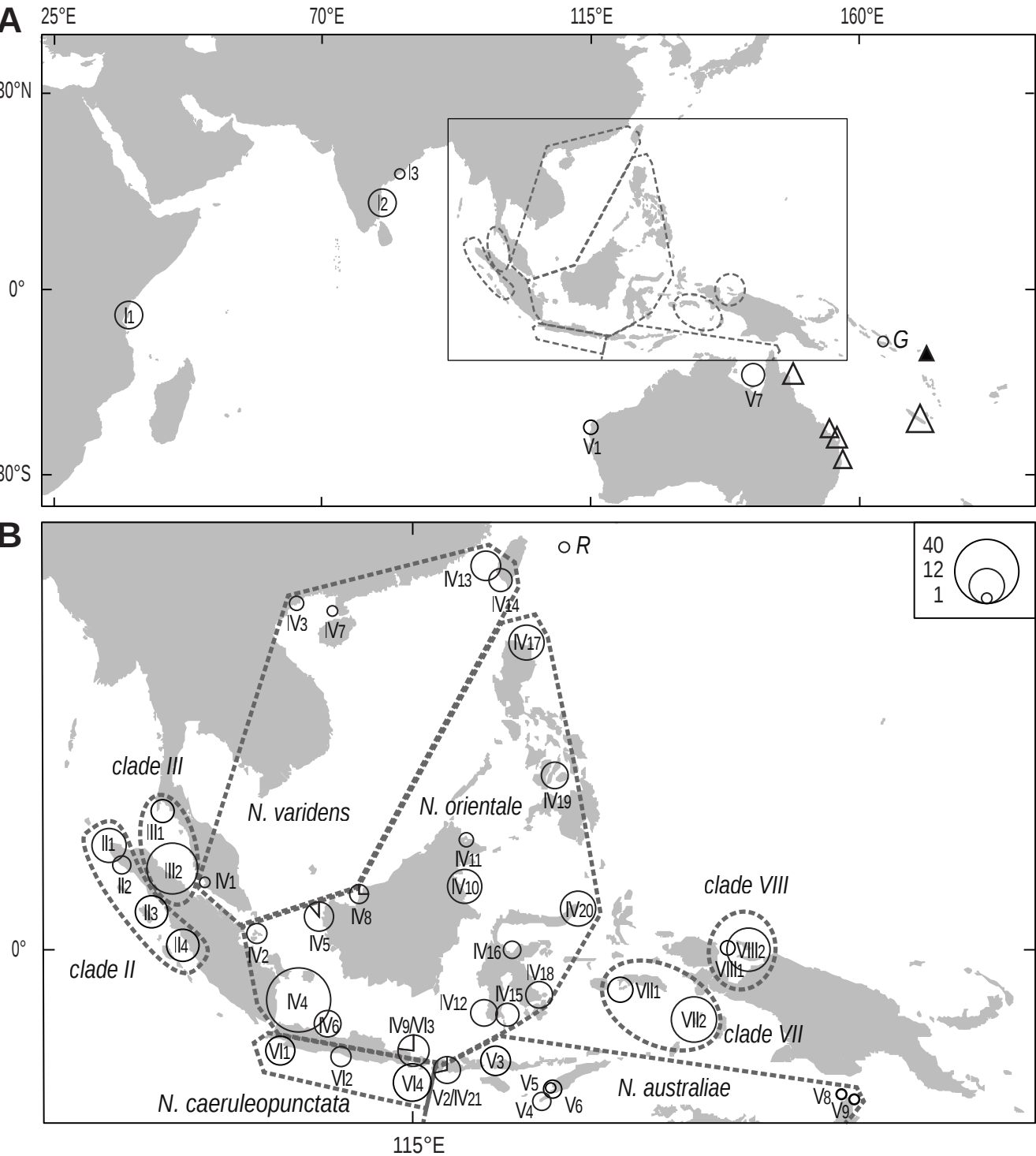


Figure 2 - Borsa et al.

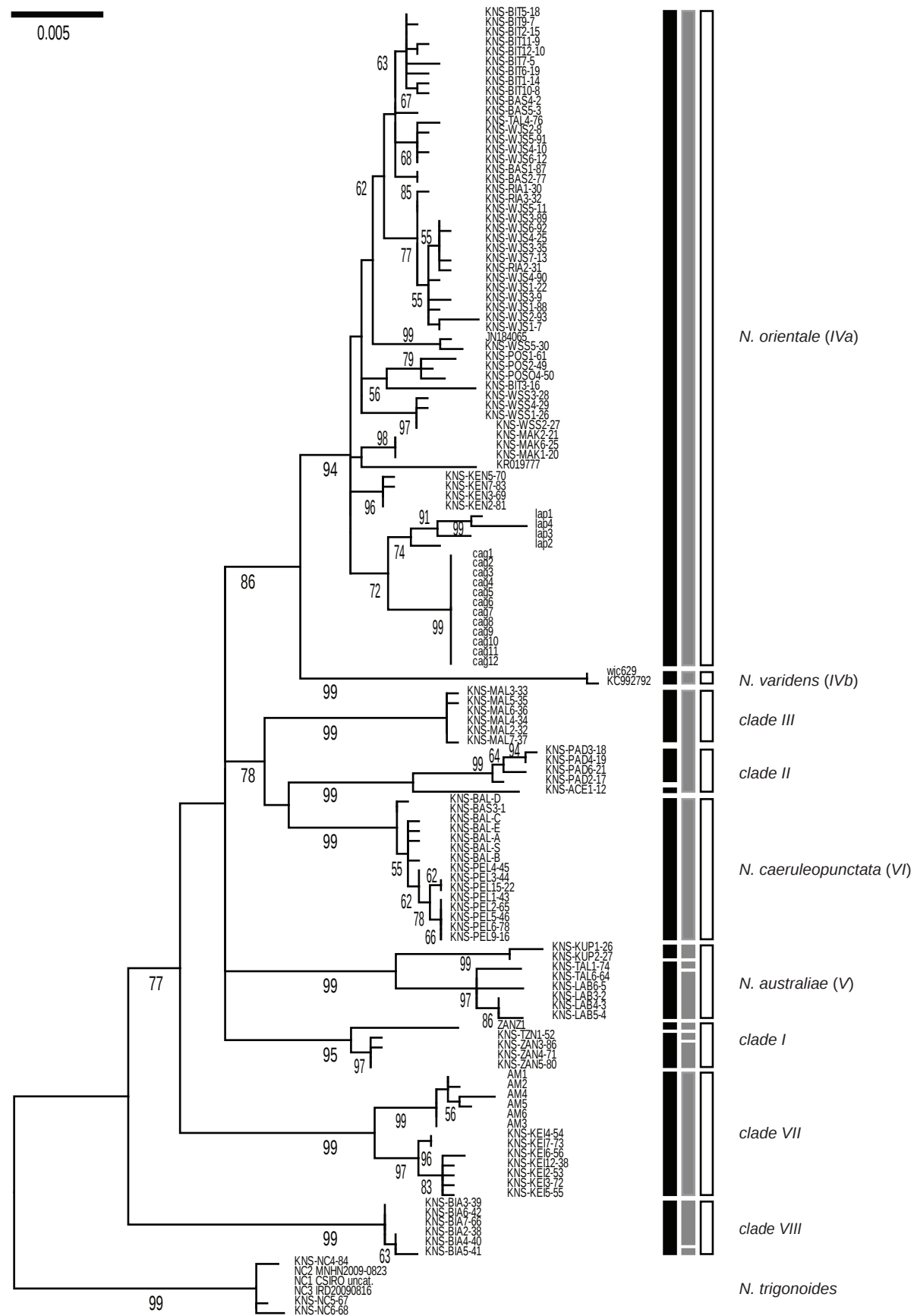
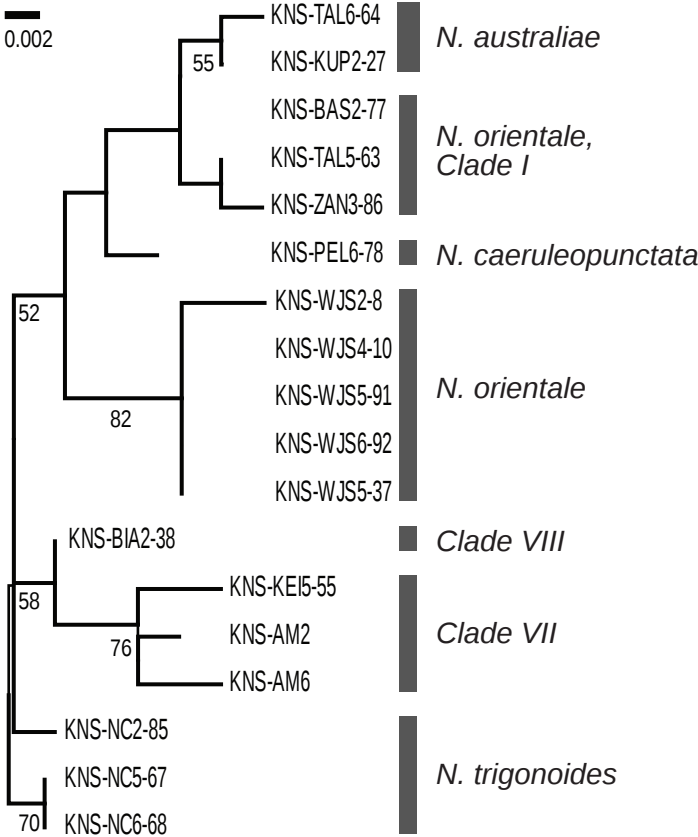
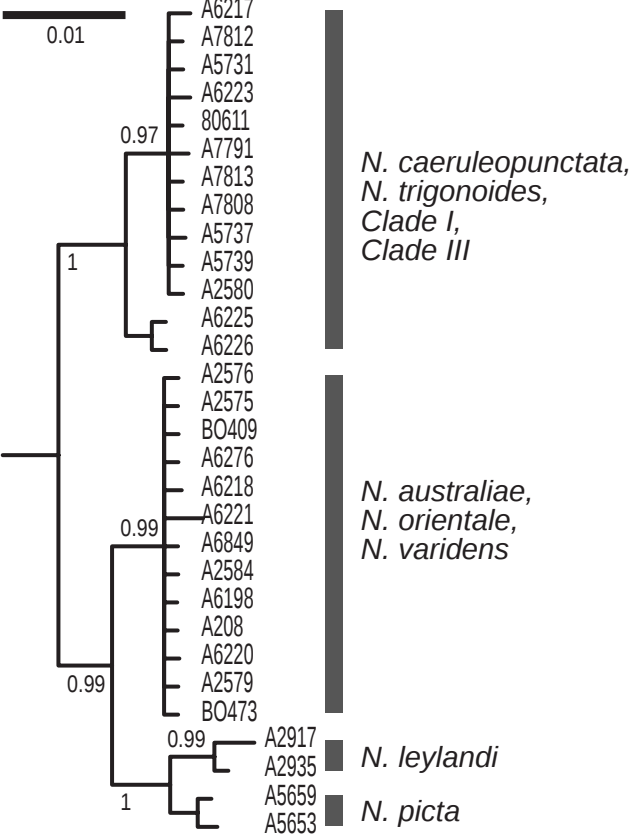


Figure 3 - Borsa et al.

A



B



Supplementary material to:

Multiple cryptic species in the blue-spotted maskray (Myliobatoidei: Dasyatidae: *Neotrygon* spp.)

Philippe Borsa, Kang-Ning Shen, Irma S. Arlyza, Thierry B. Hoareau

Supplementary Tables S1, S2 and Figs. S1, S2 here appended.

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Table S1 List of blue-spotted maskray (*Neotrygon* spp.) samples arranged by species, in alphabetical order, with sampling details, voucher details, and GenBank accession numbers

Species		Locality	Date	Latitude	Longitude	Specimen collection no.	Tissue voucher *	BOLD reg. no.	Reference / source
GenBank no. (CO1)	GenBank no. (cyt b)								
<i>N. australiae</i> Last, White and Séret 2016									
DQ108184	-	Gulf of Carpentaria	Oct. 2008	12.4667 S	141.483 E	-	BW-A208	FOA208-04	Ward et al. 2008
GU673441	-	Western Australia	-	25.7667 S	113.70 E	-	-	-	mined from GenBank
HM902468	-	Gulf of Carpentaria	-	16.2175 S	139.01 E	-	-	-	mined from GenBank
JQ765536	-	Ningaloo Reef, WA	Aug.-Sep. 2010	22.58 S	113.65 E	-	FCP-NKNR42	GBGC11903-13	Cerutti-Pereyra et al. 2012
JQ765537	-	Ningaloo Reef, WA	Aug.-Sep. 2010	21.92 S	113.89 E	-	FCP-NKNR43	GBGC11902-13	Cerutti-Pereyra et al. 2012
JX304874	-	Timor Sea: off Rote island	Sep. 2009	-	-	-	BBSJ1 (sin1)	-	Arlyza et al. 2013a
JX304875	-	Timor Sea: off Rote island	Sep. 2009	-	-	-	BBSJ2 (sin2)	-	Arlyza et al. 2013a
KC250626	-	Tanjung Luar, Lombok	Oct. 2010	8.80 S	116.483 E	CSIRO H 7853-01	BW-A5960	FOAI053-08	Puckridge et al. 2013
KC250627	-	Gulf of Carpentaria, NW of Mornington I.	Feb. 2009	16.3027 S	138.635 E	CSIRO H 7018-01 ‡	BW-A6849	FOAI942-10	Puckridge et al. 2013
KC250632	-	Tanjung Luar, Lombok	Oct. 2010	8.80 S	116.483 E	CSIRO H 7853-02	BW-A5961	FOAI054-08	Puckridge et al. 2013
KC250635	-	Gulf of Carpentaria	Feb. 2009	12.9282 S	141.214 E	CSIRO H 7016-01 †	BW-A6850	FOAI943-10	Puckridge et al. 2013
KC250642	-	Northern GBR off Turtle Head Island	Sep. 2004	10.843 S	142.87 E	-	BW-A5649	FOAH738-08	Puckridge et al. 2013
KC250645	-	Torres Strait, W of Thursday Island	Jan. 2004	10.723 S	141.722 E	-	BW-A5650	FOAH739-08	Puckridge et al. 2013
KU498029	KU497893	Kupang, Sawu Sea	Aug. 2010	-	-	-	KNS-KUP-1-26	-	present study
KU498030	KU497894	Kupang, Sawu Sea	Aug. 2010	-	-	-	KNS-KUP-2-27	-	present study
KU498031	-	Kupang, Sawu Sea	Aug. 2010	-	-	-	KNS-KUP-4-29	-	present study
KU498018	-	Labuan Bajo, Flores Sea	Oct. 2010	-	-	-	KNS-LAB2-1	-	present study
KU498019	KU497895	Labuan Bajo, Flores Sea	Oct. 2010	-	-	-	KNS-LAB3-2	-	present study
KU498020	KU497896	Labuan Bajo, Flores Sea	Oct. 2010	-	-	-	KNS-LAB4-3	-	present study
KU498021	KU497897	Labuan Bajo, Flores Sea	Oct. 2010	-	-	-	KNS-LAB5-4	-	present study
KU498022	KU497898	Labuan Bajo, Flores Sea	Oct. 2010	-	-	-	KNS-LAB6-5	-	present study
KU498023	-	Labuan Bajo, Flores Sea	Oct. 2010	-	-	-	KNS-LAB7-6	-	present study
KU498024	-	Labuan Bajo, Flores Sea	Oct. 2010	-	-	-	KNS-LAB9-2	-	present study
KU498025	-	Labuan Bajo, Flores Sea	Oct. 2010	-	-	-	KNS-LAB18-11	-	present study
-	KU497901	Rote Island, Timor Sea	Sep. 2009	-	-	-	KNS-ROT-1	-	present study
KU498026	KU497899	Tanjung Luar, Lombok	Jul. 2010	-	-	-	KNS-TAL1-74	-	present study
KU498027	-	Tanjung Luar, Lombok	Jul. 2010	-	-	-	KNS-TAL2-75	-	present study
KU498028	KU497900	Tanjung Luar, Lombok	Jul. 2010	-	-	-	KNS-TAL6-64	-	present study
KU521523	-	Tanjung Sulamo, West Timor	Aug. 2010	10°03'S	123°36'E	MZB-20863	ENTTJS2	-	present study
<i>N. caeruleopunctata</i> Last, White and Séret 2016									
EU398736	-	Kedonganan	Apr. 2004	8.75 S	115.17 E	-	BW-A2583	FOAE371-06	Ward et al. 2008
EU398742	-	Bali	Apr. 2004	8.75 S	115.17 E	-	BW-A2574	FOAE362-06	Ward et al. 2008
EU398743	-	Bali	Apr. 2004	8.75 S	115.17 E	CSIRO H 7852-01 ‡	BW-A2573	FOAE361-06	Ward et al. 2008
EU398744	-	Bali	Apr. 2004	8.75 S	115.17 E	CSIRO H 7852-03 †	BW-A2572	FOAE360-06	Ward et al. 2008
EU398745	-	Bali	Mar. 2005	8.75 S	115.17 E	-	BW-A2571	FOAE359-06	Ward et al. 2008
EF609342	-	Kedonganan	Aug. 2002	8.75 S	115.17 E	CSIRO H 6124-01 ‡	BW-A2580	FOAE368-06	Ward et al. 2008
JX304860	-	Kedonganan	Jan. 2008	08°44'S	115°11'E	-	NKBL (Bali1)	-	Arlyza et al. 2013a
KC250629	-	Kedonganan	Apr. 2004	8.75 S	115.17 E	-	BW-A2582	FOAE370-06	Puckridge et al. 2013
KC250630	-	Sadang, Central Java	Oct. 2008	7.03333 S	110.783 E	CSIRO H 7850-01 ‡	BW-A5731	FOAH820-08	Puckridge et al. 2013
KC250634	-	Sadang, Central Java	Oct. 2008	7.03333 S	110.783 E	-	BW-A5737	FOAH826-08	Puckridge et al. 2013
KC250637	-	Sadang, Central Java	Oct. 2008	7.03333 S	110.783 E	-	BW-A5739	FOAH828-08	Puckridge et al. 2013

KC250639	-	Sadang, Central Java	Oct. 2008	7.03333 S	110.783 E	-	BW-A5738	FOAH827-08	Puckridge et al. 2013
KU497925	KU497759	Kedonganan, Bali	Sep. 2013	08°44'S	115°11'E	-	KNS-BAL-A	-	present study
KU497926	KU497760	Kedonganan, Bali	Sep. 2013	08°44'S	115°11'E	-	KNS-BAL-B	-	present study
KU497927	KU497761	Kedonganan, Bali	Sep. 2013	08°44'S	115°11'E	-	KNS-BAL-C	-	present study
KU497928	KU497762	Kedonganan, Bali	Sep. 2013	08°44'S	115°11'E	-	KNS-BAL-D	-	present study
KU497929	KU497763	Kedonganan, Bali	Sep. 2013	08°44'S	115°11'E	-	KNS-BAL-E	-	present study
KU497930	KU497764	Kedonganan, Bali	Sep. 2013	08°44'S	115°11'E	MZB-22131	KNS-BAL-S	-	present study
KU497931	KU497765	Bali Strait	Oct. 2010	-	-	-	KNS-BAS3-1	-	present study
-	KU497766	Bali Strait	Oct. 2010	-	-	-	KNS-BAS12	-	present study
-	KU497767	Bali Strait	Oct. 2010	-	-	-	KNS-BAS13	-	present study
KU497932	KU497768	Pelabuhan Ratu, SW Java	Apr. 2010	06°59'S	106°32'E	-	KNS-PEL1-43	-	present study
KU497933	KU497769	Pelabuhan Ratu, SW Java	Apr. 2010	06°59'S	106°32'E	-	KNS-PEL2-65	-	present study
KU497934	KU497770	Pelabuhan Ratu, SW Java	Apr. 2010	06°59'S	106°32'E	-	KNS-PEL3-44	-	present study
KU497935	KU497771	Pelabuhan Ratu, SW Java	Apr. 2010	06°59'S	106°32'E	-	KNS-PEL4-45	-	present study
KU497936	KU497772	Pelabuhan Ratu, SW Java	Apr. 2010	06°59'S	106°32'E	-	KNS-PEL5-46	-	present study
KU497937	KU497773	Pelabuhan Ratu, SW Java	Apr. 2010	06°59'S	106°32'E	-	KNS-PEL6-78	-	present study
KU497938	KU497774	Pelabuhan Ratu, SW Java	Apr. 2010	06°59'S	106°32'E	-	KNS-PEL9-16	-	present study
KU497939	KU497775	Pelabuhan Ratu, SW Java	Apr. 2010	06°59'S	106°32'E	-	KNS-PEL15-22	-	present study
<i>N. orientale</i> Last, White and Séret 2016									
EU398737	-	Java: Muara Angke market	Apr. 2004	-	-	-	BW-A2579	FOAE367-06	Ward et al. 2008
EU398738	-	Java: Muara Angke market	Apr. 2004	-	-	-	BW-A2578	FOAE366-06	Ward et al. 2008
EU398739	-	Java: Muara Angke market	Apr. 2004	-	-	-	BW-A2577	FOAE365-06	Ward et al. 2008
EU398740	-	Java: Muara Angke market	Apr. 2004	-	-	-	BW-A2576	FOAE364-06	Ward et al. 2008
EU398741	-	Java: Muara Angke market	Apr. 2004	-	-	-	BW-A2575	FOAE363-06	Ward et al. 2008
GU673709	-	Java: Muara Angke market	Apr. 2004	-	-	CSIRO H7849-01	BW-A7737	-	Last et al. 2016
JN184065	JN184065	South China Sea: Tanjung Manis	Apr. 2004	02°07'N	111°20'E	-	BO424	ANGBF2040-12	Aschliman et al. 2012
JX304829	-	Java Sea: Pulau Pabelokan	Aug. 2009	05°27'S	106°29'E	MZB-20852	PB2 (KARpb2)	-	Arlyza et al. 2013a
JX304830	-	Java Sea: Karangantu, Banten Bay	May 2009	06°01'S	106°10'E	-	kar2 (KARkar2)	-	Arlyza et al. 2013a
JX304831	-	Java Sea: Karangantu, Banten Bay	May 2009	06°01'S	106°10'E	-	kar3 (KARkar3)	-	Arlyza et al. 2013a
JX304832	-	Java Sea: Karangantu, Banten Bay	May 2009	06°01'S	106°10'E	-	kar5 (KARkar5)	-	Arlyza et al. 2013a
JX304833	-	Java Sea: Karangantu, Banten Bay	May 2009	06°01'S	106°10'E	-	kar6 (KARkar6)	-	Arlyza et al. 2013a
JX304834	-	Java Sea: Karangantu, Banten Bay	May 2009	06°01'S	106°10'E	-	kar7 (KARkar7)	-	Arlyza et al. 2013a
JX304835	-	Java Sea: Karangantu, Banten Bay	May 2009	06°01'S	106°10'E	-	kar10 (KARkar10)	-	Arlyza et al. 2013a
JX304836	-	Java Sea: Pulau Pari	Dec. 2008	05°51'S	106°37'E	MZB-20851	PR (KARpr)	-	Arlyza et al. 2013a
JX304837	-	Java Sea: Pulau Peniki	Mar. 2009	05°46'S	106°38'E	-	PN2 (KARpn2)	-	Arlyza et al. 2013a
JX304838	-	Java Sea: Pulau Peniki	Mar. 2009	05°46'S	106°38'E	-	PN3 (KARpn3)	-	Arlyza et al. 2013a
JX304839	-	Java Sea: Pulau Peniki	Mar. 2009	05°46'S	106°38'E	-	PN4 (KARpn4)	-	Arlyza et al. 2013a
JX304840	-	Java Sea: Pulau Peniki	Mar. 2009	05°46'S	106°38'E	MZB-20850	PN5 (KARpn5)	-	Arlyza et al. 2013a
JX304841	-	Java Sea: Pulau Peniki	Mar. 2009	05°46'S	106°38'E	-	PN6 (KARpn6)	-	Arlyza et al. 2013a
JX304842	-	Java Sea: Pulau Peniki	Mar. 2009	05°46'S	106°38'E	-	PN8 (KARpn8)	-	Arlyza et al. 2013a
JX304843	-	Java Sea: Pulau Peniki	Mar. 2009	05°46'S	106°38'E	-	PN9 (KARpn9)	-	Arlyza et al. 2013a
JX304844	-	Java Sea: Pulau Peniki	Mar. 2009	05°46'S	106°38'E	-	PN10 (KARpn10)	-	Arlyza et al. 2013a
JX304845	-	Karimata Strait: Selakau, Pontianak	Aug. 2009	01°09'N	109°01'E	-	KBSU4 (sel4)	-	Arlyza et al. 2013a
JX304847	-	Karimata Strait: Selakau, Pontianak	Aug. 2009	01°09'N	109°01'E	-	KBSU6 (sel6)	-	Arlyza et al. 2013a
JX304848	-	Karimata Strait: Selakau, Pontianak	Aug. 2009	01°09'N	109°01'E	-	KBSU7 (sel7)	-	Arlyza et al. 2013a
JX304849	-	Karimata Strait: Selakau, Pontianak	Aug. 2009	01°09'N	109°01'E	-	KBSU8 (sel8)	-	Arlyza et al. 2013a
JX304850	-	Karimata Strait: Selakau, Pontianak	Aug. 2009	01°09'N	109°01'E	-	KBSU9 (sel9)	-	Arlyza et al. 2013a
JX304851	-	Karimata Strait: Selakau, Pontianak	Aug. 2009	01°09'N	109°01'E	-	KBSU10 (sel10)	-	Arlyza et al. 2013a

JX304852	-	Karimata Strait: Selakau, Pontianak	Aug. 2009	01°09'N	109°01'E	-	KBSU11 (sel11)	-	Arlyza et al. 2013a
JX304853	-	Java Sea: Batang, Central Java	Jan. 2008	06°51'S	109°47'E	-	DK5 (bat5)	-	Arlyza et al. 2013a
JX304854	-	Java Sea: Batang, Central Java	Jan. 2008	06°51'S	109°47'E	-	DK7 (bat7)	-	Arlyza et al. 2013a
JX304855	-	Java Sea: Batang, Central Java	Jan. 2008	06°51'S	109°47'E	-	DK8 (bat8)	-	Arlyza et al. 2013a
JX304856	-	Java Sea: Batang, Central Java	Jan. 2008	06°51'S	109°47'E	-	DK10 (bat10)	-	Arlyza et al. 2013a
JX304857	-	Java Sea: Batang, Central Java	Jan. 2008	06°51'S	109°47'E	-	DK20 (bat20)	-	Arlyza et al. 2013a
JX304858	-	Java Sea: Batang, Central Java	Jan. 2008	06°51'S	109°47'E	-	DK25 (bat25)	-	Arlyza et al. 2013a
JX304859	-	Java Sea: Batang, Central Java	Jan. 2008	06°51'S	109°47'E	-	DK30 (bat30)	-	Arlyza et al. 2013a
JX304861	-	Paotere, Makassar Strait	Oct. 2009	05°03'S	119°27'E	-	SSPT2 (pao2)	-	Arlyza et al. 2013a
JX304862	-	Paotere, Makassar Strait	Oct. 2009	05°03'S	119°27'E	-	SSPT3 (pao3)	-	Arlyza et al. 2013a
JX304863	-	Sulawesi Sea: Tarakan, E Kalimantan	Oct. 2009	03°38'S	117°44'E	-	KTTR1 (tar1)	-	Arlyza et al. 2013a
JX304864	-	Sulawesi Sea: Tarakan, E Kalimantan	Oct. 2009	03°38'S	117°44'E	-	KTTR2 (tar2)	-	Arlyza et al. 2013a
JX304865	-	Sulawesi Sea: Tarakan, E Kalimantan	Oct. 2009	03°38'S	117°44'E	-	KTTR3 (tar3)	-	Arlyza et al. 2013a
JX304866	-	Sulawesi Sea: Tarakan, E Kalimantan	Oct. 2009	03°38'S	117°44'E	-	KTTR5 (tar5)	-	Arlyza et al. 2013a
JX304867	-	Sulawesi Sea: Tarakan, E Kalimantan	Oct. 2009	03°38'S	117°44'E	-	KTTR6 (tar6)	-	Arlyza et al. 2013a
JX304869	-	Tonra, Bone Bay	Sep. 2009	04°44'S	120°19'E	-	BBTN2 (ton2)	-	Arlyza et al. 2013a
JX304870	-	Tonra, Bone Bay	Sep. 2009	04°44'S	120°19'E	-	BBTN4 (ton4)	-	Arlyza et al. 2013a
JX304871	-	Tonra, Bone Bay	Sep. 2009	04°44'S	120°19'E	-	BBTN5 (ton5)	-	Arlyza et al. 2013a
JX304872	-	Tonra, Bone Bay	Sep. 2009	04°44'S	120°19'E	-	BBTN6 (ton6)	-	Arlyza et al. 2013a
JX304873	-	Tonra, Bone Bay	Sep. 2009	04°44'S	120°19'E	-	BBTN29 (ton29)	-	Arlyza et al. 2013a
JX304876	KU497815	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan1 (cag1)	-	Arlyza et al. 2013a
JX304877	KU497816	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan2 (cag2)	-	Arlyza et al. 2013a
JX304878	KU497817	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan3 (cag3)	-	Arlyza et al. 2013a
JX304879	KU497818	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan4 (cag4)	-	Arlyza et al. 2013a
JX304880	KU497819	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan5 (cag5)	-	Arlyza et al. 2013a
JX304881	KU497820	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan6 (cag6)	-	Arlyza et al. 2013a
JX304882	KU497821	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan7 (cag7)	-	Arlyza et al. 2013a
JX304883	KU497822	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan8 (cag8)	-	Arlyza et al. 2013a
JX304884	KU497823	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan9 (cag9)	-	Arlyza et al. 2013a
JX304885	KU497824	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan10 (cag10)	-	Arlyza et al. 2013a
JX304886	KU497825	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan11 (cag11)	-	Arlyza et al. 2013a
JX304887	KU497826	Cagayan, Luzon, Philippines	Feb. 2012	18°17'N	121°53'E	-	Cagayan12 (cag12)	-	Arlyza et al. 2013a
JX304888	KU497827	Lapu-Lapu, Cebu Strait, Philippines	May 2011	10°17'N	124°00'E	-	HK1 (lap1)	-	Arlyza et al. 2013a
JX304889	KU497828	Lapu-Lapu, Cebu Strait, Philippines	May 2011	10°17'N	124°00'E	-	HK2 (lap2)	-	Arlyza et al. 2013a
JX304890	KU497829	Lapu-Lapu, Cebu Strait, Philippines	May 2011	10°17'N	124°00'E	-	HK3 (lap3)	-	Arlyza et al. 2013a
JX304891	KU497830	Lapu-Lapu, Cebu Strait, Philippines	May 2011	10°17'N	124°00'E	-	HK4 (lap4)	-	Arlyza et al. 2013a
KC249903	-	Tanjung Manis, Sarawak	Apr. 2004	02°07'N	111°19'E	-	BO423	GBGC11472-13	Puckridge et al. 2013
KC249904	-	Tanjung Manis, Sarawak	Apr. 2004	02°07'N	111°19'E	-	BO424	GBGC11471-13	Puckridge et al. 2013
KC249905	-	Mukah, Sarawak	Apr. 2004	02°54'N	112°06'E	-	BO473	GBGC11470-13	Puckridge et al. 2013
KM073024	-	Sandakan, Sulu Sea	Mar. 2013	-	-	-	NKUH2	ANGBF12768-15	Lim et al. 2015
KM073025	-	Sandakan, Sulu Sea	Mar. 2013	-	-	-	NKUH3	ANGBF12769-15	Lim et al. 2015
KP856772	-	Indonesia	-	-	-	-	A450a	-	mined from GenBank
KP856773	-	Indonesia	-	-	-	-	A450b	-	mined from GenBank
KR019777	KR019777	Cebu	May 2011	-	-	-	330	-	Shen et al. 2016
KU497961	KU497831	Bali Strait	Oct. 2010	-	-	-	KNS-BAS1-87	-	present study
KU497962	KU497832	Bali Strait	Oct. 2010	-	-	-	KNS-BAS2-77	-	present study
KU497963	KU497833	Bali Strait	Oct. 2010	-	-	-	KNS-BAS4-2	-	present study
KU497964	KU497834	Bali Strait	Oct. 2010	-	-	-	KNS-BAS5-3	-	present study
KU497965	-	Bali Strait	Oct. 2010	-	-	-	KNS-BAS6-4	-	present study

-	KU497835	Bali Strait	Oct. 2010	-	-	-	KNS-BAS10	-	present study
-	KU497836	Bali Strait	Oct. 2010	-	-	-	KNS-BAS11	-	present study
KU497966	KU497837	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT1-14	-	present study
KU497967	KU497838	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT2-15	-	present study
KU497968	KU497839	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT3-16	-	present study
KU497969	-	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT4-17	-	present study
KU497970	KU497840	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT5-18	-	present study
KU497971	KU497841	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT6-19	-	present study
KU497972	KU497842	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT7-5	-	present study
KU497973	-	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT8-6	-	present study
KU497974	KU497843	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT9-7	-	present study
KU497975	KU497844	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT10-8	-	present study
KU497976	KU497845	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT11-9	-	present study
KU497977	KU497846	Bitung, Molucca Sea	Oct. 2010	-	-	-	KNS-BIT12-10	-	present study
-	KU497847	Lapu-Lapu, Cebu Strait, Philippines	Nov. 2011	10°17'N	124°00'E	-	KNS-HK5	-	present study
-	KU497848	Lapu-Lapu, Cebu Strait, Philippines	Nov. 2011	10°17'N	124°00'E	-	KNS-HK6	-	present study
-	KU497849	Kendari, Banda Sea	Oct. 2010	-	-	-	KNS-KEN1	-	present study
KU497978	KU497850	Kendari, Banda Sea	Oct. 2010	-	-	-	KNS-KEN2-81	-	present study
KU497979	KU497851	Kendari, Banda Sea	Oct. 2010	-	-	-	KNS-KEN3-69	-	present study
KU497980	-	Kendari, Banda Sea	Oct. 2010	-	-	-	KNS-KEN4-51	-	present study
KU497981	KU497852	Kendari, Banda Sea	Oct. 2010	-	-	-	KNS-KEN5-70	-	present study
KU497982	KU497853	Kendari, Banda Sea	Oct. 2010	-	-	-	KNS-KEN6-82	-	present study
-	KU497854	Kendari, Banda Sea	Oct. 2010	-	-	-	KNS-KEN7-83	-	present study
KU497983	KU497855	Makassar region	Oct. 2009	-	-	-	KNS-MAK1-20	-	present study
KU497984	KU497856	Makassar region	Oct. 2009	-	-	-	KNS-MAK2-21	-	present study
KU497985	-	Makassar region	Oct. 2009	-	-	-	KNS-MAK4-23	-	present study
KU497986	-	Makassar region	Oct. 2009	-	-	-	KNS-MAK5-24	-	present study
KU497987	KU497857	Makassar region	Oct. 2009	-	-	-	KNS-MAK6-25	-	present study
KU497988	KU497858	Poso, Tomini Bay	Jan. 2011	-	-	-	KNS-POS1-61	-	present study
KU497989	KU497859	Poso, Tomini Bay	Jan. 2011	-	-	-	KNS-POS2-49	-	present study
KU497990	KU497860	Poso, Tomini Bay	Jan. 2011	-	-	-	KNS-POS4-50	-	present study
KU497991	KU497861	Riau archipelago	Dec. 2009	-	-	-	KNS-RIA1-30	-	present study
KU497992	KU497862	Riau archipelago	Dec. 2009	-	-	-	KNS-RIA2-31	-	present study
KU497993	KU497863	Riau archipelago	Dec. 2009	-	-	-	KNS-RIA3-32	-	present study
KU497994	-	Riau archipelago	Dec. 2009	-	-	-	KNS-RIA4-34	-	present study
KU497995	KU497864	Tanjung Luar, Lombok	Jul. 2010	-	-	-	KNS-TAL4-76	-	present study
-	KU497865	Tanjung Luar, Lombok	Jul. 2010	-	-	-	KNS-TAL5-63	-	present study
KU497996	KU497866	western Java Sea	Jan. 2008	-	-	-	KNS-WJS1-22	-	present study
KU497997	-	western Java Sea	Jan. 2008	-	-	-	KNS-WJS2-23	-	present study
KU497998	KU497867	western Java Sea	Jan. 2008	-	-	-	KNS-WJS4-25	-	present study
KU497999	KU497868	western Java Sea	Jan. 2008	-	-	-	KNS-WJS1-88	-	present study
KU498000	KU497869	western Java Sea	Jan. 2008	-	-	-	KNS-WJS2-93	-	present study
KU498001	KU497870	western Java Sea	Jan. 2008	-	-	-	KNS-WJS3-89	-	present study
KU498002	KU497871	western Java Sea	Jan. 2008	-	-	-	KNS-WJS4-90	-	present study
KU498003	KU497872	western Java Sea	Jan. 2008	-	-	-	KNS-WJS5-91	-	present study
KU498004	KU497873	western Java Sea	Jan. 2008	-	-	-	KNS-WJS6-92	-	present study
KU498005	KU497874	western Java Sea	Jan. 2008	-	-	-	KNS-WJS1-7	-	present study
KU498006	KU497875	western Java Sea	Jan. 2008	-	-	-	KNS-WJS2-8	-	present study
KU498007	KU497876	western Java Sea	Jan. 2008	-	-	-	KNSWJS3-9	-	present study

KU498008	KU497877	western Java Sea	Jan. 2008	-	-	-	KNS-WJS4-10	-	present study
KU498009	KU497878	western Java Sea	Jan. 2008	-	-	-	KNS-WJS5-11	-	present study
KU498010	KU497879	western Java Sea	Jan. 2008	-	-	-	KNS-WJS6-12	-	present study
KU498011	KU497880	western Java Sea	Jan. 2008	-	-	-	KNS-WJS7-13	-	present study
-	KU497881	western Java Sea	Jan. 2008	-	-	-	KNS-WJS1	-	present study
KU498012	KU497882	western Java Sea	Jan. 2008	-	-	-	KNS-WJS3-35	-	present study
-	KU497883	western Java Sea	Jan. 2008	-	-	-	KNS-WJS4-36	-	present study
KU498013	KU497884	western Sulawesi Sea	Oct. 2009	-	-	-	KNS-WSS1-26	-	present study
KU498014	KU497885	western Sulawesi Sea	Oct. 2009	-	-	-	KNS-WSS2-27	-	present study
KU498015	KU497886	western Sulawesi Sea	Oct. 2009	-	-	-	KNS-WSS3-28	-	present study
KU498016	KU497887	western Sulawesi Sea	Oct. 2009	-	-	-	KNS-WSS4-29	-	present study
KU498017	KU497888	western Sulawesi Sea	Oct. 2009	-	-	-	KNS-WSS5-30	-	present study
-	KU497889	western Sulawesi Sea	Oct. 2009	-	-	-	KNS-WSS6-31	-	present study
<i>N. trigonoides</i> (Castelnau, 1873)									
GU673434	-	New Caledonia, Magenta Beach	-	-	-	-	BW-A6217	-	Puckridge et al. 2013
HM902465	-	Australia, NSW, off Yamba	-	-	-	-	BW-A7791	-	Puckridge et al. 2013
HM902466	-	Australia, NSW	-	-	-	-	BW-A7792	-	Puckridge et al. 2013
HM902467	-	Australia, NSW	-	-	-	-	BW-A7793	-	Puckridge et al. 2013
HM902478	-	Australia, QLD, Gladstone	-	-	-	-	BW-A7808	-	Puckridge et al. 2013
HM902479	-	Australia, QLD, Gladstone	-	-	-	-	BW-A7809	-	Puckridge et al. 2013
HM902480	-	Australia, QLD, Gladstone	-	-	-	-	BW-A7810	-	Puckridge et al. 2013
HM902482	-	Australia, QLD, Moreton Bay	-	-	-	-	BW-A7813	-	Puckridge et al. 2013
HM902483	-	Australia, QLD, Moreton Bay	-	-	-	-	BW-A7814	-	Puckridge et al. 2013
HM902484	-	Australia, QLD, Moreton Bay	-	-	-	-	BW-A7815	-	Puckridge et al. 2013
HM902485	-	Australia, QLD, Moreton Bay	-	-	-	-	BW-A7816	-	Puckridge et al. 2013
JQ765533	-	Great Barrier Reef	Dec. 2008	14.69 S	145.45 E	-	FCP-NKGBR39	GBGC11906-13	Cerrutti-Pereyra et al. 2012
JQ765534	-	Great Barrier Reef	Dec. 2008	14.69 S	145.45 E	-	FCP-NKGBR40	GBGC11905-13	Cerrutti-Pereyra et al. 2012
JQ765535	-	Lizard Island	Dec. 2008	-	-	-	FCP-NKNR41	GBGC11904-13	Cerrutti-Pereyra et al. 2012
JX263420	KC493691	New Caledonia, St Vincent Bay	Mar. 2009	21°57'S	166°02'E	MNHN 2009-0823	Dkuh 20090307, NC2	-	Borsa et al. 2013a
JX304916	KU497902	New Caledonia, St Vincent Bay	Aug. 2008	21°56'S	165°55'E	CSIRO uncat.	Dkuh 20080816, NC1	-	Borsa et al. 2013a
JX304917	KU497903	New Caledonia, St Vincent Bay	Aug. 2009	21°57'S	166°02'E	IRDN20090816	Dkuh 20090816, NC3	-	Borsa et al. 2013a
KC250643	-	Australia, QLD, Lizard Island	Sep. 2008	-	-	-	UGA008	LIFS993-08	Puckridge et al. 2013
KU498032	KU497904	New Caledonia, St Vincent Bay	Aug. 2009	21°57'S	166°02'E	-	KNS-NC4-84	-	present study
KU498033	KU497905	New Caledonia, St Vincent Bay	Aug. 2009	21°57'S	166°02'E	-	KNS-NC5-67	-	present study
KU498034	KU497906	New Caledonia, St Vincent Bay	Aug. 2009	21°57'S	166°02'E	-	KNS-NC6-68	-	present study
<i>N. varidens</i> (Garman 1885)									
EU398733	-	PengHu	May 2005	~23°37'N	~119°36'E	-	BW-A2586	FOAE374-06	Ward et al. 2008
EU398734	-	PengHu	May 2005	~23°37'N	~119°36'E	-	BW-A2585	FOAE373-06	Ward et al. 2008
EU398735	-	PengHu	May 2005	~23°37'N	~119°36'E	-	BW-A2584	FOAE372-06	Ward et al. 2008
-	EU870496	Taiwan	June 2006	-	-	-	-	-	Su et al. 2011
JQ681494	-	Beibu Gulf, South China Sea	Feb.-Jul. 2011	19°52'N	108°14'E	-	FBGCG040-11 (bei)	GBGC11848-13	Wang et al. 2012
JQ765561	-	Haiphong, Vietnam	Sep. 2010	20°46'N	106°52'E	-	FCP-NKVN74	GBGC11878-13	Cerutti-Pereyra et al. 2012

KM073023	-	Kuala Selangor, Malaysia	Oct. 2012	-	-	-	NKU1	ANGBF12767-15	Lim et al. 2015
NA	-	Taiwan	Apr. 2013	23.96194	120.29436	ASIZP0073535	AS1-C-1-12-33	-	CryoBank
NA	-	Taiwan	July 2013	23.97167	120.2934	ASIZP0806153	AS1-C-1-13-22	-	CryoBank
NA	-	Taiwan	July 2013	23.97167	120.2934	ASIZP0806154	AS1-C-1-13-23	-	CryoBank
-	KU497891	western coast of Taiwan	Oct. 2010	-	-	-	wjc625	-	present study
-	KU497892	western coast of Taiwan	Oct. 2010	-	-	-	wjc628	-	present study
clade I									
HM467799	-	Chennai, India	-	-	-	-	NBFGR:CHN 156	GBGC10627-13	mined from GenBank
JX263421	KU497907	Pemba Island, Tanzania	May 2010	06°09'S	39°10'E	-	ZANZ 1	GBGC12836-13	Arlyza et al. 2013b
JX978329	^b -	Vizakhapatnam, India	-	17.6833	83.2833	-	VIZ-DK1	GBGC11459-13	mined from GenBank
KC249906	-	Tanga, Tanzania	-	-	-	-	80611	GBGC11469-13	Puckridge et al. 2013
KC295416	^c -	Pemba Island, Tanzania	May 2010	06°09'S	39°10'E	-	-	GBGC12789-13	Borsa et al. 2013a
KF899609	-	Chennai, India	-	-	-	-	NBFGR:CHN:R24	ANGBF11513-15	mined from GenBank
KF899610	-	Chennai, India	-	-	-	-	NBFGR:CHN:R25	ANGBF11512-15	mined from GenBank
KF899611	-	Chennai, India	-	-	-	-	NBFGR:CHN:R165	ANGBF11511-15	mined from GenBank
KF899612	-	Chennai, India	-	-	-	-	NBFGR:CHN:NK13	ANGBF11510-15	mined from GenBank
KF899613	-	Chennai, India	-	-	-	-	NBFGR:CHN:199	ANGBF11509-15	mined from GenBank
KR003770	-	Tamil Nadu, India	-	-	-	-	-	-	mined from GenBank
KU498035	KU497908	Tanzania	May 2010	-	-	-	KNS-TZN1-52	-	present study
KU498036	KU497909	Pemba Island, Tanzania	May 2010	06°09'S	39°10'E	-	KNS-ZAN3-86	-	present study
KU498037	KU497910	Pemba Island, Tanzania	May 2010	06°09'S	39°10'E	-	KNS-ZAN4-71	-	present study
KU498038	KU497911	Pemba Island, Tanzania	May 2010	06°09'S	39°10'E	-	KNS-ZAN5-80	-	present study
clade II									
JX304798	-	Andaman Sea: Pulau Breueh, Aceh	Apr. 2009	05°53'N	95°02'E	-	BA15 (bre15)	-	Arlyza et al. 2013a
JX304799	-	Andaman Sea: Pulau Breueh, Aceh	Apr. 2009	05°53'N	95°02'E	-	BA16 (bre16)	-	Arlyza et al. 2013a
JX304800	-	Andaman Sea: Pulau Breueh, Aceh	Apr. 2009	05°53'N	95°02'E	-	BA18 (bre18)	-	Arlyza et al. 2013a
JX304801	-	Andaman Sea: Pulau Breueh, Aceh	Apr. 2009	05°53'N	95°02'E	-	BA19 (bre19)	-	Arlyza et al. 2013a
JX304802	-	Andaman Sea: Pulau Breueh, Aceh	Apr. 2009	05°53'N	95°02'E	-	BA20 (bre20)	-	Arlyza et al. 2013a
JX304803	-	Andaman Sea: Pulau Breueh, Aceh	Apr. 2009	05°53'N	95°02'E	-	BA22 (bre22)	-	Arlyza et al. 2013a
JX304804	-	NE Indian Ocean: Meulaboh, Aceh	Apr. 2009	04°07'N	96°08'E	-	ME2 (meu2)	-	Arlyza et al. 2013a
JX304805	-	NE Indian Ocean: Meulaboh, Aceh	Apr. 2009	04°07'N	96°08'E	MZB-20843	ME3 (meu3)	-	Arlyza et al. 2013a
JX304806	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS18 (sib18)	-	Arlyza et al. 2013a
JX304807	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS19 (sib19)	-	Arlyza et al. 2013a
JX304808	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS20 (sib20)	-	Arlyza et al. 2013a
JX304809	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS22 (sib22)	-	Arlyza et al. 2013a
JX304810	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS23 (sib23)	-	Arlyza et al. 2013a
JX304811	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS24 (sib24)	-	Arlyza et al. 2013a
JX304812	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS25 (sib25)	-	Arlyza et al. 2013a
JX304813	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS26 (sib26)	-	Arlyza et al. 2013a
JX304814	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS28 (sib28)	-	Arlyza et al. 2013a
JX304815	-	NE Indian Ocean: Sibolga, NW Sumatra	Mar. 2009	01°45'N	98°46'E	-	WIOS30 (sib30)	-	Arlyza et al. 2013a
JX304828	-	Padang, Western Sumatra	Aug 2009	00°56'S	100°21'E	MZB-20845	WIOPD1 (pad1)	-	Arlyza et al. 2013a
-	KU497752	Aceh	Apr. 2009	-	-	-	KNS-ACE-M	-	present study
KU497912	KU497753	Banda Aceh, Andaman Sea	Dec. 2013	-	-	-	KNS-ACE1-12	-	present study
KU497913	-	Banda Aceh, Andaman Sea	Dec. 2013	-	-	-	KNS-ACE2-13	-	present study
KU497914	-	Banda Aceh, Andaman Sea	Dec. 2013	-	-	-	KNS-ACE3-14	-	present study
-	KU497754	Banda Aceh, Andaman Sea	Dec. 2013	-	-	-	KNS-ACE4-15	-	present study
KU497915	-	Banda Aceh, Andaman Sea	Apr. 2009	-	-	-	KNS-ACE12-60	-	present study
KU497916	-	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD1-16	-	present study

KU497917	KU497755	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD2-17	-	present study
KU497918	KU497756	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD3-18	-	present study
KU497919	KU497757	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD4-19	-	present study
KU497920	KU497758	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD6-21	-	present study
KU497921	-	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD9-48	-	present study
KU497922	-	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD10-49	-	present study
KU497923	-	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD12-50	-	present study
KU497924	-	Padang, Western Sumatra	Aug. 2009	-	-	-	KNS-PAD14-52	-	present study
clade III									
GU673423	-	Thailand, Andaman Coast	-	-	-	-	BW-A6226	FOAI319-09	Puckridge et al. 2013
GU673425	-	Thailand, Andaman Coast	-	-	-	-	BW-A6225	FOAI318-09	Puckridge et al. 2013
GU673426	-	Thailand, Andaman Coast	-	-	-	-	BW-A6224	FOAI317-09	Puckridge et al. 2013
GU673427	-	Thailand, Andaman Coast	-	-	-	-	BW-A6222	FOAI315-09	Puckridge et al. 2013
GU673428	-	Thailand, Andaman Coast	-	-	-	-	BW-A6223	FOAI316-09	Puckridge et al. 2013
JX304816	-	Kuala Lama, Malacca Strait	Dec. 2008-Mar. 2009	03°26'N	99°16'E	-	MSKL1 (PAGmskl1)	-	Arlyza et al. 2013a
JX304817	-	Kuala Lama, Malacca Strait	Dec. 2008-Mar. 2009	03°26'N	99°16'E	-	MSKL2 (PAGmskl2)	-	Arlyza et al. 2013a
JX304818	-	Kuala Lama, Malacca Strait	Dec. 2008-Mar. 2009	03°26'N	99°16'E	MZB-20847	MSKL3 (PAGmskl3)	-	Arlyza et al. 2013a
JX304819	-	Kuala Lama, Malacca Strait	Dec. 2008-Mar. 2009	03°26'N	99°16'E	-	MSKL30 (PAGmskl30)	-	Arlyza et al. 2013a
JX304820	-	Pagurawan, Malacca Strait	Mar. 2009	03°39'N	98°59'E	-	PAG9 (PAGpag9)	-	Arlyza et al. 2013a
JX304821	-	Pagurawan, Malacca Strait	Mar. 2009	03°39'N	98°59'E	-	PAG10 (PAGpag10)	-	Arlyza et al. 2013a
JX304822	-	Pagurawan, Malacca Strait	Mar. 2009	03°39'N	98°59'E	-	PAG11 (PAGpag11)	-	Arlyza et al. 2013a
JX304823	-	Pagurawan, Malacca Strait	Mar. 2009	03°39'N	98°59'E	-	PAG12 (PAGpag12)	-	Arlyza et al. 2013a
JX304824	-	Pagurawan, Malacca Strait	Mar. 2009	03°39'N	98°59'E	-	PAG17 (PAGpag17)	-	Arlyza et al. 2013a
JX304825	-	Pagurawan, Malacca Strait	Mar. 2009	03°39'N	98°59'E	-	PAG18 (PAGpag18)	-	Arlyza et al. 2013a
JX304826	-	Pagurawan, Malacca Strait	Mar. 2009	03°39'N	98°59'E	-	PAG19 (PAGpag19)	-	Arlyza et al. 2013a
JX304827	-	Pagurawan, Malacca Strait	Mar. 2009	03°39'N	98°59'E	-	PAG21 (PAGpag21)	-	Arlyza et al. 2013a
-	KU497786	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL1-31	-	present study
KU497946	KU497787	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL2-32	-	present study
KU497947	KU497788	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL3-33	-	present study
KU497948	KU497789	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL4-34	-	present study
KU497949	KU497790	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL5-35	-	present study
KU497950	KU497791	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL6-36	-	present study
KU497951	KU497792	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL7-37	-	present study
-	KU497793	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL10	-	present study
-	KU497794	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL11	-	present study
-	KU497795	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL12	-	present study
-	KU497796	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL14	-	present study
-	KU497797	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL16	-	present study
-	KU497798	Malacca Strait	Dec. 2008-Mar. 2009	-	-	-	KNS-MAL17	-	present study
clade VII									
JX304892	KU497799	Ambon	Oct.-Dec. 2008	03°40'S	128°11'E	MZB-20864	AM1 (amb1)	-	Arlyza et al. 2013a
JX304893	KU497800	Ambon	Oct.-Dec. 2008	03°40'S	128°11'E	-	AM2 (amb2)	-	Arlyza et al. 2013a
JX304894	KU497801	Ambon	Oct.-Dec. 2008	03°40'S	128°11'E	-	AM3 (amb3)	-	Arlyza et al. 2013a
JX304895	KU497802	Ambon	Oct.-Dec. 2008	03°40'S	128°11'E	-	AM4 (amb4)	-	Arlyza et al. 2013a
JX304896	KU497803	Ambon	Oct.-Dec. 2008	03°40'S	128°11'E	-	AM5 (amb5)	-	Arlyza et al. 2013a
JX304897	KU497804	Ambon	Oct.-Dec. 2008	03°40'S	128°11'E	-	AM6 (amb6)	-	Arlyza et al. 2013a
JX304898	-	Tual	Mar.- May 2009	05°38'S	132°44'E	MZB-20866	ARA1 (ara1)	-	Arlyza et al. 2013a
JX304899	-	Tual	Mar.- May 2009	05°38'S	132°44'E	-	ARA23 (ara23)	-	Arlyza et al. 2013a
JX304900	-	Tual	Mar.- May 2009	05°38'S	132°44'E	-	ARA24 (ara24)	-	Arlyza et al. 2013a

JX304901	-	Tual	Mar.- May 2009	05°38'S	132°44'E	-	ARA25 (ara25)	-	Arlyza et al. 2013a
JX304902	-	Tual	Mar.- May 2009	05°38'S	132°44'E	-	ARA27 (ara27)	-	Arlyza et al. 2013a
JX304903	-	Tual	Mar.- May 2009	05°38'S	132°44'E	-	ARA29 (ara29)	-	Arlyza et al. 2013a
JX304904	-	Tual	Mar.- May 2009	05°38'S	132°44'E	-	ARA30 (ara30)	-	Arlyza et al. 2013a
JX304905	-	Tual	Mar.- May 2009	05°38'S	132°44'E	-	ARA31 (ara31)	-	Arlyza et al. 2013a
KU497952	KU497805	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI2-53	-	present study
KU497953	KU497806	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI3-72	-	present study
KU497954	KU497807	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI4-54	-	present study
KU497955	KU497808	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI5-55	-	present study
KU497956	KU497809	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI6-56	-	present study
KU497957	KU497810	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI7-73	-	present study
-	KU497811	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI8-34	-	present study
-	KU497812	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI10-36	-	present study
KU497958	KU497813	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI12-38	-	present study
KU497959	-	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI13-39	-	present study
KU497960	-	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI15-41	-	present study
-	KU497814	Kei Islands	Mar.- May 2009	-	-	-	KNS-KEI18-44	-	present study
clade VIII									
JX304906	-	Biak, West Papua	May 2009	00°58'S	136°16'E	-	BK1 (bia1)	-	Arlyza et al. 2013a
JX304907	-	Biak, West Papua	May 2009	00°58'S	136°16'E	-	BK2 (bia2)	-	Arlyza et al. 2013a
JX304908	-	Biak, West Papua	May 2009	00°58'S	136°16'E	-	BK4 (bia4)	-	Arlyza et al. 2013a
JX304909	-	Biak, West Papua	May 2009	00°58'S	136°16'E	MZB-20867	BK5 (bia5)	-	Arlyza et al. 2013a
JX304910	-	Biak, West Papua	May 2009	00°58'S	136°16'E	-	BK6 (bia6)	-	Arlyza et al. 2013a
JX304911	-	Biak, West Papua	May 2009	00°58'S	136°16'E	-	BK7 (bia7)	-	Arlyza et al. 2013a
JX304912	-	Biak, West Papua	May 2009	00°58'S	136°16'E	-	BK8 (bia8)	-	Arlyza et al. 2013a
JX304913	-	Biak, West Papua	May 2009	00°58'S	136°16'E	-	BK9 (bia9)	-	Arlyza et al. 2013a
JX304914	-	Biak, West Papua	May 2009	00°58'S	136°16'E	-	BK23 (bia23)	-	Arlyza et al. 2013a
JX304915	-	Pulau Numfor, West Papua	May 2009	01°09'S	134°55'E	-	BK24 (bia24)	-	Arlyza et al. 2013a
-	KU497776	Biak, West Papua	May 2009	-	-	-	KNS-BIA1-37	-	present study
KU497940	KU497777	Biak, West Papua	May 2009	-	-	-	KNS-BIA2-38	-	present study
KU497941	KU497778	Biak, West Papua	May 2009	-	-	-	KNS-BIA3-39	-	present study
KU497942	KU497779	Biak, West Papua	May 2009	-	-	-	KNS-BIA4-40	-	present study
KU497943	KU497780	Pulau Numfor, West Papua	May 2009	-	-	-	KNS-BIA5-41	-	present study
KU497944	KU497781	Biak, West Papua	May 2009	-	-	-	KNS-BIA6-42	-	present study
KU497945	KU497782	Biak, West Papua	May 2009	-	-	-	KNS-BIA7-66	-	present study
-	KU497783	Biak, West Papua	May 2009	-	-	-	KNS-BIA9	-	present study
-	KU497784	Biak, West Papua	May 2009	-	-	-	KNS-BIA10	-	present study
-	KU497785	Biak, West Papua	May 2009	-	-	-	KNS-BIA13	-	present study
Guadalcanal blue-spotted maskray									
-	-	Honiara, Guadalcanal Island	May 2015	-	-	CSIRO H7723-01	-	-	Last et al. 2016
Ryukyu blue-spotted maskray									
AB485685	-	Ishigaki-shima, Ryukyu archipelago	Nov. 2004	-	-	NSMT: P-91858	-	GBGC10609-13	Yagishita et al. 2009

^a sequence no. KC992792 (BOLD GBGC12667-13) synonymous with NC_021767

^b *N. kuhlii* specimens VIZ-DK1 (GenBank JX978329; BOLD GBGC11459-13) and VIZNK-01 (BOLD INELA012-12) have identical sequences and apparently belong to the same individual

^c *N. kuhlii* sequence with GenBank accession no. KC295416 is identical to that with no. JX263421 and represents the same individual

* in brackets: sample abbreviation in Arlyza et al. 2013a

† holotype

‡ paratype

Blue-spotted maskray, *Neotrygon* spp. Alignment of partial *CO1* + cytochrome *b* gene sequences. The concatenated fragment is 1864 bp long. The *CO1* gene sub-fragment spans the first 722 nucleotides of the alignment (nucleotides 5517–6238 of the mitochondrial genome of *N. varidens* (GenBank NC_021767). The cytochrome *b* gene sub-fragment starts at nucleotide site 727 and spans nucleotide sites 14434–15577 of GenBank NC_021767. There are 133 sequences in total, arranged by species or lineage

[illegible]

[illegible]

N. australiae

[illegible]

N. australiae

[illegible]

[illegible]

Table S2
(continued)

	605	615	625	635	645	655	665	675	685	695	705	715	725	735	745	755	765	775	785	795
N. australiae																				
KNS-KUP1-26	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGAGGAGG	GGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	--AAAAACACA	TCOCCTATT	AAAAATTATCA	ACAACCTCACT	AATTGATCTA	CCAGCTCCAA
KNS-LAB3-2	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGAGGAGG	GGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----TCC	GTA AAAACACA	TCOCCTATT	AAAAATTATCA	ACAACCTCACT	AATTGATCTA	CCAGCTCCAA
KNS-LAB3-3	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGAGGAGG	GGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----CA	TCOCCTATT	AAAAATTATCA	ACAACCTCACT	AATTGATCTA	CCAGCTCCAA
KNS-LAB5-4	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGAGGAGG	GGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----CA	ACAACCTCACT	AATTGATCTA	-----	CTCCAA
KNS-LAB5-5	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGAGGAGG	GGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-TAL1-74	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGAGGAGG	GGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	--AAATCTC	GTA AAAACACA	TCOCCTATT	AAAAATTATCA	ACAACCTCACT	CCAGCTCCAA
KNS-TAL6-64	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGAGGAGG	GGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	--AAATCTC	GTA AAAACACA	TCOCCTATT	AAAAATTATCA	ACAACCTCACT	CCAGCTCCAA
N. caeruleopunctata																				
KNS-BAL-A	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	--AAAAACACA	TCOCCTATT	AAAAATTATCA	ACAACCTCACT	GATTGATCTA
KNS-BAL-B	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----ACACA	TCOCCTATT	AAAAATTATCA	ACAACCTCACT	GATTGATCTA
KNS-BAL-C	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-BAL-D	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-BAL-E	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-BAL-S	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-BAS3-1	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-PEL1-43	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-PEL2-65	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-PEL3-44	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-PEL4-45	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-PEL5-46	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TTGACCCAG	CTGGGGGAGG	AGATCCCAT	CTTTACCAAC	ACCTCTTCTG	ATTCTTTGGC	CA-----	-----	-----	-----	-----	-----	-----	-----
KNS-PEL6-78	CCAGTCTCTAG	CAGCTGGCAT	TACTATACTC	CTCAGACAGC	GAATCTTTAA	TACAACITTTT	TT													

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Table S2
(continued)

Table S2
(continued)

[illegible]

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N. australiae

[illegible]

Table S2
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Table S2
(continued)

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[illegible]

Table S2
(continued)

	1805	1815	1825	1835	1845	1855	
<i>N. australiae</i>							
KNS-KUP1-26	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-KUP2-27	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCT-
KNS-LAB3-2	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTC
KNS-LAB4-3	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-LAB5-4							
KNS-LAB6-5	CTTCTCCTTC	TTCTCAT--					
KNS-TAL1-74	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-TAL6-64	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
<i>N. caeruleopunctata</i>							
KNS-BAL-A	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BAL-B	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BAL-C	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BAL-D	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BAL-E	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATG-----	
KNS-BAL-S	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BAS3-1	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-PEL1-43	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-PEL2-65	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-PEL3-44	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-PEL4-45	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-PEL5-46	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-PEL6-78	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-PEL9-16	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCC-----			
KNS-PEL15-22	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCT-
<i>N. orientale</i>							
UN184065	CTTCTCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap1	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap2	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap3	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap4	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap5	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap6	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap7	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap8	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap9	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap10	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap11	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
cap12	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
lap1	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
lap2	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
lap3	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
lap4	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KR019777	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGCTAAA	CCTT
KNS-BAS1-87	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BAS2-77	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BAS4-2	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BAS5-3	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BIT1-14	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BIT2-15	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGA-----		
KNS-BIT3-16	CTTCTCCTTC	TTCTCATCT	TTTTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BIT5-18	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BIT6-19	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BIT7-5	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	C-----			
KNS-BIT9-7	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BIT10-8	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BIT11-9	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-BIT12-10	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-KEN2-81	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTT--	
KNS-KEN3-69	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-KEN5-70	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-KEN7-83	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-MAK1-20	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-MAK2-21	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-MAK6-65	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-POS1-61							
KNS-POS2-49							
KNS-POS04-50							
KNS-RIA1-30	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT
KNS-RIA2-31	CTTCTCCTTC	TTCTCATCT	TATTCCCAAT	CGCCGGATGA	TGAGAAAAACA	AAATGTTAAA	CCTT

KNS-RIA3-32	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-TAL4-76	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS1-22	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CC--
KNS-WJS4-25	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS1-88	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS2-93	-----	-----	-----	-----	-----	-----	----
KNS-WJS3-89	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CC--
KNS-WJS4-90	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS5-91	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCAT
KNS-WJS6-92	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS1-7	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS2-8	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS3-9	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS4-10	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS5-11	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS6-12	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS7-13	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WJS3-35	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAA-	----
KNS-WSS1-26	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AA-----	----
KNS-WSS2-27	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WSS3-28	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WSS4-29	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-WSS5-30	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
<i>N. trigonoides</i>							
NC2 MNHN2009-0823	CTTCTCCTTC	TTCTCATCC	TATTCCTCAAT	CGCTGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
NC1 CSIRO uncat.	CTTCTCCTTC	TTCTCATCC	TATTCCTCAAT	CGCTGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
NC3 IRD20090816	CTTCTCCTTC	TTCTCATCC	TATTCCTCAAT	CGCTGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-NC4-84	CTTCTCCTTC	TTCTCATCC	TATTCCTCAAT	TG-----	-----	-----	----
KNS-NC5-67	CTTCTCCTTC	TTCTCATCC	TATTCCTCAAT	CGCTGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-NC6-68	CTTCTCCTTC	TTCTCATCC	TATTCCTCAAT	CGCTGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
<i>N. varidens</i>							
wjc629	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KC992792	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
<i>clade I</i>							
ZANZ1	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCTGGATGG	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-TZN1-52	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCTGGATGG	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-ZAN3-86	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCTGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-ZAN4-71	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCTGGATGG	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-ZAN5-80	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCTGGATGG	TGAGAAAACA	AAATGTTAAA	CCTT
<i>clade II</i>							
KNS-ACE1-12	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-PAD2-17	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-PAD3-18	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-PAD4-19	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-PAD6-21	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
<i>clade III</i>							
KNS-MAL2-32	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGCTAAA	CCTT
KNS-MAL3-33	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGCTAAA	CCTT
KNS-MAL4-34	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGCTAAA	CCTT
KNS-MAL5-35	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGCTAAA	CCTT
KNS-MAL6-36	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGCTAAA	CCTT
KNS-MAL7-37	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGCTAAA	CCTT
<i>clade VII</i>							
AM1	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGG	TGAGAAAATA	AAATGTTAA-	----
AM2	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGG	TGAGAAAATA	AAATGTTAAA	CCTT
AM3	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGG	TGAGAAAATA	AAATGTTAAA	CCTT
AM4	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGG	TGAGAAAATA	AAATGTTAAA	CCTT
AM5	CTTCTCCTTC	TTCTCATCT	TATTCCTCA-	-----	-----	-----	----
AM6	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGG	TGAGAAAATA	AAATGTTAAA	CCTT
KNS-KEI2-53	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGGTGG	TGAGAAAATA	AAATGTTAAA	CCT-
KNS-KEI3-72	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGGTGG	TGAGAAAATA	AAATGTTAAA	CCTT
KNS-KEI4-54	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGG	TGAGAAAATA	AAATGTTAAA	CCTT
KNS-KEI5-55	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGGTGG	TGAGAAAATA	AAATGTTAAA	CCTT
KNS-KEI6-56	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGGTGG	TGAGAAAATA	AAATGTTAAA	CC--
KNS-KEI7-73	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGG	TGAGAAAATA	AAATGTTAAA	CCTT
KNS-KEI12-38	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGGTGG	TGAGAAAATA	AAATGTTAAA	CCTT
<i>clade VIII</i>							
KNS-BIA2-38	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-BIA3-39	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-BIA4-40	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-BIA5-41	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-BIA6-42	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT
KNS-BIA7-66	CTTCTCCTTC	TTCTCATCT	TATTCCTCAAT	CGCCGGATGA	TGAGAAAACA	AAATGTTAAA	CCTT

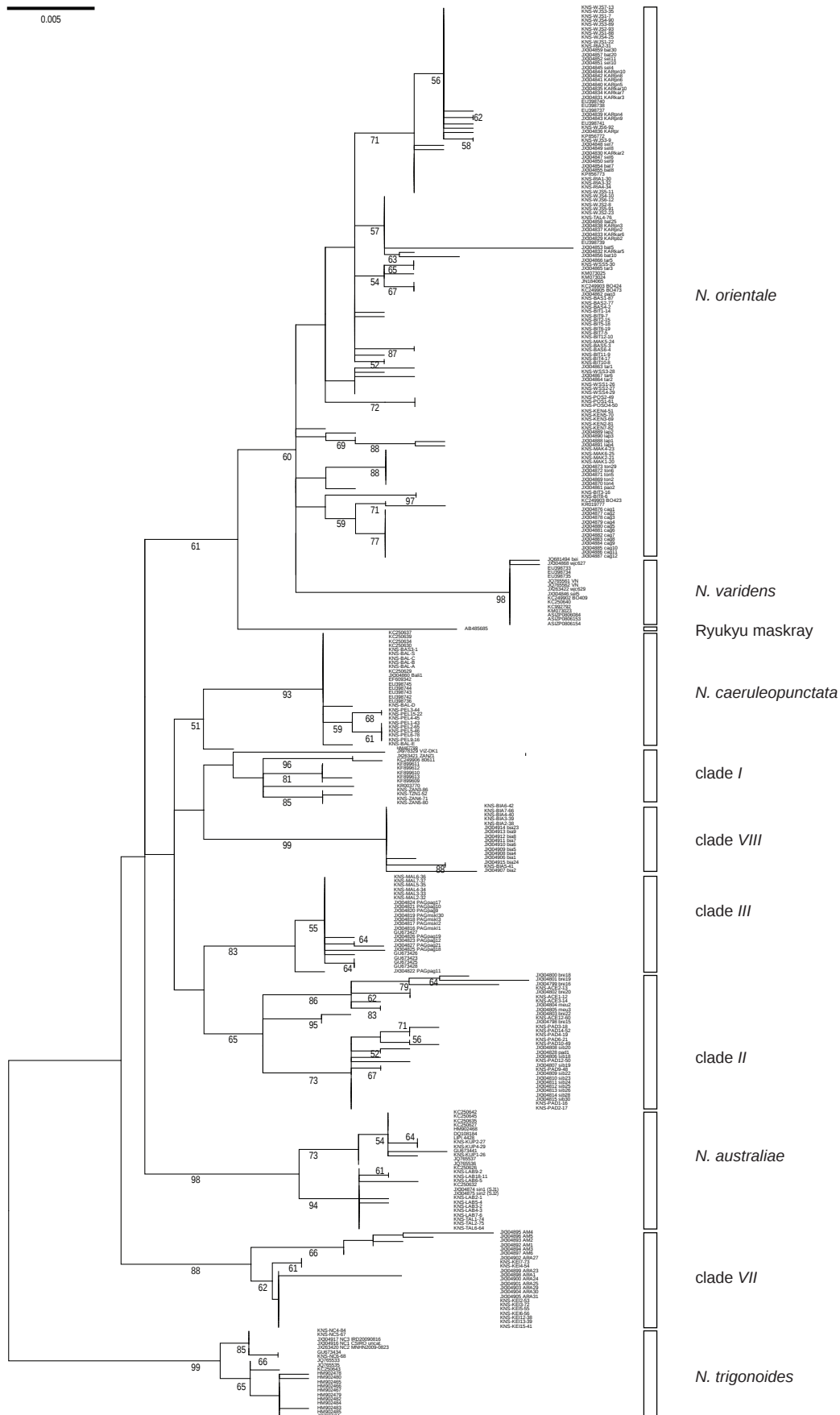


Fig. S1 Maximum-likelihood phylogeny [MEGA6 (Tamura et al. 2013); Tamura 3-parameter model (Tamura 1992) with gamma-distributed rate differences among sites; partial deletion] of *Neotrygon* spp., including four new species formerly under *N. kuhlii*, based on nucleotide sequences of the *CO1* gene. A total of 330 sequences aligned over 519 bp [nucleotide sites nos. 106-624 of the *CO1* gene; JN184065 (Aschliman et al. 2012)] was retained in the final dataset, after all positions with less than 95% site coverage had been eliminated. *N. trigonoides* was designated as outgroup (Borsa et al. 2013a). Numbers at a node are bootstrap scores (from 600 bootstrap resampling runs).

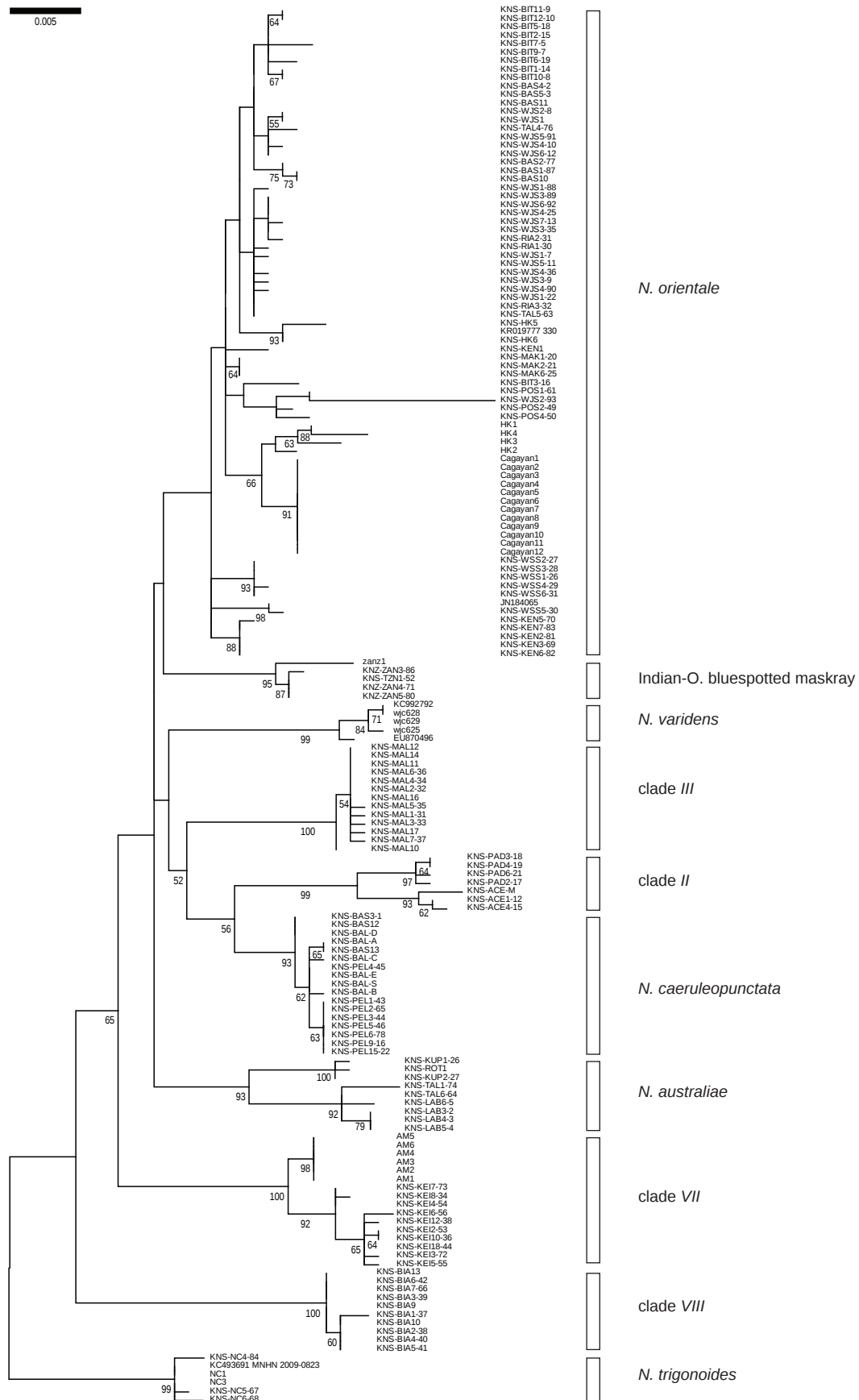


Fig. S2 Maximum-likelihood phylogeny [MEGA6 (Tamura et al. 2013); Tamura-Nei model (Tamura & Nei 1993) with gamma-distributed rate differences among sites, invariable sites allowed; partial deletion] of *Neotrygon* spp., including 9 lineages of the blue-spotted maskray (previously *N. kuhlii*), based on 165 nucleotide sequences of the *cytochrome b* gene. A total of 1059 nucleotide positions was retained in the final dataset, after all positions with less than 95% site coverage had been eliminated. *N. trigonoides* was designated as outgroup (Borsa et al. 2013a). Numbers at a node are bootstrap scores (from 600 bootstrap resampling runs).