***Neotrygon vali*, a new species of the blue-spotted maskray complex (Myliobatoidei: Dasyatidae)**

Philippe Borsa

Institut de recherche pour le développement (IRD), UMR 250 "Ecologie marine tropicale des océans Pacifique et Indien", BP A5, 98848 Nouméa, New Caledonia
Email: philippe.borsa@ird.fr; Tel: +33 6 46838763

ABSTRACT

The blue-spotted maskray from Guadalcanal Island (Solomon archipelago) is distinct by its colour patterns from *Neotrygon kuhlii* with which it was previously confused, and belongs to a genetic lineage clearly separate from all other known species in the genus *Neotrygon*. It is here described as a new species, *Neotrygon vali* sp. nov., on the basis of its nucleotide sequence at the *cytochrome oxidase 1* (*CO1*) gene locus. It is diagnosed from all other known species in the genus *Neotrygon* by the possession of nucleotide T at nucleotide site 420 and nucleotide G at nucleotide site 522 of the *CO1* gene.

Key words: new species, *CO1* gene, molecular diagnosis, taxonomy

INTRODUCTION

Genetic studies of the dasyatid genus *Neotrygon* Castelnau, 1873 or maskrays have pointed to the possible occurrence of several species complexes (Ward et al., 2008; Naylor et al., 2012; Borsa et al., 2016a and references therein). This genus currently comprises 10 nominal species: *N. annotata* (Last, 1987), *N. australiae* Last, White and Séret, 2016, *N. caeruleopunctata* Last, White and Séret, 2016, *N. kuhlii* (Müller and Henle, 1841), *N. leylandi* (Last, 1987), *N. ningalooensis* Last, White and Puckridge, 2010, *N. orientale* Last, White and Séret, 2016, *N. picta* (Last, 1987), *N. trigonoides* (Castelnau, 1873) and *N. varidens* (Garman, 1885). The blue-spotted maskray, previously *N. kuhlii*, consists of up to eleven lineages representing separate species (Arlyza et al., 2013a; Puckridge et al., 2013; Borsa et al., 2016a, 2016b) of which four (*N. australiae*, *N. caeruleopunctata*, *N. orientale*, *N. varidens*) have so far been formally described. One of the paratypes of *N. kuhlii*, a specimen from Vanikoro in the Santa Cruz archipelago, has been recently designated as lectotype (Last et al., 2016), although the pigmentation patterns of the Vanikoro maskray, thus now the typical *N. kuhlii*, do not fit those of the original description of the species by J. Müller and F.G.J. Henle (Müller and Henle, 1841; Borsa and Béarez, 2016). In their re-description of *N. kuhlii*, Last et al. (2016) hastily included a fresh specimen collected from Guadalcanal Island in the Solomon archipelago, over 800 km away from Vanikoro, the type-locality. Pigmentation patterns clearly distinguish the Guadalcanal maskray from *N. kuhlii* from Vanikoro (Borsa and Béarez 2016), but not from other species previously under *N. kuhlii* except *N. varidens* (Garman 1885).

In contrast, mitochondrial DNA sequence information contributes valuable diagnostic characters to the taxonomic description of species and is fundamental to the description of cryptic species (Jörger and Schrödl, 2013). The taxonomic value of mitochondrial DNA sequences has been demonstrated in morphologically intractable species complexes in Elasmobranchs such as *Himantura uarnak* and *N. kuhlii* (Naylor et al., 2012; Arlyza et al., 2013a; Borsa et al., 2013a, 2013b; Puckridge et al., 2013; Borsa, 2017).

It is here emphasized that after careful re-examination of Last et al.'s (2016) work, Borsa et al. (in press) found no diagnostic morphological character that clearly distinguished any of the three new species described from the two others or from *N. kuhlii*. Thus, Last et al.'s (2016) morphological diagnoses were found to be invalid. The objectives of the present paper, which follows up Borsa and Béarez (2016), are the following: (1) to identify diagnostic characters that distinguish the Guadalcanal maskray from other species in the genus *Neotrygon*; (2) to describe it as a new maskray species, a necessary step towards clarifying the intricate taxonomy of species in this species complex.

METHODS

Because *N. kuhlii* from Vanikoro, the type-locality, has not yet been analyzed genetically, pigmentation patterns were used to distinguish it from the Guadalcanal maskray, following Borsa et al. (2013a). Three

specimens of the Guadalcanal maskray were examined including specimen no. CSIRO H 7723-01 (p. 539 of Last et al., 2016) and two live specimens photographed underwater, one by Randall (2005) and the other one by M.A. Rosenstein (Fig. 1). The diameter of ocellated blue spots on the dorsal side of the disk, relative to disk width, was measured on the photographs. Ocellated blue spots were qualified as "small" when their maximum diameter was ≤ 2% disk width (DW), "medium" when ≤ 4% DW and "large" when > 4% DW (Borsa et al., 2013a). On Randall's (2005) picture and on Fig. 1, DW was deduced from disk length (DL; measured from tip of snout to rear tip of pelvic fin) from the relationship DW = 1.13 DL, obtained from measurements on specimen no. CSIRO H 7723-01. Dark speckles (≤ 1% DW) and dark spots (> 1% DW) were also counted on the dorsal surface of the disk (Borsa et al., 2013a). The counts did not include those speckles and spots located within the dark band around eyes that forms the mask. The presence or absence of a scapular blotch was also checked.

The Guadalcanal maskray was compared to other species in the genus *Neotrygon* based on nucleotide sequences of the *CO1* gene. A total of 205 complete or partial *CO1* gene sequences were found in the literature (Ward et al., 2008; Yagishita et al., 2009; Aschliman et al., 2012; Arlyza et al., 2013a; Borsa et al., 2013a; Puckridge et al., 2013; Last et al., 2016) and compiled into a single FASTA file which was edited under BIOEDIT (Hall, 1999). The recently-described *N. australiae* and *N. caeruleopunctata* correspond to, respectively, clades *V* and *VI* of Arlyza et al. (2013a). Clade *IV* of Arlyza et al. (2013a) included a distinct sub-clade that corresponds to *N. varidens*. All other haplotypes of clade *IV* of Arlyza et al. (2013a), together with GenBank no. JN184065 (Aschliman et al., 2012) correspond to *N. orientale*, except a distinct haplotype (GenBank no. AB485685; Yagishita et al., 2009) here referred to as the Ryukyu maskray. Two haplotypes from the Indian Ocean (GenBank nos. JX263421 and KC249906) belonging to Haplogroup *I* of Arlyza et al. (2013a) are here referred to as the Indian Ocean maskray. Sample sizes were: N = 8 for *N. annotata*; N = 11 for *N. australiae*; N = 12 for *N. caeruleopunctata*; N = 7 for *N. leylandi*; N = 1 for *N. ningalooensis*; N = 68 for *N. orientale*; N = 5 for *N. picta*; N = 18 for *N. trigonoides*; N = 11 for *N. varidens*; N = 19 for clade *II* of Arlyza et al. (2013a); N = 17 for clade *III* of Arlyza et al. (2013a); N = 14 for clade *VII* of Arlyza et al. (2013a); N = 10 for clade *VIII* of Arlyza et al. (2013a); N = 1 for the Guadalcanal maskray; N = 2 for the Indian Ocean maskray; and N = 1 for the Ryukyu maskray. GenBank accession numbers for all the foregoing sequences are provided in Supplementary Table S1.

Average nucleotide divergences between pairs of sequences within a lineage and net nucleotide divergences between lineages were estimated according to the Tamura-3 parameter substitution model (Tamura, 1992), the most likely model as inferred from the Bayesian information criterion using MEGA6 (Tamura et al., 2013). Variable nucleotide sites were determined automatically using MEGA6. Diagnostic nucleotide sites at the *CO1* gene locus that distinguish the Guadalcanal maskray from all other lineages in the genus *Neotrygon* were then selected visually on the EXCEL (Microsoft Corporation, Redmond WA) file generated by MEGA6.

RESULTS AND DISCUSSION

Last et al. (2016) have claimed that the Guadalcanal maskray specimen they had in hands was "very similar in coloration and shape to Müller and Henle's Solomon Island types" but this statement was shown to be unwarranted (Borsa and Béarez, 2016). Pigmentation patterns on the dorsal side of each pectoral fin in the Guadalcanal maskray consisted of a variable number (N = 2-21) of small ocellated blue spots, a small number (N = 1-6) of medium-sized ocellated blue spots, and 3-7 dark speckles (Table 1). All three Guadalcanal maskray specimens available for the present study thus lacked the dark spots and the scapular blotch that are present in the Vanikoro maskray, i.e. *N. kuhlii* (Borsa and Béarez, 2016). Given the relevance of pigmentation patterns in diagnosing species in the genus *Neotrygon* (Last and White, 2008; Last et al., 2010; Borsa et al.,

2013a) and more generally in stingrays (Arlyza et al., 2013b; Borsa, 2017), this observation alone suffices to reject the hypothesis that the Guadalcanal maskray is synonymous with *N. kuhlii*. Other measurements, expressed as percentage of disc length (DL), also showed strong differences between the Guadalcanal maskray and the type material of *N. kuhlii* including the lectotype (MNHN-IC-0000-2440, smaller of two) and the paralectotype (MNHN-IC-0000-2440, larger of two). For instance, the distance from pectoral fin insertion to sting origin was substantially larger in the Guadalcanal maskray (5.4% DL) than in *N. kuhlii* (4.2% DL), as was the nostril length (5.0% DL *vs.* 3.4-3.9% DL). The inter-orbital width was substantially narrower (9.2% DL *vs.* 10.3-11.6% DL), as were the inter-ocular width (19.7% DL *vs.* 21.3-22.6% DL), the distance between first-gill slits (19.2% DL *vs.* 21.9% DL), and the distance between fifth-gill slits (9.8% DL *vs.* 11.1% DL).

The maximum-likelihood tree of *CO1* haplotypes (Fig. 2) confirmed the monophyly of species in the genus *Neotrygon*, except *N. picta* which was paraphyletic with *N. leylandi*. Also, no distinction was evident between haplotypes of *N. annotata* and those previously assigned to a related undescribed lineage provisionally referred to as "*Neotrygon* cf. *annotata*" (Puckridge et al., 2013). Estimates of nucleotide divergence at the *CO1* locus among species and deep lineages [i.e. cryptic species remaining undescribed; Borsa et al. (2016b)] in the genus *Neotrygon* ranged from 0.015 to 0.301 (Table 2). They ranged from 0.015 to 0.038 among the four already-described blue-spotted maskray species previously under *N. kuhlii*, i.e. *N. australiae*, *N. caeruleopunctata*, *N. orientale* and *N. varidens* (Table 2). Nucleotide divergence between the Guadalcanal maskray and other species in the genus *Neotrygon* was ≥ 0.049 (Table 2). Meanwhile, nucleotide divergence estimates within lineages ranged from 0 in *N. caeruleopunctata* to 0.011 in *N. orientale* and in clade *II* of Arlyza et al. (2013a) (Table 2), thus systematically lower than inter-specific estimates, and largely so. The single Guadalcanal maskray haplotype belonged to a lineage clearly distinct from all other *Neotrygon* spp. lineages sampled so far. At two sites at the *CO1* locus, it possessed nucleotides that were absent in *N. annotata*, *N. australiae*, *N. caeruleopunctata*, *N. leylandi*, *N. ningalooensis*, *N. orientale*, *N. picta*, *N. trigonoides*, *N. varidens*, and in six yet-undescribed blue-spotted maskray species sampled from the Indian Ocean, the western and northern costs of Sumatra, the Malacca strait, the Banda sea, the Ryukyu archipelago and West Papua (Arlyza et al., 2013a; Borsa et al., 2016a, 2016b) (Supplementary Table S1). Nucleotide sequences at the *CO1* locus therefore provided diagnostic characters for the Guadalcanal maskray, relative to all other species in the genus *Neotrygon*. The Guadalcanal maskray is here considered to represent a distinct species, based on its colour patterns, its distinct phylogenetic placement, its level of nucleotide distance with other species in the genus *Neotrygon*, and its unique nucleotide composition at the *CO1* locus. No name being available for the Guadalcanal maskray (Eschmeyer et al., 2016), it is here described as a new species.

TAXONOMY

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

Neotrygon vali sp. nov. http://zoobank.org/A5BE7B5D-64A3-40C2-AD44-63ECAE060FF6. Previously referred to as: Guadalcanal maskray (Borsa and Béarez, 2016; Borsa et al., 2016b; Borsa et al., in press); erroneously placed under *Neotrygon kuhlii* by Last et al. (2016).

*Holotype***.** Specimen CSIRO H 7723-01, a female 295 mm DW, is here designated as the holotype of *Neotrygon vali* sp. nov. This specimen was obtained on 7 May 2015 from the Plaza fish market, Honiara, Guadalcanal Island (Last et al., 2016). Based on the assumption that fishes sold at the local fish market in Honiara have

been captured along the shores of Guadalcanal Island, the type locality is Guadalcanal Island in the Solomon archipelago.

Description. The morphological description of the holotype of *Neotrygon vali* sp. nov. has been published previously (pp. 535-541 of Last et al., 2016). This includes 11 meristic counts and 40 measurements made on the body (table 1 of Last et al., 2016). In addition, pigmentation patterns on the dorsal side of disk consist of a variable number of small ocellated blue spots and a moderate number of medium-sized ocellated blue spots, few dark speckles and no scapular blotch. The *CO1* gene sequence of *Neotrygon vali* sp. nov. is unique among species in the genus *Neotrygon* as it clusters with no one of its homologues in congeneric species (Fig. 2). The partial *CO1* gene sequence of the holotype, comprised between homologous nucleotide sites nos. 95 and 696 of the *CO1* gene in *N. orientale* (GenBank no. JN184065; Aschliman et al., 2012) is 5'- C T G G C C T C A G T T T A C T T A T C C G A A C A G A A C T A A G C C A A C C A G G C G C T T T A C T G G G T G A T G A T C A G A T T T A T A A T G T A A T C G T T A C T G C C C A C G C C T T C G T A A T A A T C T T C T T T A T A G T A A T A C C A A T T A T A A T C G G T G G G T T T G G T A A C T G A C T A G T G C C C C T G A T G A T T G G A G C T C C G G A C A T A G C C T T T C C A C G A A T A A A C A A C A T A A G T T T C T G A C T T C T G C C T C C C T C C T T C C T A T T A C T G C T A G C C T C A G C A G G A G T A G A A G C C G G A G C C G G A A C A G G T T G A A C A G T T T A T C C T C C A T T A G C T G G T A A T C T A G C A C A T G C T G G A G C T T C T G T G G A C C T T A C A A T C T T C T C T C T T C A C C T A G C A G G T G T T T C C T C T A T T C T G G C A T C C A T C A A C T T T A T C A C A A C A A T T A T T A A T A T A A A A C C G C C T G C A A T C T C C C A A T A T C A A A C C C C A T T A T T C G T C T G A T C C A T C C T T G T T A C A A C T G T G C T T C T C C T G C T A T C C C T A C C A G T C C T A G C A G C T G G C A T T A C T A T A C T C C T C A C A G A C C G A A A T C T T A A T A C A A C T T T C T T T G A T C C A G C T G G A G G A G G A G A T C C T A T T C T T T A C -3' (Last et al., 2016).

Diagnosis. Based on Supplementary Table S1, *Neotrygon vali* sp. nov. is distinguished from all other species in the genus *Neotrygon* except *N. kuhlii* for which no genetic information is available yet, by the possession of nucleotide T at nucleotide site 420 and G at nucleotide site 522 of the *CO1* gene. In addition, the Guadalcanal maskray is distinct from *N. kuhlii* by the lack of dark spots (> 1% DW) and by the lack of a pair of scapular blotches on the dorsal side.

Distribution. Apart from the type locality (Honiara on the northern coast of Guadalcanal Island in the Solomon archipelago), the distribution of *Neotrygon vali* sp. nov. is likely to be confined within the part of Melanesia east of Cenderawasih Bay in West Papua, where the lineage present is *Neotrygon* clade *VIII* (Arlyza et al., 2013a) and west of the Santa Cruz archipelago, where the species present is *N. kuhlii*.

Etymology. "Vali" is the word for stingray in Gela, one of the languages spoken in Guadalcanal (Froese and Pauly, 2016). Epithet *vali* is intended to refer to the common name of the species among Guadalcanal fishers and it is a noun in apposition (Truper and De'Clari, 1997). Proposed vernacular names: Guadalcanal maskray (English); vali Guadalcanal (Gela); pastenague masquée à points bleus de Guadalcanal (French).

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CONFLICTS OF INTEREST

No one.

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Table 1. Pigmentation patterns on left or right dorsal side of disk in Guadalcanal maskray *Neotrygon vali* sp. nov. including numbers of ocellated blue spots, number of dark speckles or spots and presence or absence of a scapular blotch. Ocellated blue spots qualified as *small* when diameter ≤ 2% disk width (DW); *medium* when > 2% DW and ≤ 4% DW and *large* when > 4% DW; *dark speckles* ≤ 1% DW; *dark spots* > 1% DW (Borsa et al., 2013a). *N*: number of speckles or spots.

Specimen,	*N* ocellated spots			*N* dark	*N* dark	Scapular
Side of disk	Small	Medium	Large	speckles	spots	blotch
CSIRO H7723-01						
left	2	1	0	3	0	no
right	4	1	0	6	0	no
Randall (2005: 18)						
left	11	4	0	6	0	no
Fig. 1						
left	21	6	0	7	0	no

Table 2. *Neotrygon* spp. Estimates of net nucleotide divergence (Tamura-3 parameter model; MEGA6) between lineages. Clades *II*, *III*, *VII* and *VIII* were defined by Arlyza et al. (2013a). *N* sample size; *ns* number of base substitutions per site from averaging over all sequence pairs within each lineage (Tamura-3 parameter model; MEGA6).

No.	Lineage	*N*	*ns*	Lineage no.														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*N. annotata*	8	0.004															
2	*N. australiae*	11	0.006	0.268														
3	*N. caeruleopunctata*	12	0.000	0.278	0.028													
4	*N. leylandi*	7	0.002	0.243	0.167	0.179												
5	*N. ningalooensis*	1	-	0.229	0.271	0.267	0.201											
6	*N. orientale*	68	0.011	0.236	0.029	0.028	0.160	0.233										
7	*N. picta*	5	0.001	0.286	0.190	0.205	0.034	0.213	0.174									
8	*N. trigonoides*	18	0.003	0.250	0.047	0.044	0.178	0.212	0.036	0.174								
9	*N. vali* sp. nov.	1	-	0.301	0.054	0.049	0.192	0.235	0.053	0.193	0.054							
10	*N. varidens*	11	0.001	0.240	0.036	0.038	0.161	0.269	0.015	0.189	0.047	0.064						
11	Clade *II*	19	0.011	0.288	0.027	0.021	0.199	0.263	0.029	0.214	0.035	0.034	0.050					
12	Clade *III*	17	0.003	0.282	0.027	0.021	0.198	0.266	0.024	0.203	0.044	0.038	0.016	0.043				
13	Clade *VII*	14	0.008	0.262	0.028	0.027	0.154	0.220	0.028	0.194	0.039	0.039	0.027	0.027	0.050			
14	Clade *VIII*	10	0.002	0.249	0.028	0.022	0.150	0.251	0.034	0.175	0.037	0.044	0.025	0.027	0.025	0.056		
15	Indian O. maskray	2	0.002	0.271	0.031	0.026	0.169	0.245	0.031	0.201	0.052	0.043	0.024	0.025	0.018	0.020	0.049	
16	Ryukyu maskray	1	-	0.246	0.039	0.038	0.173	0.250	0.024	0.187	0.039	0.032	0.028	0.038	0.039	0.038	0.049	0.041

Figure 1. Guadalcanal maskray *Neotrygon vali* sp. nov. showing the pigmentation patterns that differentiate it from *N. kuhlii* from Vanikoro (Borsa and Béarez 2016). Photographed by M.A. Rosenstein near Mbike Wreck (09°06'S 160°11E), November 2014.

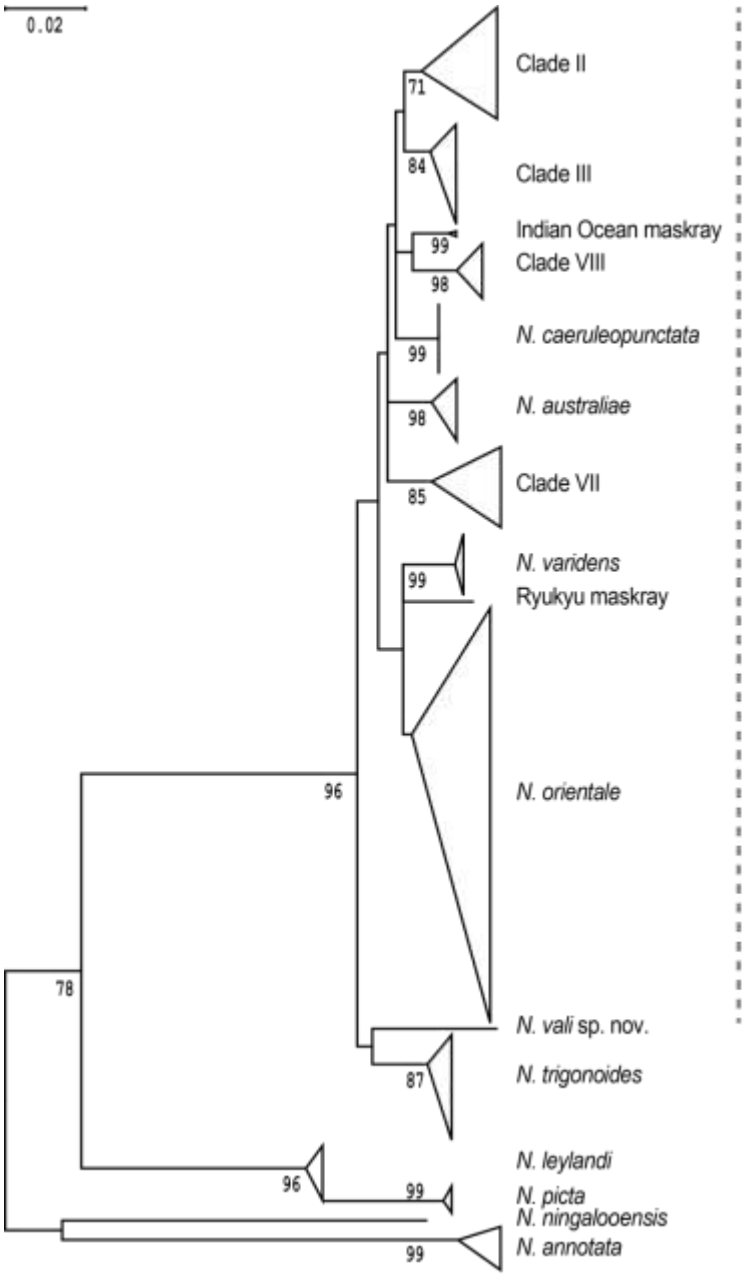

Figure 2. *Neotrygon* spp. Maximum-likelihood tree (Tamura 3-parameter model; MEGA6) of nucleotide sequences at the *CO1* locus (*N* = 205), compiled from several sources (Ward et al., 2008; Yagishita et al., 2009; Arlyza et al., 2013a; Borsa et al., 2013a; Puckridge et al., 2013; Last et al., 2016; Aschliman et al., 2012) showing the phylogenetic placement of the Guadalcanal maskray *Neotrygon vali* sp. nov. Numbers at nodes are bootstrap scores (500 bootstrap resampling runs under MEGA6). Dotted vertical line: blue-spotted maskrays previously under *N. kuhlii* (Borsa et al., 2016b).

Appendices

Supplementary Table S1. Variable nucleotide sites at the *CO1* locus that distinguish *Neotrygon vali* sp. nov. from congeneric species.

Supplementary Table S2. Reviews received by this manuscript, from four peer-reviewed journals to which it has been successively submitted, and each time rejected.

Supplementary Table S1. Variable nucleotide sites at the *CO1* locus that distinguish *Neotrygon vali* sp. nov. from congeneric species. Nucleotides diagnostic of *N. vali* sp. nov. are highlighted in blue. 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.

| Species, GenBank no. | Nucleotide site no. | | |

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JX304891   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C . G . . . C C . . . . A A . G G . . T G C A . . . . . C . . . A
KC249903   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C . G . . . C . . . . . A A . G . . . T G C A . . . . . C . . . A
KC249904   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C . G . . . C . A . . T A A . G G . . T G C A . . . . . C . . . A
KC249905   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C . G . . . C . A . . T A A . G G . . T G C A . . . . . C . . . A
N. picta
DQ108172   T . . . . . C . . . A T T . . . T . . T . . T T . . . . . G . . G . C C A . . . A . . . . . A . . C A . . . . . G
DQ108173   T . . . . . C . . . A T T . . . T . . T . . T T . . . . . G . . G . C C A . . . A . . . . . A . . C A . . . . . G
DQ108174   T . . . . . C . . . A T T . . . T . . T . . T T . . . . . G . . G . C C A . . . A . . . . . A . . C A . . . . . G
DQ108175   T . . . . . C . . . A T T . . . T . . T . . T T . . . . . G . . G . C C A . . . A . . . . . A . . C A . . . . . G
DQ108185   T . . . . . C . . . A T T . . . T . . T . . T T . . . . . G . . G . C C A . . . A . . . . . A . . C A . . . . . G
N. trigonoides
GU673434   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . T . C C . . . A
HM902465   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902466   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902467   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902478   T . . . . . C . . . C T T . G C T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902479   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902480   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902482   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902483   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902484   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
HM902485   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
JQ765533   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
JQ765534   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
JQ765535   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
JX263420   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C C . . . A
JX304916   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C C . . . A
JX304917   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C C . . . A
KC250643   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . C . . . . . A A . . G G A T G C A . . . . . C . . . A
N. vali sp. nov.
XX000000   T . . . . . C . . . C T T . G . T G . T . . . T C . . . C . . . . . C . . . . . A A . G G G A T G C A . . . . . C C . . C G
N. varidens
EU398733   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
EU398734   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
EU398735   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
JQ681494   T . . . . . C . . . C T T . G . T . . T G . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
JQ765561   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
JQ765562   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
JX263422   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
JX304846   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
JX304868   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
KC249902   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
KC250640   T . . . . . C . . . C T T . G . T . . T . . . T C T . . C . G . . . C . . . . . A A . G G . . T G C A . . . . . C . . . A
Arlyza et al.'s (2013) clade II
JX304798   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G C . . . . . C . A A . . G . . T G C A . . . . . C C . . . A
JX304799   T . . . . . C . . . C T T . G . T G . T . . . . C . . . C G G . . . . . C . A . . G . . . T G C A . . . . . C C . . . A
JX304800   T . . . . . C . . . C T T . G . T G . T . . . . C . A . C G G . . . . . C . A . . G . . . T G C A C . . . . C C . . . A
JX304801   T . . . . . C . . . C T T . G . T T . T . . . . C . . . C G G . . . . . C . A . . G . . . T G C A C . . . . C C . . . A
JX304802   T . . . . . C . . . . T T . G . T . . T . . . . C . . . C G G . . . . . C . A . . G G . . T G C A . . . . . C C . . . A
JX304803   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G C . . . . . C . A A . . G . . T G C A . . . . . C C . . . A
JX304804   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . . . C . A . . G G . . T G C A . . . . . C C . . . A
JX304805   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . . . C . A . . G G . . T G C A . . . . . C C . . . A
JX304806   T . . . . . C . . . C T T . G . T G . T . . . . C T . . C G G . . . . . C . A A . G G . . T G C A . . . . . C C . . . A
JX304807   T . . . . . C . . . C T T . G . T G . T . . . . C . . . C G G . . . . . C . A A . G G . . T G C A . . . . . C C . . . A
JX304808   T . . . . . C . . . C T T . G . T . . T . . . . C . . . C G G . . . . . C . A A . G G . . T G C A . . . . . C C . . . A
JX304809   T . . . . . C . . . C T T . G . T G . T . . . . C . . . C G G . . . . . C . A A . G G . . T G C A . . . . . C C . . . A
JX304810   T . . . . . C . . . C T T . G . T G . T . . . . C . . . C G G . . . . . C . A A . G G . . T G C A . . . . . C C . . . A
JX304811   T . . . . . C . . . C T T . G . T G . T . . . . C . . . C G G . . . . . C . A A . G G . . T G C A . . . . . C C . . . A
JX304812   T . . . . . C . . . C T T . G . T G . T . . . . C . . . C G G . . . . . C . A A . G G . . T G C A . . . . . C C . . . A
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Table S1. (continued)

| Species, GenBank no. | Nucleotide site no. | | |

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Table S1. (continued)

Species, GenBank no.	Nucleotide site no.																											
	516	522	525	531	537	540	549	550	552	558	561	564	567	573	579	582	588	589	591	592	597	598	600	606	607	610	615	618	624
N. annotata																													
EU398727	A	A	A	T	A	T	C	T	A	T	A	C	T	C	C	A	T	T	A	T	C	C	A	T	T	G	T	C	T
EU398728	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EU398729	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EU398730	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EU398731	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KC250622	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
KC250623	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
KC250628	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
N. australiae																													
DQ108184	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JQ765536	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JQ765537	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304874	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304875	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250626	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250627	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250632	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250635	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250642	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250645	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
N. caeruleopunctata																													
EU398736	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
EU398742	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
EU398743	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
EU398744	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
EU398745	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
EF609342	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304860	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250629	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250630	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250634	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250637	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC250639	.	.	T	C	G	.	A	.	.	C	.	T	C	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
N. leylandi																													
EU398746	G	.	C	.	G	.	A	C	.	.	.	T	C	.	T	.	C	.	G	C	.	.	.	C	C	.	C	.	.
EU398747	G	.	C	.	G	.	A	C	.	.	.	T	C	.	T	.	C	.	G	C	.	.	.	C	C	.	C	.	.
EU398748	G	.	C	.	G	.	A	C	.	.	.	T	C	.	T	.	C	.	.	C	.	.	.	C	C	.	C	.	.
EU398749	G	.	C	.	G	.	A	C	.	.	.	T	C	.	T	.	C	.	G	C	.	.	.	C	C	.	C	.	.
EU398750	G	.	C	.	G	.	A	C	.	.	.	T	C	.	T	.	C	.	G	C	.	.	.	C	C	.	C	.	.
EU398751	G	.	C	.	G	.	A	C	.	.	.	T	C	.	T	.	C	.	G	C	.	.	.	C	C	.	C	.	.
JQ765538	G	.	C	.	G	.	A	C	.	.	.	T	C	.	T	.	C	.	G	C	.	.	.	C	C	.	C	.	.
N. ningalooensis																													
JQ765539	.	.	C	C	.	C	A	C	.	C	.	.	.	T	T	C	C	.	.	.	T	T	.	.	C	.	.	A	C
N. orientale																													
EU398737	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
EU398738	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
EU398739	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
EU398740	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
EU398741	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.

GU673709	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JN184065	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304829	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304830	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304831	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304832	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304833	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304834	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304835	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304836	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304837	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304838	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304839	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	.	.	.	.
JX304840	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304841	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304842	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304843	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	.	.	.	.
JX304844	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304845	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304847	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304848	.	.	C	C	.	.	A	C	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304849	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304850	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304851	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304852	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304853	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304854	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304855	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304856	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304857	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304858	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304859	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304861	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304862	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304863	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304864	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304865	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304866	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.
JX304867	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304869	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304870	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304871	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304872	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304873	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304876	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304877	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304878	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304879	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304880	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304881	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304882	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304883	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304884	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304885	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304886	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304887	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304888	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304889	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.
JX304890	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.

JX304891	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC249903	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
KC249904	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
KC249905	.	.	C	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
N. picta																													
DQ108172	G	.	C	.	.	.	.	C	.	.	G	T	C	T	T	.	C	.	G	C	A	.	.	C	C	.	C	.	.
DQ108173	G	.	C	.	.	.	.	C	.	.	G	T	C	T	T	.	C	.	G	C	A	.	.	C	C	.	C	.	.
DQ108174	G	.	C	.	.	.	.	C	.	.	G	T	C	T	T	.	C	.	G	C	A	.	.	C	C	.	C	.	.
DQ108175	G	.	C	.	.	.	.	C	.	.	G	T	C	T	T	.	C	.	G	C	A	.	.	C	C	.	C	.	.
DQ108185	G	.	C	.	.	.	.	C	.	.	G	T	C	T	T	.	C	.	G	C	A	.	.	C	C	.	C	.	.
N. trigonoides																													
GU673434	.	.	T	C	G	.	A	.	.	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902465	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902466	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902467	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902478	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902479	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902480	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902482	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902483	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902484	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
HM902485	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
JQ765533	.	.	T	C	G	.	A	.	.	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
JQ765534	.	.	T	C	G	.	A	.	G	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
JQ765535	.	.	T	C	G	.	A	.	.	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
JX263420	.	.	T	C	G	.	A	.	.	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
JX304916	.	.	T	C	G	.	A	.	.	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
JX304917	.	.	T	C	G	.	A	.	.	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
KC250643	.	.	T	C	G	.	A	.	.	C	.	.	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	C
N. vali sp. nov.																													
XX000000	.	G	T	C	.	.	A	.	.	C	.	.	C	T	T	G	C	C	G	C	.	.	.	C	C	.	.	.	.
N. varidens																													
EU398733	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
EU398734	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
EU398735	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
JQ681494	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
JQ765561	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
JQ765562	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
JX263422	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
JX304846	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
JX304868	.	.	T	C	G	.	A	.	.	.	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
KC249902	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
KC250640	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	.	.	.	.	C	C	.	.	.	.
Arlyza et al.'s (2013) clade II																													
JX304798	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304799	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	G	C	C	G	C	.	.	.	C	C	A	.	.	C
JX304800	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	G	C	C	G	C	.	.	.	C	C	.	.	.	C
JX304801	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	G	C	C	G	C	.	.	.	C	C	.	.	.	C
JX304802	.	.	T	C	G	.	A	C	.	C	.	T	.	T	T	G	C	C	G	C	.	.	.	C	C	.	.	.	C
JX304803	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304804	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	G	C	C	G	C	T	.	.	C	C	.	.	.	C
JX304805	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	G	C	C	G	C	T	.	.	C	C	.	.	.	C
JX304806	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304807	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304808	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304809	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304810	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304811	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304812	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.

JX304813	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304814	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304815	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304828	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
Arlyza et al.'s (2013) clade III																													
GU673423	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
GU673425	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
GU673426	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
GU673427	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
GU673428	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304816	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304817	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304818	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304819	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304820	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304821	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304822	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304823	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304824	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304825	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304826	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304827	.	.	T	C	.	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
Arlyza et al.'s (2013) clade VII																													
JX304892	.	.	T	C	G	.	A	.	.	.	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304893	.	.	T	C	G	.	A	.	.	.	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304894	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304895	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304896	.	.	T	C	G	.	A	.	.	.	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304897	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304898	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304899	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304900	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304901	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304902	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304903	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304904	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304905	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
Arlyza et al.'s (2013) clade VIII																													
JX304906	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304907	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304908	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304909	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304910	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304911	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304912	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304913	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304914	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
JX304915	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	C	.	.	.	.
Indian Ocean maskray																													
JX263421	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	.	.	.	.	.
KC249906	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	.	C	.	.	.	.	.
Ryukyu maskray																													
AB485685	.	.	T	C	G	.	A	.	.	C	.	T	.	T	T	.	C	C	G	C	.	.	G	C	C	.	.	.	.

Supplementary Table S2. Reviews received by this manuscript, from four peer-reviewed journals to which it has been successively submitted, and each time rejected

Journal	Responsible editor	Time elapsed from submission to decision	Number of reviews	Review	Editor's decision and eventual comment
Biochemical Systematics and Ecology	Monique SJ Simmonds	42 days	1	"The CO1 sequence of this new species should be ok but not for author's analytic method. Based on my understanding, CO1 has no many phylogenetic information site. Thus, CO1 sequence only appropriate for using Neighbor-Joining tree (NJ) method,not Maximum Likelihood tree (ML) method. It is strange that in text, he used ML to construct tree but use Tamura 3-parameter model. This is in contradictory. My suggestions are: 1. Using Tamura 2-parameter model (not 3-parameter model) to construct NJ tree and using outgroup of other genus. 2. If author still like to use ML method, then he should use modeltest to find the best model (GTR, HKY,…) after including outgroup. Then, use PAUP, RaxML or PhyML to construct tree, On taxonomy part, this paper has not followed traditional format to describe new species either. The diagnostic morphological character only use pigmentation pattern seems insufficient to pursue other taxonomists. The author need to have more data and further examination and find more external or internal differences and make character comparison table with their congeneric species."	"Reviewers' comments on your work have now been received. You will see that they are advising against publication of your work. Therefore I must reject it."
Turkish Journal of Zoology	Nusret Ayyildiz	189 days	1	"i can?t understanding why the holotype of Neotrygon vali sp. nov. has been provided by Last et al., (2016: 535-541), and now described again as a new species? (pg.7, line 140-141). Please do have a comparative materials (Neotrygon kuhlii, N. annotata, N. leylandi, N. picts, N. ningalooensis, N. caeruleopunctata, N. australiae, N. varidens, N. orientale, N. trigonoides) and the body size, because the spots may available changes with the size of body or in female and male. Pg.3, line 31: Arlyza et al., 2013 are not in the reference list; Pg.7, line 141, Description: what was the 11 meristic counts? the citations (p.536 Laste et al. 2016) are not in the reference list? Pg.9, line 174, How can the distribution of Neotrygon vali sp. nov. is unknown? Some reference citations are not in the text: Pg.12, line 237-239: Cerutti.....e36479; Pg.12, line 243-244: Frose.....March 2016) Please list the key of the genus of Neotrygon."	"Your manuscript has been reviewed, and we regret to inform you that it has not been found suitable for publication in our journal. […]". "It is not acceptable to describe a new species on a genetic sequence only."
DNA Barcodes	Andrew Mitchell	190 days	2	Reviewer 1 "In the present paper author describe a new species of maskray Genus Neotrygon from the Solomon archipelago, on the basis of its nucleotide sequence at the cytochrome oxidase 1 (CO1) gene locus. The description is based on three specimens: a specimen from CSIRO Collection (CSIRO H7723-01), and two live specimens photographed underwater. Major inconveniences: - There is no formal description of the species, at least in the way species are described since last century. Apart from some weak data regarding colour pattern, no information regarding the morphology of this new species is showed. According to the author "The morphological description of the holotype of Neotrygon vali sp. nov. has been published previously (pp. 535-541 of [9]). This includes 11 meristic counts and 41 measurements made on the body (table 1 of [9])". Is the first time I find this kind of description of new species, using the information provided by other authors and without making his observations and measurements. The other type specimens are solely two photographs!...from which author describe the colour pattern. Some morphometric were taken from these photographs. - Author have made a molecular analysis based on DNA barcoding (COI). The molecular data (sequences) were obtained from the available literature (and Genbank). The methodology applied is correct. However, author stated "Based on Supplementary Table S1, Neotrygon vali sp. nov. is distinguished from all other species in the genus Neotrygon except N. kuhlii for which no genetic information is available yet, by the possession of nucleotide T at nucleotide site 420 and G at nucleotide site 522 of the CO1 gene". The COI sequences used by the author to characterize the holotype of the new species is that of specimen (CSIRO H7723-01). A specimen identified as N. kuhlii by Last et al 2016. I understand that author consider that these specimen is different from all other specimens included by Last et al 2016 into N. kuhlii, but no evidence of genetic divergence between both supposed entities are showed by the author in the present manuscript. - Describing species is an urgent task also in this century, and nowadays description should be based on both morphological and molecular approaches. The present manuscript suggest that some specimens included within N. kuhlii by other authors, should be a different species. However as described previously, the description is rather poor, based on a specimen apparently not observed by the author and two photos. If he consider is a different species, he should revise carefully the specimens included within N. kuhlii, making strong morphological comparisons	[no comment from editor]

				(and not using a table made by other scientist), taking his own data. Besides, he should collect new specimens including some "N. kuhlii like", in order to asses also the molecular differences. On the 1800 scientist could describe a new species based on one specimen, and the description could be a sentences (there are several cases). Nowadays, the description of a new species should be based on several individuals, in order to evaluate in some way, the intraspecific variability. - In conclusion I consider that the manuscript is not adequate for the description of a new species, therefore I suggest reject it." Reviewer 2 "The manuscript deals with the description a new species of maskray, Neotrygon vali based on genetic differences (position of nucleotide T at nucleotide site 420 and G at nucleotide site 522 of the CO1 gene). The author based his results in one female non-type specimen (CSIRO H 7723-01) published in a recent paper on the taxonomic status of maskrays of the Neotrygon kuhlii species complex by Last et al. (2016). From my view point the ms sent by Borsa fails in many aspects. Firstly, it is not a formal description of a new species since he does not present a Table with morphological features, morphometrics and meristics of the material examined. He just only used the data from the female specimen collected by Last et al. in their paper published in Zootaxa and also used a good photograph (CSIRO H 7723-01) from which he obtained some measurements (e.g., disc width and colour pattern) and he said that"the morphological description of the holotype of Neotrygon vali sp. nov. has been published previously (pp. 535-541". This includes 11 meristic counts and 41 measurements made on the body (table 1 of Last et al. paper. But, the author of this ms did not take any data by himself. I wonder if he had the specimen in his hands in orde to tahe the same or other data that Last et al. have taken. In addition, in his ms there is neither a diagnosis nor a description of the new species. Moreover, all the information (colour pattern, and some measurements) has been obtained from the published paper on Zootaxa regarding the complex species of N. kuhlii. At least, the author could have said that the specimen from Guadalcanal has genetic differences from other Neotrygon species, but further information is necessary to surely confirmed that N. kuhlii from Vanikoro is genetically distinct from the new proposed species (the author affirmed that specimens from the type locality of Vanikoro have not yet been analyzed genetically), regardless the pigmentation patterns used to distinguish it from the Guadalcanal maskray."	
Journal of Asia-Pacific Biodiversity	Bong-Kyu Byun	34 days	1	"This paper is the worst piece of science I have ever witnessed. In no possible way could this paper be considered anything but a reject and it is a disgrace that it was submitted to a journal with the authors knowledge that it has already been made available online back in February this year. Where to begin - 1) this paper has already essentially been published and the name has been entered into the Catalog of Fishes. Borsa, P. 2017 Neotrygon vali (Myliobatoidei: Dasyatidae), a new blue-spotted maskray from the Solomon archipelago described from its DNA barcode. bioRxiv: 1-14 + supplementary table S1. While this is not peer-reviewed, edited, or typeset before being posted online, it does unfortunately meet the ICZN requirements and therefore it is an available name according to Cat of Fishes. 2) The holotype is a CSIRO collected specimen, yet the author has never seen this specimen himself or requested to see this specimen at all. This is imperative. Although he refers to a photograph of this specimen in someone else's (Last et als) paper, he has not verified that this is correct, or the image matches the specimen, or that the specimen is still intact, etc. Standard collection procedures. 3) The description (well there is no description) is pathetic. There are no useful characters presented in the paper to warrant description of a new species. The author mentions colour, but based on one specimen this is next to useless as does not account for any variation whatsoever. Describing a new ray species based on a single specimen is lazy. Members of this genus are common where they occur, so why did the author not go and collect more specimens? 4) Genetics - I would have thought from the authors previous papers that he had a gross understanding of DNA barcoding but it is obvious from this paper that he has limited knowledge of genetics in general. To suggest that 2 base pair differences warrant a new species based on a single specimen is probably the most ludicrous claim I have read in a paper ever. Single genetic marker approaches are dangerous to rely on unless there is sufficient morphological data to back it up. In this case, a single specimen does not make this possible. There are instances where CO1 sequences can vary up to 5% within a species, with other mDNA markers showing 0% divergence! Thus, the genetic findings of this paper equate to nothing and cannot be used as a distinguishing character. 5) Validity of species - extremely questionable yet I will concede, it is impossible to judge anything from this paper to be able to ascertain either way. What can be said is that there is almost zero evidence currently presented in this paper to confirm this species is separate to N. kuhlii. Unfortunately, there is not a single positive thing to say about this paper. I find it quite depressing that this is the quality of science we are beginning to see creep into mainstream science and in taxonomy it is particularly unfortunate given that this is already an available name based on a non peer-reviewed article the author posted online in February."	"I regret to inform you that the reviewers of your manuscript have advised against publication, and I must therefore reject it."