SC/67A/SM/23

Confirmed occurrence of the Australian snubfin dolphin, Orcaella heinsohni in Papua New Guinea

Isabel Beasley, Maria Jedensjo, Jim Anamiato

INTERNATIONAL
WHALING COMMISSION

Confirmed Occurrence of the Australian snubfin dolphin, *Orcaella heinsohni* in Papua New Guinea

Isabel Beasley[1], Maria Jedensjö[2], and Jim Anamiato[3]

[1]. *Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville, Australia*

[2]. *South Australian Museum, Adelaide, South Australia, 0500, Australia and Australia and Evolutionary Genetics Group, Anthropology, University of Zurich, Switzerland*

[3]. *National Museum and Art Gallery, Port Moresby, Papua New Guinea*

Abstract

Genetic analyses were conducted for the first time on *Orcaella* sp. from Papua New Guinea waters, and new samples from northern Australia, including the type specimen of the Australian snubfin dolphin *Orcaella heinsoni*. Nineteen *Orcaella* samples were included in this study; eleven samples from Papua New Guinea and eight new *Orcaella heinsohni* samples from northern Australia. The mtDNA dataset was analyzed using Neighbor-Joining, Maximum Likelihood, and Bayesian Inference clustering algorithms to infer phylogenetic relationships. Phylogenetic reconstruction showed that the Papua New Guinea samples clustered with *O. heinsohni*, thereby confirming the species occurrence in southern Papua New Guinea. There are no confirmed records of *Orcaella sp.* from other regions of the Pacific Islands or New Guinea. The demarcation between *O. heinsohni* and *O. brevirostris* therefore remains unknown, although is located within the Wallacea region. The viability of the small, apparently isolated southern Papua New Guinea *O. heinsohni* population is uncertain. The most significant threat to inshore dolphins in southern Papua New Guinea appears to be accidental bycatch in subsistence fisheries, and anecdotal reports of direct catch. Conservation strategies that focus on maintaining corridors to preserve gene flow and prevent further population fragmentation and loss of genetic diversity will be an essential priority to assist with conserving this small, apparently remnant population.

Introduction

Understanding the genetic structure of populations is important for the assessment of conservation status and effective management of a species, particularly species that appear widespread but actually occur in fragmented, genetically isolated subpopulations (Hamner et al. 2012). The Irrawaddy dolphin, *Orcaella brevirostris*, and Australian snubfin dolphin (hereafter snubfin dolphin), *Orcaella heinsonhi* are two species recognized within the genus *Orcaella*. Both have a relatively widespread distributions but little is known about the genetic status of populations, or gene flow between regions.

The Irrawaddy dolphin was first described from the Ayeyarwady River of Myanmar (Owen 1866) and is known to occur along the Sunda Shelf of southeast Asia from the northwest Bay of Bengal southeast to east Kalimantan, Indonesia (Beasley et al. 2005). There have been few studies to estimate population size, where numbers have ranged from 48-55 in the Mahakam River population (Kreb 2004), to 5,400 in the Bay of Bengal, Bangladesh (Smith et al. 2008). The Irrawaddy dolphin is listed as '*Vulnerable*' by the IUCN Red List (Reeves et al. 2008a), although subpopulations in the Ayeyarwady, Mahakam and Mekong Rivers, Malampaya Sound and Songkhla Lake are listed as '*Critically Endangered'* (Reeves et al. 2008a).

Previously considered to be the Irrawaddy dolphin, the snubfin dolphin was described in 2005 (Beasley et al. 2005) and is known to occur in coastal waters of northern Australia from the Fitzroy River, Queensland on the east coast to Roebuck Bay, Western Australia on the west coast. The few studies that have been conducted on the snubfin dolphin have found that nearly all subpopulations are small, numbering no more than 100-150 individuals (Brown et al. 2014; Cagnazzi et al. 2013; Parra et al. 2006). Genetic analysis conducted across two nearby populations (Roebuck Bay and Cygnet Bay ~ 250km apart) in Western Australia and three populations in Central Queensland (Keppel Bay, Whitsundays ~ 400 km and Cleveland Bay ~ 250km from the Whitsundays) suggest that, at least at the extremity of their range, snubfin dolphins exist as a metapopulation of small and relatively isolated subpopulations with limited gene flow (Brown et al. 2014; DoE 2013). In Western Australia, contemporary migration rates revealed an estimated proportion of 0.04 (95% CI 0.01– 0.10) of snubfin dolphins in Cygnet Bay derived from Roebuck Bay and 0.03 (0.00–0.08) of Roebuck Bay individuals derived from Cygnet Bay (Brown et al. 2014). There is subsequently increased concern about the long-term viability of the snubfin dolphin, which is listed as '*Near Threatened'* by the IUCN Red List (Reeves et al. 2008b).

It has been proposed that the snubfin dolphin occurs along the Sahul Shelf of northern Australia and New Guinea (Beasley et al. 2005), however, until recently, no direct observations with photographs, genetic samples or skeletal specimens have been available for comparison from New Guinea or the Pacific Islands, despite numerous cetacean studies throughout this region (Beasley et al. 2015b). One skull reportedly from Daru, southwest Papua New Guinea (PNG) was described by Beasley et al. (2002), however, it was possible that this skull could have originated from northern Australia. Based solely on interview data, snubfin dolphins' were reported from Malaita, Isabel, Western and Choiseul Provinces of the Solomon Islands, however, no further confirmation details were provided and these records remain unconfirmed (Bass 2010). Dawbin (1972) reported anecdotal observations of cetaceans in PNG waters, where he described the Irrawaddy dolphin as likely to be recorded in the future based on records in neighbouring waters (i.e. northern Australia and Borneo) and apparent bycatch in the Gulf Prawn Fishery; although no further details were provided about this bycatch. Previous to this study, throughout New Guinea and the Pacific Islands the only confirmed records of *Orcaella* sp. were from the Kikori Delta of southern PNG, where six groups consisting of 14 individuals were sighted on 15 and 17 December 1999 (Bonaccorso et al. 2000).

In this study, genetic analyses were conducted for the first time on *Orcaella* sp. from PNG waters, and new samples from Queensland and northern Australia, including the type specimen of *O. heinsoni*, to assist towards confirming which species of *Orcaella* occurs in the New Guinea/Pacific Islands region.

Materials and Methods

Study Site and sample collection

This study focuses on investigating *Orcaella* sp. genetic status in the waters of the Kikori Delta of southern PNG. PNG is located on the eastern portion of New Guinea and considered to be part of the Pacific Islands. Papua and West Papua are Provinces of Indonesia, and located on the western side of New Guinea (Figure 1). Nineteen *Orcaella* samples were included in this study (Figure 1). Eleven genetic bone samples and three tissue samples were collected from *Orcaella* sp. carcasses opportunistically discovered during recent field surveys in the Kikori Delta of PNG (Beasley et al. 2014; Beasley et al. 2015a), along with eight new *Orcaella* sp. samples; four from Queensland and four from the northern Territory, Australia, including the type specimen (*Orcaella heinsohni* Beasley 2005) and a syntype. The mtDNA sequences generated from these samples were compared to 43 *Orcaella* sp. sequences from GenBank (Figure 1, Appendix 1), based on previous studies that included *Orcaella* samples (Beasley et al. 2005; Brown et al. 2014; Caballero et al. 2008; Jayasankar et al. 2011; Palmer et al. 2011; Vilstrup et al. 2011).

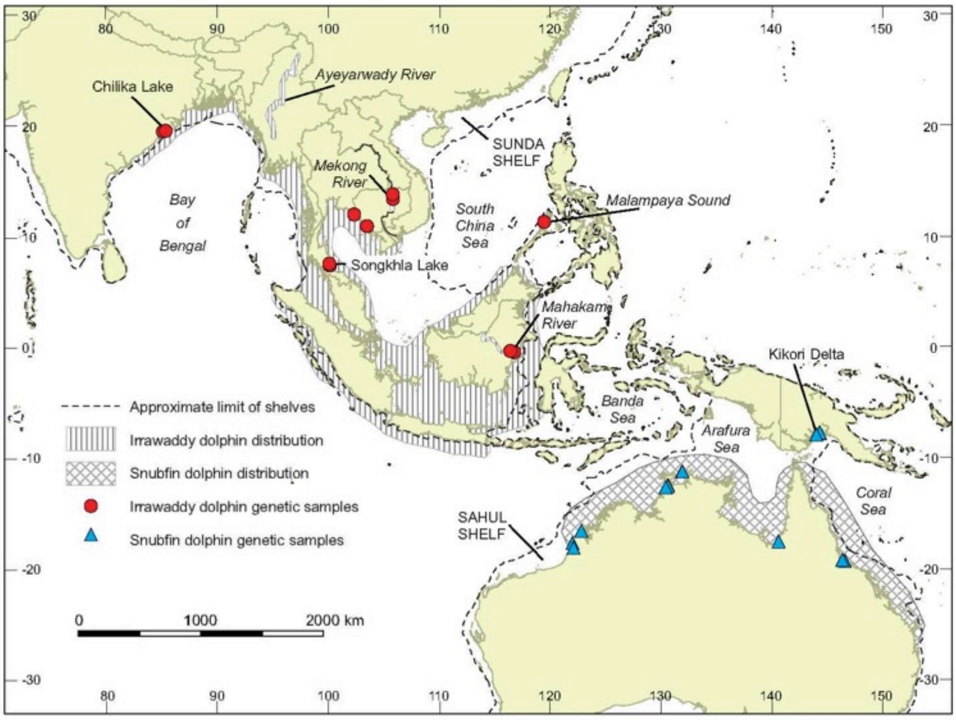

Figure 1. Distribution of Irrawaddy and Australian snubfin dolphin samples analysed in this study, where black circles = Irrawaddy dolphin samples and black triangles = Australian snubfin dolphin samples (see Appendix 1 for complete sample list). The distribution of each species is adapted, with permission, from the IUCN Red List species distributions.

Genetic analyses

Genomic DNA was extracted and PCA amplified from bone (Boessenkool et al. 2009) and tissue samples (Austin et al. 2013). Amplification of approximately 500-base pairs of the mtDNA control region was amplified using the polymerase chain reaction (PCR) and primers dlp1.5 (5′-TCA CCC AAA GCT GRA RTT CTA-3′) and dlp5R (5′-CCA TCG WGA TGT CTT ATT TAA GRG GAA-3) (Baker 2009). DNA sequencing was performed by the Australian Genome Research Facility. Sequence chromatograms were edited and assembled using Geneious 8.1.5 (Biomatters).

The mtDNA dataset was analysed using Neighbour-Joining (NJ), Maximum likelihood (ML) (MEGA 5) and Bayesian Inference (BI) (Mr Bayes) clustering algorithms to infer phylogenetic relationships. For BI, Monte Carlo Markov Chain (MCMC) was run over 10,000 000 iterations with a sampling frequency of 500 and run over two replicates. All other parameters were set to default in Mr Bayes. For the NJ and ML trees, 1,000 bootstrap replications were used. *Orcinus orca* (EU714123) was used as outgroup for all analyses. Sequences were downloaded from Genebank for a comparison with *Orcaella* sp. from other localities.

Mutational distance between haplotypes and their frequencies were shown by generating a median-joining network, using Network v4.6 and Network Publisher v1.2.0; http://www.fluxus-engineering.com (Bandelt et al. 1999).

Results

A 489-base sequence of the mtDNA control region was obtained from the new *Orcaella* sp. samples. When aligned with other *Orcaella* sp. haplotypes from other localities, a 346-base sequence was comparable. Thirty-five diagnostic fixed-base pair differences were found between the sequences compared.

Twenty-five different haplotypes were found. Four haplotypes could be found in PNG (H1–H4, Table 1). Haplotype 1 was unique for PNG while the other three were identical to Australian haplotypes. Fifteen haplotypes could be found in Australia (H2–H15), where 12 of them were unique for Australia. Two haplotypes could be found in Indonesia (H16–H17), three in Thailand (H18–H19), three in Cambodia (H21–H23), and two in India (H24–H25). None of the haplotypes were shared between PNG/Australian, Indonesia, Thailand, Cambodia and India. The generated mtDNA phylogenetic network shows two geographical clusters (Figure 3), reflecting structuring between the two *Orcaella* spp. species and shared haplotypes between PNG and Australia.

Phylogenetic reconstruction by NJ, ML and BI showed that the PNG samples clustered with *O. heinsohni* (Figure 2), while *O. brevirostris* samples from Cambodia, Philippines, Thailand, Indonesia and India clustered together. All three methods showed similar topologies with high node support between species.

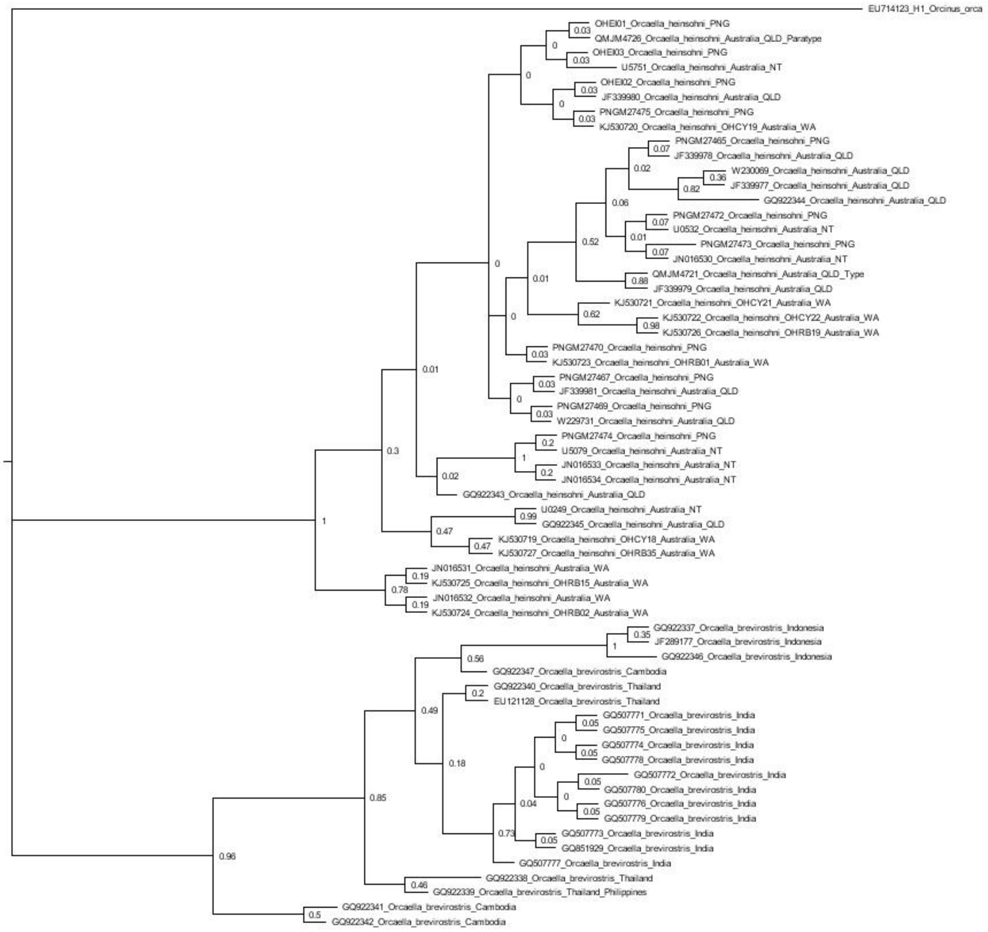

Figure 2. Bayesian phylogenetic tree based on sequences of 346 bp of the mtDNA control region of *Orcaella* sp. samples from PNG, Australia, Indonesia, Thailand, Philippines, Cambodia and India. Bootstrap support is shown at each branch. The top clade representing *Orcaella* sp. from PNG and Australia, and the bottom clade *Orcaella* sp. from Indonesia, Thailand, Philippines, Cambodia and India.

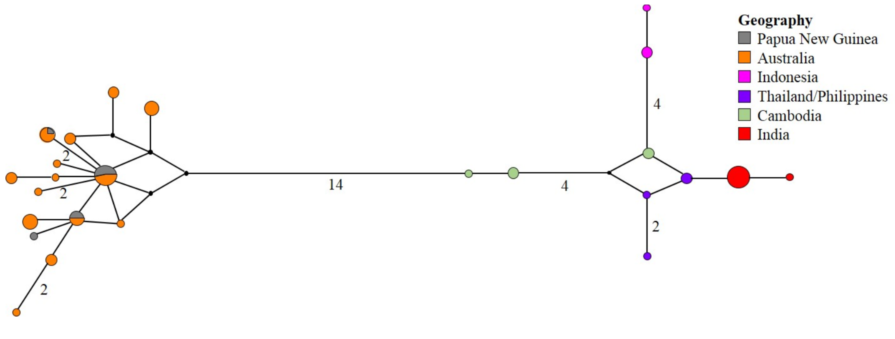

Figure 3. Median joining network of *Orcaella* spp. mtDNA HVRI haplotypes. Each different haplotype, shown as a circle, represents the haplotype frequency. The colors represent the geographical location, Papua New Guinea = grey, Australia = orange, Indonesia = pink, Thailand/Philippines = purple, Cambodia = green and India = red. Mutational steps are one unless indicated by the numbers.

Discussion

Confirmed range extension of Australian snubfin dolphins to Papua New Guinea waters

This study has confirmed that *O. heinsohni* occurs in the Kikori Delta of PNG, rather than the Irrawaddy dolphin previously recorded by Bonaccorso et al. (2000). We found identical haplotypes in PNG to the Northern Territory (NT), Western Australia (WA) and Queensland (QLD) in Australia. This confirmation extends the known range of the snubfin dolphin to include the southern Gulf region of Papua New Guinea. Despite this range extension, the taxonomic relationship of *O. heinsohni* in PNG to those in northern Australia remains unknown. The Kikori Delta is situated only 400km from the tip of Cape York, mainland northern Australia. However, it is assumed that the deep-water straits of the Torres Straits may prevent significant movement of *O. heinsoni* between PNG and northern Australia. No *Orcaella* or Australian humpback dolphins have yet been recorded from the Torres Strait region, despite numerous research projects on turtles and dugongs in the straits (Limpus et al. 1992; Marsh et al. 2004; Sheppard et al. 2006). The nearest known *O. heinsohni* sightings have been from the Port Musgrave/Weipa region of western Cape York, northern Australia (GHD 2015), approximately 700km southwest of Kikori.

The recent rediscovery of the river sharks, *Glyphis garricki* and *Glephis glyphis* in the Western province of Papua New Guinea resulted in similar findings to this study. Although these shark species have not been recorded to occur in Torres Strait, genetic analysis confirmed the two species clustered well within samples from these species collected in northern Australia (White et al. 2015).

Although *O. heinsohni* samples from southern PNG cluster with *O. heinsohni* from northern Australia, genetic differentiation between these regions may become apparent with increased sampling effort and inclusion of microsatellite analyses (Hayano et al. 2004; Hoelzel et al. 1998).

Proposed distribution of *O. heinsohni* in New Guinea and Pacific Island coastal waters

Given the occurrence of *O. heinsohni* in southern PNG waters, there is potential for the species distribution to extend to the Indonesian Provinces of Papua and West Papua based on known habitat preferences; i.e., shallow coastal waters with significant freshwater output. There is limited probability for *O. heinsohni* to be found along the coasts of other Pacific Islands due to a lack of shallow coastal waters in most regions. A similar distribution throughout New Guinea and a lack of sightings for the Pacific Islands has also been described for the Australian humpback dolphin, *Sousa sahulensis* (see Beasley et al. 2015b).

As with the Australian humpback dolphin, it is proposed that *Orcaella* distribution appears to be related to the deep water bio-geographic barrier between the Sahul and Sunda shelves, where *O. heinsohni* distribution is likely to follow either Wallace's line (i.e. including Lombok, Sulawesi and Timor-Leste), Weber's line (i.e. excluding Lombok, Sulawesi and Timor-Leste), or Lydekker's Line (i.e. excluding Lombok, Sulawesi, Timor-Leste, Halmahera and Ceram/Buru (Beasley et al. 2015b).

The closest known *Orcaella* records west/north west of New Guinea are Irrawaddy dolphin records from Kalimantan, Indonesia and southern Philippines (Kreb 2004; Smith et al. 2004). No *Orcaella* records are currently known from Sulawesi, west of Java or Timor-Leste.

Conservation Implications

The Kikori Delta *O. heinsohni* population appears to be a small remnant population. Small isolated populations are particularly sensitive to anthropogenic threats (Huang et al. 2012), where a significant loss in genetic variation may decrease fitness of limit the long-term capacity of a population to respond to environmental challenges (Westemeier et al. 1998). For inshore dolphins in southern PNG, accidental bycatch in subsistence fisheries, and anecdotal reports of direct catch seem to be the most significant threat (Beasley et al. 2014; Beasley et al. 2015a). As an example, during a 15-day field trip to the Kikori Delta in 2015, six inshore dolphins (5 snubfin and 1 humpback dolphins) were confirmed as accidentally bycaught in large mesh gillnets during the 15 days.

Gene flow among small fragmented populations is critical for maintaining genetic diversity, and the evolutionary potential of a species (Hamner et al. 2012). Conservation strategies that focus on maintaining corridors to preserve gene flow and prevent further population fragmentation and loss of genetic diversity will be an essential priority to assist with conserving this small, apparently remnant population.

Based on the results of this study, it is recommended that further research is needed to clarify the taxonomic relationship of *O. heinsohni* between PNG and northern Australia and establish whether any movement is occurring between these two regions. An additional focus should be to clarify the geographical spread of *O. heinsohni,* by conducting surveys in key coastal regions of the Indonesian Provinces of Papua and West Papua, and Wallace Region. A lack of sightings along these Indonesian Provinces will subsequently heighten the conservation status of the Kikori *O. heinsohni* population, which is currently considered a small population of high conservation concern.

Acknowledgements

Thanks to Jeremy Austin from the Australian Centre for Ancient DNA, The University of Adelaide, for analysing the Papua New Guinea and new Australian *Orcaella* samples. Many thanks to Gavin Dally from the Museum and Art Gallery of the Northern Territory, and Robyn Cumming from the Museum of Tropical Queensland for providing access to new Australian *Orcaella* samples. Particular thanks to Kelly Robertson from the Southwest Fisheries Science Centre for assisting to provide previously published *Orcaella* sequences. Thanks also to Adella Edwards for assisting with Figure 1. IB would like to sincerely thank PNG LNG Community Investment Program and the Australian Marine Mammal Centre for providing funding for the fieldwork component of this manuscript and genetic analysis. JA would like to thank the National Museum and Art Gallery Director Dr. Andrew Moutu, for allowing JA's participation on the PIDU trips. Fieldwork in Papua New Guinea was conducted under Papua New Guinea Research Research Visa 10350006555. CITES permits were obtained to export (015034) and import (PWS2015-AU-000607) samples to Australia.

References

Austin JJ, Joseph L, Pedler LP, Black AB (2013) Uncovering cryptic evolutionary diversity in extant and extinct populations of the southern Australian arid zone Western and Thick-billed Grasswrens (Passeriformes: Maluridae: Amytornis) Conservation Genetics 14:1173-1184

Baker A (2009) Molecular methods in ecology. John Wiley & Sons,

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies Molecular biology and evolution 16:37-48

Bass DK (2010) Status of Dugong Dugong dugon and Australian Snubfin Dolphin Orcaella heinsohni, in the Solomon Islands Pacific Conservation Biology 16:133-143

Beasley I, Arnold P, Heinsohn G (2002) Geographical variation in skull morphology of the Irrawaddy dolphin, Orcaella brevirostris (Owen in Gray, 1866) Raffles Bulletin of Zoology:15-34

Beasley I, Golding M, Anamiato J (2014) Looking for Pidu (Dolphins and Dugongs) in the Kikori Delta of Papua New Guinea—December 2013 College of Marine and Environmental Sciences, James Cook University, AustraliaUnpublished report available from IB

Beasley I, Golding M, Anamiato J (2015a) Looking for Pidu (Dolphins and Dugongs) in the Kikori Delta of Papua New Guinea—February 2015 College of Marine and Environmental Sciences, James Cook University, AustraliaUnpublished report available from IB

Beasley I, Jedensjö M, Wijaya GM, Anamiato J, Kahn B, Kreb D (2015b) Observations on Australian Humpback Dolphins (Sousa sahulensis) in Waters of the Pacific Islands and New Guinea Advances in Marine Biology

Beasley I, Robertson KM, Arnold P (2005) Description of a new dolphin, the Australian snubfin dolphin Orcaella heinsohni sp. n.(Cetacea, Delphinidae) Marine Mammal Science 21:365-400

Boessenkool S, Austin JJ, Worthy TH, Scofield P, Cooper A, Seddon PJ, Waters JM (2009) Relict or colonizer? Extinction and range expansion of penguins in southern New Zealand Proceedings of the Royal Society of London B: Biological Sciences 276:815-821

Bonaccorso F, Anamiato J, Leary T (2000) The marine mammals of the Kikori ICAD: a rapid assessment: Unpublished report submitted to the National Museum and Art Gallery and World Wildlife Fund. 16 pp Unpublished report available from IB

Brown AM et al. (2014) Population differentiation and hybridisation of Australian snubfin (Orcaella heinsohni) and Indo-Pacific humpback (Sousa chinensis) dolphins in north-western Australia PloS one 9:e101427

Caballero S, Jackson J, Mignucci-Giannoni AA, Barrios-Garrido H, Beltrán-Pedreros S, Robertson KM, Baker CS (2008) Molecular systematics of South American dolphins Sotalia: Sister taxa determination and phylogenetic relationships, with insights into a multi-locus phylogeny of the Delphinidae Molecular phylogenetics and evolution 46:252-268

Cagnazzi D, Parra GJ, Westley S, Harrison PL (2013) At the heart of the industrial boom: Australian snubfin dolphins in the Capricorn Coast, Queensland, need urgent conservation action PloS one 8:e56729

Dawbin W (1972) Dolphins and whales Encyclopaedia of Papua and New Guinea (P Ryan, ed) Melbourne University Press, Australia 3:1-1231

DoE (2013) Coordinated research framework to assess the national conservation status of Australian snubfin dolphins (Orcaella heinsohni) and other tropical inshore dolphins. Department of the Environment

GHD (2015) South of Embley Inshore Dolphin Project December 2014 Baseline Survey. Rio Tinto Alcan,

Hamner RM, Pichler FB, Heimeier D, Constantine R, Baker CS (2012) Genetic differentiation and limited gene flow among fragmented populations of New Zealand endemic Hector's and Maui's dolphins Conservation Genetics 13:987-1002

Hayano A, Yoshioka M, Tanaka M, Amano M (2004) Population differentiation in the Pacific white-sided dolphin Lagenorhynchus obliquidens inferred from mitochondrial DNA and microsatellite analyses Zoological science 21:989-999

Hoelzel AR, Potter CW, Best PB (1998) Genetic differentiation between parapatric 'nearshore'and 'offshore'populations of the bottlenose dolphin Proceedings of the Royal Society of London B: Biological Sciences 265:1177-1183

Huang S-L, Hao Y, Mei Z, Turvey ST, Wang D (2012) Common pattern of population decline for freshwater cetacean species in deteriorating habitats Freshwater Biology 57:1266-1276

Jayasankar P, Patel A, Khan M, Das P, Panda S (2011) Mitochondrial DNA diversity and PCR-based sex determination of Irrawaddy dolphin (Orcaella brevirostris) from Chilika Lagoon, India Molecular biology reports 38:1661-1668

Kreb D (2004) Abundance of freshwater Irrawaddy dolphins in the Mahakam River in East Kalimantan, Indonesia, based on mark-recapture analysis of photo-identified individuals Journal of Cetacean Research and Management 6:269-277

Limpus C, Miller J, Paramenter C, Reimer D, McLachlan N, Webb R (1992) Migration of green (Chelonia mydas) and loggerhead (Caretta caretta) turtles to and from eastern Australian rookeries Wildlife Research 19:347-357

Marsh H, Lawler IR, Kwan D, Delean S, Pollock K, Alldredge M (2004) Aerial surveys and the potential biological removal technique indicate that the Torres Strait dugong fishery is unsustainable Animal Conservation 7:435-443

Owen P (1866) On some Indian Cetacea collected by Walter Elliot, Esq The Transactions of the Zoological Society of London 6:17-47

Palmer C, Murphy SA, Thiele D, Parra GJ, Robertson KM, Beasley I, Austin CM (2011) Analysis of mitochondrial DNA clarifies the taxonomy and distribution of the Australian snubfin dolphin (Orcaella heinsohni) in northern Australian waters Marine and Freshwater Research 62:1303-1307

Parra GJ, Corkeron PJ, Marsh H (2006) Population sizes, site fidelity and residence patterns of Australian snubfin and Indo-Pacific humpback dolphins: Implications for conservation Biological conservation 129:167-180

Reeves RR et al. (2008a) Orcaella brevirostris The IUCN Red List of Threatened Species 2008 e.T15419A4579987:Downloaded on 27 February 2016

Reeves RR et al. (2008b) Orcaella heinsohni The IUCN Red List of Threatened Species 2008 e.T136315A4273414

Sheppard JK, Preen AR, Marsh H, Lawler IR, Whiting SD, Jones RE (2006) Movement heterogeneity of dugongs, Dugong dugon (Müller), over large spatial scales Journal of Experimental Marine Biology and Ecology 334:64-83

Smith BD, Ahmed B, Mowgli RM, Strindberg S (2008) Species occurrence and distributional ecology of nearshore cetaceans in the Bay of Bengal, Bangladesh, with abundance estimates for Irrawaddy dolphins Orcaella brevirostris and finless porpoises Neophocaena phocaenoides Journal of Cetacean Research and Management 10:45-58

Smith BD et al. (2004) Status, ecology and conservation of Irrawaddy dolphins(Orcaella brevirostris) in Malampaya Sound, Palawan, Philippines Journal of cetacean research and management 6:41-52

Vilstrup JT et al. (2011) Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae BMC evolutionary biology 11:65

Westemeier RL et al. (1998) Tracking the long-term decline and recovery of an isolated population Science 282:1695-1698

White WT et al. (2015) Rediscovery of the Threatened River Sharks, Glyphis garricki and G. glyphis, in Papua New Guinea PloS one 10:e0140075