

## Analysis of mitochondrial DNA clarifies the taxonomy and distribution of the Australian snubfin dolphin (*Orcaella heinsohni*) in northern Australian waters

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**Abstract.** Conservation management relies on being able to identify and describe species. Recent morphological and molecular analyses of the dolphin genus *Orcaella* show a species-level disjunction between eastern Australia and South-east Asia. However, because of restricted sampling, the taxonomic affinities of the geographically intermediate populations in the Northern Territory and Western Australia remained uncertain. We sequenced 403 base pairs of the mitochondrial control region from five free-ranging *Orcaella* individuals sampled from north-western Western Australia and the Northern Territory. Low net nucleotide divergence (0.11–0.67%) among the Australian *Orcaella* populations show that populations occurring in the Northern Territory and Western Australia belong to the Australian snubfin (*O. heinsohni*) rather than the Asian Irrawaddy dolphin (*O. brevirostris*). Clarifying the distribution of *Orcaella* is an important first step in the conservation and management for both species; however, an understanding of the metapopulation structure and patterns of dispersal among populations is now needed.

**Additional keywords:** distribution, mitochondrial DNA, northern Australia.

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### Introduction

Accurate taxonomic identification of organisms is essential to making informed decisions about conservation and implementation of protective legislation (Mace 2004) and for undertaking on-ground management. Recently, the Australian snubfin dolphin (*Orcaella heinsohni*) was described as a new species and is currently the only cetacean known to be endemic to Australian waters, although the species may also occur in Papua New Guinean waters (Beasley *et al.* 2002, 2005). The species was split from the Asian Irrawaddy dolphin (*O. brevirostris*) on morphological characteristics and 17 fixed nucleotide

differences in a 403 base-pair (bp) segment of the mitochondrial control region (mtDNA; Beasley *et al.* 2005). The genetic component of the study by Beasley *et al.* (2005) was based on 24 samples from South-east Asia and four samples from Australia (three from north-eastern Queensland and one from the Northern Territory). Since the publication of the study of Beasley *et al.* (2005), uncertainties have arisen about the collection location of the sample from the Northern Territory (i.e. Darwin). The Darwin sample was received by South-west Fisheries Science Centre, USA, by a secondary party and the origin of the sample has not been confirmed through museum

records (K. M. Robertson, pers. comm., Protected Resources Division, South-west Fisheries Science Centre, National Marine Fisheries Services, National Oceanic and Atmospheric Administration).

*Orcaella* populations in the Northern Territory and Western Australian waters are approximately midway between the known distributions of *O. brevirostris* and *O. heinsohni*. Additionally, with changing sea levels for ~90% of the past 250 000 years, and the formation of the land-bridge between Australia and New Guinea during the Pleistocene, *Orcaella* populations on the eastern coast of Queensland would have been isolated at varying times from populations in the Northern Territory and Western Australia (Voris 2000). Given the doubt surrounding the 'Darwin' sample in the study of Beasley *et al.* (2005), and the historical separation of eastern coast Queensland from northern Australian waters, it remains unclear whether these northern Australian populations are composed of *O. brevirostris*, *O. heinsohni* or a mixture of both species.

In the present study, as part of an ongoing multi-species coastal dolphin program, we analysed mtDNA sequence data from *Orcaella* individuals from the Northern Territory and north-western Western Australia. Our results corroborate the

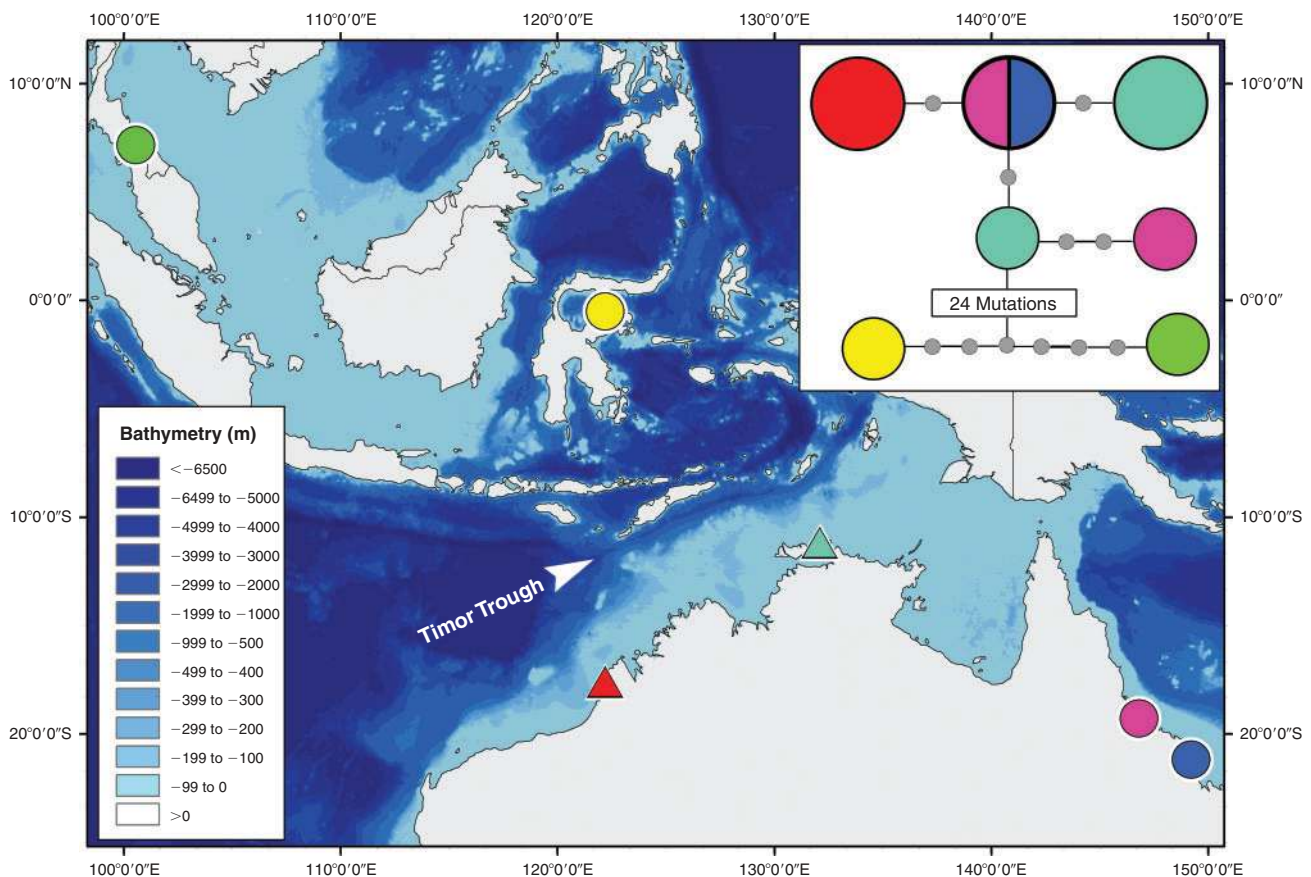
findings of Beasley *et al.* (2005) and confirm the presence of the Australian snubfin dolphin across northern Australia.

## Materials and methods

### Tissue sampling

During 2008, the following two areas were targeted for collection of biopsy samples from free-ranging *Orcaella*: Cobourg Peninsula (Northern Territory) and Roebuck Bay (Western Australia) (Fig. 1, Table 1). Skin samples were collected using a biopsy system specially designed for small cetaceans (PAXARMS, Krützen *et al.* 2002).

Sampling was attempted only during calm sea conditions (Beaufort <2), with no swell. Biopsy sampling of *Orcaella* presents some challenges, in comparison with sampling other coastal dolphins. The species is wary of vessels (Dhandapani 1992; Parra and Corkeron 2001; Krebs 2004) and typically their surfacing patterns are unpredictable. Individuals tend to maintain a low profile on surfacing and inhabit turbid in-shore waters (Parra and Corkeron 2001; Parra *et al.* 2002); thus, sampling opportunities are limited. We were able to approach *Orcaella* schools to within sampling distance (2–12 m) more successfully



**Fig. 1.** *Orcaella* sample locations (coloured symbols on map) used to investigate taxonomy and distribution. Background map shows elevation, including bathymetry (darker shading = lower elevation). The white arrow indicates the position of the Timor Trough. The inset shows the haplotype network, colour-coded to match sample locations. Circle sizes in the network are proportional to the number of individuals with that haplotype. Grey circles represent single-step mutations.



**Table 2.** Percentage pairwise nucleotide divergence for each *Orcaella* haplotype (the present study and Beasley *et al.* 2005)

Sample	Population	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]
2905Qld	Queensland	[1]								
2906Qld	Queensland	[2]	0.8							
2907Qld	Queensland	[3]	0.0	0.8						
Co6COB	Northern Territory	[4]	0.5	1.3	0.5					
Co7COB	Northern Territory	[5]	0.5	1.3	0.5	0.0				
Co8COB	Northern Territory	[6]	0.3	0.5	0.3	0.8	0.8			
Ro1ROE	Western Australia	[7]	0.5	1.3	0.5	1.0	1.0	0.8		
Ro4ROE	Western Australia	[8]	0.5	1.3	0.5	1.0	1.0	0.8	0.0	
1436INDO	SE Asia	[9]	6.6	6.9	6.6	7.2	7.2	6.3	6.6	6.6
EU121128.1	SE Asia	[10]	6.3	7.2	6.3	6.9	6.9	6.6	6.3	6.3

**Table 3.** Percentage net nucleotide divergence (Da) among *a priori* populations in the present study

Population	Queensland	Northern Territory	Western Australia
Northern Territory	0.11		
Western Australia	0.50	0.67	
South-east Asia	5.52	5.71	5.58

### Haplotypes

A 403-bp section of the control region from our five samples revealed five variable sites, defining three haplotypes (Table 1). In the full analysis involving all sequences from 10 individuals, there were 32 variable sites (excluding gaps at two positions), which defined seven haplotypes.

Our study reports three novel haplotypes defined by only one or two point mutations (Table 1). Pairwise genetic distances among haplotypes are given in Table 2. Da values between South-east Asian and all Australian individuals from sampled areas were high, ranging from 5.5 to 5.7%. In contrast, Da among the Australian individuals from sampled areas was very low, ranging from 0.1% to 0.7%. Our sequences were clearly closely allied to *O. heinsohni* from Queensland. Table 3 shows Da values for our *a priori* groupings. These patterns are reflected in the haplotype network, which shows a relatively small number of mutational steps separating Australian haplotypes, compared with the differences between the Australian and South-east Asian haplotypes consisting of 24 and 25 nucleotide differences (Fig. 1).

### Phylogeography

During the last 1.7 million years of the Pleistocene, it is thought that up to 28 stadial and interstadial cycles occurred, during which time the configuration of land and sea would have changed repeatedly (Shackleton and Opdyke 1973; Stringer and Gamble 1993). For northern Australia, these cycles caused repeated isolation of Queensland in-shore waters from those in the Northern Territory and Western Australia. In total, this configuration accounts for ~90% of the past 250 000 years (Voris 2000). It is therefore surprising that we observed low

genetic differentiation between Queensland and Northern Territory/Western Australia (Da values from 0.11% to 0.67%) because this evidence suggests that there has been effective dispersal between these areas during periods of connectivity. In contrast, there has been continuous oceanic connectivity between Australia and South-east Asia throughout the Pleistocene. However, the narrow Timor Trough, which reaches depths of 1500–3200 m (Fig. 1) is likely to have provided a long-term and stable barrier to dispersal and gene flow for a near-shore shallow-water specialist such as *Orcaella* (Voris 2000; Parra 2006).

### Conservation management

Conservation decision-making is inextricably linked to taxonomy (Mace 2004) and our results have helped resolve uncertainty about the taxonomy and distribution of *O. heinsohni* in Australian waters. *O. heinsohni* is listed as *Near Threatened* at an international level (Reeves *et al.* 2008); however, population trends at a national scale are unknown (Parra *et al.* 2006a; Reeves *et al.* 2008). Estimates for local areas in Queensland suggest that populations of *O. heinsohni* appear to be small and localised (Parra *et al.* 2006a). The low reproductive rate (i.e. maximum recorded age of 28 years: Marsh *et al.* 1989; estimated age at reproduction of 9 years and one calf every 2–3 years: Taylor *et al.* 2007), combined with the low numbers and continued threats (i.e. accidental catch in shark nets set for bathers protection: Paterson 1990; Gribble *et al.* 1998) are some of the key reasons why the species is susceptible to local extinction. The Northern Territory and north-western Western Australia's maritime estate is situated in a region of high marine biodiversity and low human impact (Halpern *et al.* 2008). Despite encompassing some of Australia's remote and sparsely populated coastlines, the Northern Territory and north-western Western Australia face rapid development in some areas. As is common for remote areas, there is little baseline information with which to support marine planning and develop conservation plans in relation to coastal dolphins. Future studies on metapopulation structure and gene flow among different populations across their range, and taxonomic identification of potential populations (i.e. Papua New Guinea) are needed to establish conservation status and management actions at national and regional scales.

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