New insights on the taxonomy and population structure of "Bryde's whale" species across the Indo-Western Pacific

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ABSTRACT

A species- and population-level analysis was conducted for two species of Bryde's whales, *Balaenoptera brydei* ('ordinary' form) and *B. edeni* ('small' form), using new genetic data from across the Northern Indian Ocean (Oman, Maldives, Bangladesh) combined with existing data from the Eastern Indian Ocean (EIO), Coast of Japan (CoJ), and the north and central west North Pacific (NWNP, CWNP). The objectives of this study were i) to determine the putative taxonomic units of each region and their relationship to one another through preliminary phylogenetic analyses and ii) undertake a population-level analysis to provide updated management recommendations. A total of 31 biopsy samples from Bangladesh, eight samples from the Maldives and 18 from beach-cast whales in Oman were combined with Genbank data from the EIO (n=27), CoJ (n=16), NWNP (n=194) and CWNP (n=116). A total of 49 haplotypes were identified from a total sample of 410 individuals. Nine discrete diagnostic characters were detected via Population Aggregation Analysis and used to define operational taxonomic units for *B. brydei* and *B. edeni* in the Indo-Western Pacific. Population-level analyses consisting of haplotype reconstruction using a Maximum Parsimony network and genetic diversity and differentiation indices, provide evidence of strong differences in the genetic diversity and structure between *B. brydei* and *B. edeni*. Recommendations are made for the recognition of two species of Bryde's whale in the Indo-Western Pacific and the independent designation of multiple management units for each species both within and across ocean basins.

INTRODUCTION

Almost a century has passed since the Bryde's whale (*Balaenoptera brydei*) was first described (Olsen, 1913) from a specimen taken off Durban, South Africa, yet the phylogeny of the species complex remains to be resolved (Perrin & Brownell, 2007). The holotype of *B. edeni* (Anderson, 1879) is based on a skeleton from Sittang River, Burma. Andrews (1918) examined the type specimen of *B. edeni* and two others from the region that were referred to as this species and suggested they were conspecific with *B. brydei*. The nominal species, *B. brydei*, was later synonymized with *B. edeni* (Junge, 1950) based on a specimen from Pulu Sugi Island, Rhio Archipelago, Singapore, but the holotype of *B. edeni* originated from Burma. However, the taxonomic relationships of these nominal species has been further obscured by the discovery that populations in several parts of their range exhibit differences in body size, including one or more smaller forms with a predominantly coastal distribution (Best, 1977, 2001; Perrin et al., 1996; Perrin & Brownell, 2007).

Early genetic work demonstrated that coastal Bryde's whales are differentiated at the sub-specific level from those inhabiting offshore waters (Yoshida & Kato, 1999). Later, researchers advocated for the recognition of two species based on morphological and genetic evidence (mtDNA), recommending the name *B. brydei* be used for the 'ordinary' form and *B. edeni* for the 'small', presumably coastal, form (Wada et al., 2003). Subsequent SINE and mtDNA genome maximum likelihood (ML) and maximum parsimony (MP) analysis supported this designation, resolving *B. edeni* as a sister taxa to *B. brydei*, although with shallow divergence (Sasaki et al., 2006). However, the genetics of the holotype of *B. edeni* had not yet been determined leading to recommendations by the Scientific Committee of the International Whaling Commission to retain the name *B. edeni* provisionally for both forms, on the understanding that further research would be needed to justify the future recognition of two species (Perrin & Brownell, 2007, SC-59-O15). More recently there has been confirmation that *B. brydei* is differentiated from the smaller *B. edeni* at the species level (Kanda et al., 2007). A new species, *B. omurai*, which is similar in appearance to *B. brydei* and *B. edeni*, was also recently described in the Indo-Pacific (Wada et al., 2003) and has been confirmed to represent an ancient lineage in the Balaenopteridae clade (Sasaki et al., 2006).

In addition to resolving the taxonomy and phylogenetic relationships of *B. brydei* and *B. edeni*, population level analyses provide additional knowledge on genetic divergence within each species. This enables higher resolution inferences to be made on the structure of populations across geography and on the relationships between them, information vital for informing appropriate management (Kanda et al., 2007).

MATERIALS AND METHODS

Samples

Seventy-nine DNA sequences of the mitochondrial control region were obtained for individuals biopsied from the Swatch-of-No-Ground (SoNG) off the coast of Bangladesh (BAN). One sample originated from a stranding in southeastern Bangladesh at Cox's Bazaar (Smith, 2009). 31 of the sequences are used in the current analysis and the remaining samples await DNA sequencing and analysis. Sequences were assembled and exported using Geneious ver 5.3.5 (Drummond et al., 2011).

Sequences were also obtained for 18 whales stranded on the coast of Oman (OMA) and 8 individuals sampled off the Maldives (MAL). Haplotypes from the Eastern Indian Ocean (EIO), Coast of Japan (CoJ) and the north and central west North Pacific (NWNP, CWNP) were obtained from Genbank (ACCN:146378-388, Yoshida & Kato, 1999; EF068013-048, EF068060-063, Kanda et al., 2007). NWNP and CWNP samples are labeled following the nomenclature in Kanda et al. (2007).

Reference sequences based on best available knowledge for *B. brydei* and *B. edeni*, as well as for *B. omurai*, were obtained from Genbank (ACCN: AB201159, AB201258, AB201256 respectively).

Analyses

Population Aggregation Analysis

All sequences were aligned using ClustalW under default settings in MEGA5 (Tamura et al., 2011) and trimmed to the 299bp mitochondrial control region. Without *a priori* designations, a Population Aggregation Analysis (PAA) based on diagnostic nucleotide characters (Davis & Nixon, 1992; DeSalle & Amato, 2004) and using references sequences, was undertaken in order to differentiate individuals of *B. brydei* from *B. edeni*.

Haplotype Network

All sequences were collapsed to haplotypes using in DnaSP ver 5 (Librado & Rosaz, 2009). EIO, NWNP and CWNP haplotypes were replicated to reflect the frequencies specified in Yoshida & Kato (1999) and Goto et al. (SC/56/PF15) respectively. The entire dataset was converted to a Roehl data format using DnaSP and Median Joining haplotype networks (Bandelt et al., 1999), both with and without Maximum Parsimony post-processing, were created using NETWORK ver 4.6.0.0 (Fluxus Technology Ltd, 1999-2010).

Genetic Divergence and Diversity

For the statistical analysis, haplotypes for *B. brydei* and *B. edeni* were treated separately and only haplotypes where n>5 were included.

Samples were grouped based on their geographic sampling site and analyzed using DnaSP. Hierarchical pairwise tests of genetic differentiation between samples were conducted in Arlequin ver 3.5 (Excoffier & Lischer, 2010). A heterogeneity test for haplotype frequencies was calculated using Fisher's exact test of population differentiation (implemented with 10,000 Markov chain steps and 1000 dememorization steps) at the 0.05 significance level. Heterogeneity among the samples would indicate individuals in the samples originate from genetically different populations. Pairwise genetic distance values using haplotype frequencies (F_{ST}) and molecular distance based methods (ϕ_{ST}) with 1000 permutations at the 0.05 significance level (Weir & Cockerham, 1984) were also calculated. The sampling sites that were not found to be significantly differentiated from one another were grouped to a single population and the pairwise tests were re-run between the new samples.

Genetic diversity (number of haplotypes, haplotype diversity, nucleotide diversity with Jukes-Cantor correction, number of nucleotide differences) for each sample derived through the hierarchical pairwise tests described above were calculated in DnaSP.

RESULTS AND DISCUSSION

Defining Taxonomic Units of Bryde's whales in the Indo-Western Pacific

Nine discrete diagnostic sites were detected through the PAA indicating species-level differentiation between *B. brydei* ('ordinary' form) and *B. edeni* ('small' form) whales (Table 1).

The genetic analysis of the mtDNA control region for all samples resulted in the identification of 49 unique haplotypes across the Indo-Western Pacific. Specifically 45 haplotypes (H1-H45) were identified as *B. brydei* and only four haplotypes (H46-H49) were identified for *B. edeni* (see Figure 1 for relative haplotype frequencies across sampling locations). These results provide evidence from the mitochondrial control region for two species of Bryde's whale in the Indo-Western Pacific, findings that are consistent with previous phylogenetic analyses that clearly separate the two taxa (Yoshida & Kato, 1999; LeDuc & Dizon, 2002; Wada et al., 2003) into two species.

Haplotype reconstruction

Median-Joining networks showed comparable results irrespective of whether Maximum Parsimony (MP) post-processing was included. Median-Joining networks have been recommended over Maximum Parsimony approaches in intraspecific studies as they capture a greater degree of ambiguity thus enabling more realistic interpretation (Cassens et al., 2005). The Median-Joining network in this analysis did capture a higher number of inferred nodes and reticulations; however, as the fundamental relationships between the haplotypes were not affected, only the simpler network with MP post-processing is shown (Figure 1).

The non-overlapping genetic distributions observed between *B. brydei* and *B. edeni*, as separated by the long central branch, under some species concepts may provide additional support for the recognition of two species of Bryde's whales in the Indo-Western Pacific. A proper phylogenetic analysis to evaluate the phylogenetic relationship among this species complex is forthcoming.

For the 45 haplotypes identified as *B. brydei*, two main clusters are shown: the NIO (OMA, MAL, BAN) and the western North Pacific (NWNP, CWNP). Haplotypes from the EIO are represented across the network. Given its disproportionately high frequency, H11 appears to represent an ancestral haplotype within the western North Pacific. Additionally, the high frequency of haplotypes that occur at lower frequencies as well as the high degree of reticulation between NWNP, CWNP and EIO samples are indicative of relatively recent genetic divergence among these regions. Two clusters are also evident for *B. edeni*, however, a single individual from the CoJ was found to share a haplotype with NIO individuals.

Genetic Divergence and Diversity

The hierarchical pairwise F_{ST}, ϕ_{ST} and Fisher's exact tests showed no significant differentiation for the NWNP and CWNP samples for *B. brydei* so these samples were grouped to a single western North Pacific (WNP) population for the remainder of the analysis. Similarly no significant differentiation was observed between OMA and BAN samples for *B. edeni* which similarly were subsequently grouped for exploratory purposes in further analysis (OMB).

For *B. brydei*, where sample sizes exceeded five individuals, 44 unique haplotypes were derived from 345 sequences with 34 polymorphic sites (2 singletons, 32 parsimony informative) in the 297bp control region after the removal of gaps and missing data. Genetic diversity (Table 2) was relatively high (*Hd*: 0.843; π(JC): 0.01270; *k*: 3.719) over all samples. Haplotype diversity was generally comparable between samples with MAL exhibiting relatively lower *k* values, likely relating to small sample size, and the EIO

exhibiting a relatively lower *Hd* value. Pairwise F_{ST} and ϕ_{ST} values (Table 3) were highly significant between all sampling sites (P<0.001) indicating that populations in the MAL, EIO, and WNP can be considered genetically discrete populations.

B. edeni showed remarkably low genetic diversity with only four haplotypes being derived from 62 sequences with 4 polymorphic sites (1 singleton, 3 parsimony informative) in the 299bp control region (*Hd*: 0.411; π(JC): 0.00375; *k*: 1.115). Pairwise F_{ST} and ϕ_{ST} results also showed highly significant differentiation between the provisionally combined OMB sample and coastal Japan (F_{ST}: 0.86442, p<0.001; ϕ_{ST}:0.93184, p<0.001). However, one haplotype (H46) is shared between all three sampling locations.

These results, taken collectively, provide additional evidence for strong differences in the mtDNA diversity and structure between *B. brydei* and *B. edeni* confirming that independent management recommendations should be formulated for each.

The genetic structure observed for *B. brydei* provides evidence for discrete management units in the NIO, EIO and WNP. The results support previous findings designating the WNP as a single management unit (Kanda et al., 2007; Goto et al., SC/56/PF15). The differences between the EIO sample is least clear as three of the five EIO haplotypes were found to be shared with either the NIO sample (n=1) or the WNP sample (n=2). Further sub-structure in the NIO may become apparent with additional data analysis from the existing sample collection. Additional sampling is recommended in this region in order to elucidate these relationships.

In contrast, the low degree of genetic diversity and structure found for *B. edeni* across the entire NIO is not consistent with previous observations of the species in southern Africa (Best, 2001). However, if the form is highly migratory and there are no significant environmental barriers to dispersal (e.g., Mendez et al., 2010) it is feasible that there could be mixing of individuals along NIO coastal waters. Signals of low genetic diversity and relative isolation have also been observed for other cetacean species in the NIO including the humpback whale (Minton et al., 2010; Pomilla et al., 2010, 2006; Rosenbaum et al., 2009) and blue whale (Perrin et al., 2010; Rice, 1998; Brownell & Donahue, 1994) indicating that the genetic structure exhibited by *B. edeni* represents a more generalized pattern. Comparative genetic analyses are invaluable for informing comprehensive regional management plans capable of responding to general and specific management needs for these whales.

Alternatively, inconsistent sampling effort or any resampling of individuals within Bangladesh may also provide an explanation for the low degree of diversity observed. What can be inferred from this analysis is that there is strong genetic differentiation between populations of *B.edeni* in the NIO and those sampled from the CoJ and thus, two distinct broad management units are recommended. Given the low diversity and additional samples to be included in the analysis, the number of management units within each region cannot be fully evaluated and for precautionary regions, it is important maintain geographic populations as management targets for the time being. Pooling of datasets, however, was illustrative for a comparative analysis with samples from Japan.

The need for additional genetic sampling, both geographically and across the genome (using other molecular markers), is clear (Perrin & Brownell, 2007). Microsatellite analysis would provide additional insights into population structure and, importantly, patterns of both male and female dispersal (see Kanda et al., 2007). Additionally, integration of genetic data with morphological studies will further assist the operational identification of these taxa in the field (see Smith, 2009).

RECOMMENDATIONS

1. Additional genetic sampling in the coastal Indo-Western Pacific region especially along the coast of the Malay Peninsula and the Gulf of Thailand.
2. Recognition of two species of Bryde's whales in the Indo-Western Pacific: *B. brydei* and *B. edeni*.
3. Designation of provisional multiple management units across and within ocean regions for each species, integrating findings for other whale species in the region.
4. Further resolution and provisional designation of multiple management units across ocean regions for *B. edeni* for consideration in national and regional conservation planning.

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TABLES

Table 1. Results of the Population Aggregation Analysis (PAA) for the 299bp mitochondrial control region for all sequences included in the analysis. Reference sequences for *B. brydei* and *B. edeni* are shown in bold type in the first rows on the left and right respectively. Only pure diagnostic sites are shown. All haplotypes have been collapsed under one representative haplotype for each region color coded to correspond to the Maximum Parsimony network in Figure 1. Corresponding haplotype numbers are as follows: Oman (OMA): *B. brydei* – H39; *B. edeni* – H46; Maldives (MAL), *B. brydei*: H1, H39, H42-43; Bangladesh (BAN): *B. brydei* – H1; *B. edeni* - H46-47; Coast of Japan (CoJ), *B. edeni*: H48-49; Eastern Indian Ocean (EIO), *B. brydei*: H37-41; Western North Pacific (WNP, including samples from CWNP and NWNP), *B. brydei*: H2-H37.

	28	57	74	79	81	146	187	191	234		28	57	74	79	81	146	187	191	234
B. brydei	**T**	**T**	**T**	**G**	**G**	**C**	**T**	**A**	**A**	**B. edeni**	**C**	**C**	**C**	**A**	**T**	**T**	**C**	**G**	**T**
OMA	**T**	**T**	**T**	**G**	**G**	**C**	**T**	**A**	**A**	**OMA**	**C**	**C**	**C**	**A**	**T**	**T**	**C**	**G**	**T**
MAL	**T**	**T**	**T**	**G**	**G**	**C**	**T**	**A**	**A**	**BAN**	**C**	**C**	**C**	**A**	**T**	**T**	**C**	**G**	**T**
BAN	**T**	**T**	**T**	**G**	**G**	**C**	**T**	**A**	**A**	**COJ**	**C**	**C**	**C**	**A**	**T**	**T**	**C**	**G**	**T**
EIO	**T**	**T**	**T**	**G**	**G**	**C**	**T**	**A**	**A**										
WNP (CW+ NWNP)	**T**	**T**	**T**	**G**	**G**	**C**	**T**	**A**	**A**										

Table 2. Genetic diversity indices for *B. brydei and B. edeni* haplotypes for samples of five or more individuals. Results shown for the total sample and each individual sampling location. N, number of sequences; S, number of segregating sites; *H*, number of haplotypes; *Hd*, haplotype diversity; π(JC), nucleotide diversity with Jukes Cantor correction; *k*, number of nucleotide differences.

Species	Sample	N	S	*H*	*Hd*	π(JC)	*k*
B. brydei	All	345	34	44	0.843	0.01270	3.719
	MAL	8	3	4	0.750	0.00520	1.536
	EIO	27	12	5	0.396	0.00721	2.108
	WNP	310	32	37	0.810	0.01041	3.054
B. edeni	All	62	4	4	0.411	0.00375	1.115
	OMB	46	1	2	0.043	0.00015	0.043
	CoJ	16	3	3	0.342	0.00193	0.575

Table 3. Pairwise F_{ST} and ϕ_{ST} values for *B. brydei* and *B. edeni*. F_{ST} values are shown on the top row above the diagonal, ϕ_{ST} results are shown on the bottom row above the diagonal, significance tests for Fisher's exact test are shown below the diagonal. Significance values are indicated as follows: *, p<0.05; **, p<0.01; ***, p<0.001.

B. brydei		MAL	EIO	WNP
	MAL	-	0.47938*** 0.74537***	0.21115*** 0.70287***
	EIO	0.00000+-0.0000***	-	0.33374*** 0.48165***
	WNP	0.00000+-0.0000***	0.00000+-0.0000***	-

B. edeni		OMB	CoJ
	OMB	-	0.86442*** 0.93184***
	CoJ	0.00000+-0.0000***	-

FIGURES

Figure 1. Sampling locations and haplotype network for all sequences included in the analysis. Size of node is proportional to the number of individuals for each haplotype. Nodes are color coded according to sampling location.