



Rare or cryptic? The first report of an Omura's whale (*Balaenoptera omurai*) in the South Atlantic Ocean

ANA LÚCIA CYPRIANO-SOUZA,¹ Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga 6681, 90619-900 Porto Alegre, Rio Grande do Sul, Brazil; ANA CAROLINA OLIVEIRA DE MEIRELLES AND VITOR LUZ CARVALHO, Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (AQUASIS), Rua José de Alencar, 150, 61627-210 Caucaia, Ceará, Brazil; SANDRO LUIS BONATTO, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga 6681, 90619-900 Porto Alegre, Rio Grande do Sul, Brazil.

ABSTRACT

The Omura's whale (*Balaenoptera omurai*), previously referred to as a small form of Bryde's whale, was described in 2003 as a distinct baleen whale species of the family Balaenopteridae. Omura's whales are currently confirmed to occur in three of the world's oceans; the western Pacific, Indian, and northeastern Atlantic. Here we report the genetic identification and a morphological description of a 4.16 m female calf of an Omura's whale found stranded in Pecém beach (03°32'11.6"S, 38°47'51.8"W), northeastern Brazil, in 2010. The three mitochondrial DNA markers (control region, cytochrome *b*, and cytochrome *c* oxidase subunit 1) unequivocally identified the specimen as a *B. omurai*, providing the first report of this species in the South Atlantic Ocean. The morphological identification of the specimen was limited due to the absence of the skull and loss of color pattern, but the proportions of the body were not consistent with the other balenopterid species and a single rostrum ridge distinguished it from Bryde's whales. This, together with the record of another juvenile in Mauritania, suggests the existence of an autochthonous Atlantic population of Omura's whales and indicates a necessity of reassessment of specimens indicated as Bryde's whale in the region.

Key words: Omura's whale, genetic identification, morphological description, distribution, South Atlantic Ocean.

Knowledge of the taxonomy, population structure, and distribution of whale species is of high importance for management and recovery, especially for the taxonomic groups subject to hunting under scientific permit from the International Whaling Commission or those overexploited by commercial whaling (Clapham *et al.* 2008). Rare or cryptic species are difficult to identify because they often show scarcity of data on biology and distribution, also hampering conservation efforts (Vine *et al.* 2009). The Omura's whale (*Balaenoptera omurai*) is the most recent baleen whale species described, having previously been mistaken for and classified as a small form of Bryde's whale (*B. edeni*) (Wada *et al.* 2003, Sasaki *et al.* 2006). This erroneous

¹Corresponding author: (e-mail: anacypriano@yahoo.com.br).

classification raises the question of whether this species is truly rare or primarily difficult to identify (cryptic).

The description of Omura's whale was initially based on the analysis of mitochondrial DNA (mtDNA) control region sequences and on the morphological differences of specimens collected near the Solomon Islands in the southwestern Pacific Ocean (six specimens collected in 1976), off the Cocos Islands in the eastern Indian Ocean (two specimens collected in 1978), and a stranded whale found in the Tsunoshima Island, in the Sea of Japan, in 1998 (Wada *et al.* 2003). Later, the species' status was confirmed based on the molecular analysis of complete mtDNA sequences and short interspersed repetitive elements (Sasaki *et al.* 2006). This study indicated that Omura's whale represents an ancient independent lineage that diverged earlier (around 17 million years ago) within the Balaenopteridae, and thus the species was included in the Society for Marine Mammalogy's official list of marine mammal species (Committee on Taxonomy 2014).

In addition to the first reports of the species, studies based on skull morphology further reported specimens of the Omura's whale in Taiwan (seven specimens), Philippines (24 specimens), Thailand (three specimens) in the Andaman Sea (Yamada *et al.* 2006a, 2008), Malaysia (one specimen) (Ponnampalam 2012), and South Australia (Yamada *et al.* 2006b). Records of live whales (photographic evidence) have been reported for Thailand (Yamada 2009, Adulyanukosol *et al.* 2012) and New Caledonia (Garrigue and Poupon 2013). While this manuscript was in review, a potentially resident population was discovered off northwest Madagascar, and a genetically identified neonate was found stranded on the Mauritania coast, in west Africa, in 2013, extending the Omura's whale distribution to the southwestern Indian Ocean and the North Atlantic Ocean (Fig. 1) (Cerchio *et al.* 2015, Jung *et al.* 2016).

The Omura's whale is listed as "data deficient" on the International Union for Conservation of Nature (IUCN) Red List (Reilly *et al.* 2008). However, since whaling records have not distinguished Omura's from Bryde's whales, the latter of which was a targeted species of commercial whaling (Kanda *et al.* 2007), and the previously

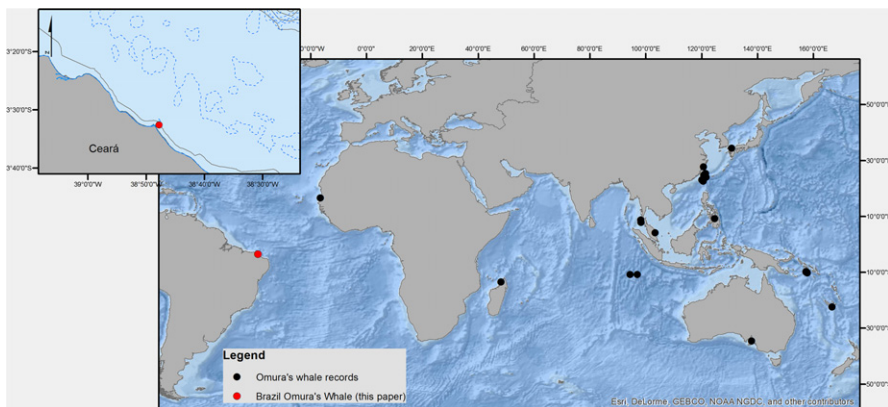


Figure 1. Map showing the known published (black circles) Omura's whale records and the new (red circle) record of an Omura's whale in the central coast of the Ceará State, northeastern Brazil.

known distribution of the Omura's whale was limited to the western Pacific Ocean and the eastern Indian Ocean, it is likely that an unknown number of Omura's whales have been hunted. Therefore, it is important to identify correctly this species and its actual distribution to avoid overexploitation and loss of undetected conservation units.

Here we report the molecular identification (mtDNA sequence) and morphological description of an Omura's whale found in 2010 stranded in Pecém beach, northeastern Brazil, South Atlantic Ocean, and discuss the consequences of the recent findings on the geographic distribution and abundance of the species.

METHODS

Specimen Record and Sample Collection

Since 1992, beach surveys have been conducted along the 573 km of Ceará State coastline by Aquasis (Associação de Pesquisa e Preservação de Ecossistemas Aquáticos), a nongovernmental organization that is part of the Brazilian Aquatic Mammals Network (Meirelles *et al.* 2009, 2010). On 10 September 2010, a carcass of an unidentified baleen whale species was found in the breakwater of the Pecém Harbour ($03^{\circ}32'11.6''\text{S}$, $38^{\circ}47'51.8''\text{W}$), São Gonçalo do Amarante municipality, situated in the central coast of the Ceará State, northeastern Brazil (Fig. 1). The carcass (Fig. 2A) was in advanced decomposition (Code 4, Geraci and Lounsbury 2005) and most of the animal's skin was absent, precluding the discrimination of the color pattern. During carcass examination, it was verified that most of the skull was lost, and only the two premaxilla and the right mandible were recovered and deposited in Aquasis's collection (#02C0172/421). Body measurements were taken with a tape measure in a straight line from point to point according to Geraci and Lounsbury (2005). Girth measurements were not made due to postmortem meteorism, including rectal and umbilicus prolapse. A tissue sample from the epaxial muscle of the caudal peduncle was collected and preserved in 90% ethanol for molecular analysis. External measurements of the head were taken in a straight line with a tape-measure or a 50 cm caliper according to Omura (1975) for minke whales, detailed in Nakamura and Kato (2014).

DNA Extraction and mtDNA Sequencing

The DNA extraction and PCR reactions were performed at the Laboratory for Genomic and Molecular Biology of Pontifical Catholic University of Rio Grande do Sul (PUCRS), in Brazil. No sample or DNA of Bryde's or Omura's whale was present in the laboratory previously. Genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen) according to the manufacturer's protocol, and quantified using a Low DNA Mass Ladder (Invitrogen). Two independent DNA extractions were performed to verify the initial result and total DNA concentrations averaged 50 ng/ μL . Negative (no DNA) controls were included in all PCR reactions. DNA of a humpback whale sample was used as positive control in all PCR reactions and sequencing.

A fragment of approximately 550 bp of the mtDNA control region was amplified (primers Dlp-1.5 and Dlp-5, Baker *et al.* 1993) by PCR with 50 ng of DNA and the conditions described in Engel *et al.* (2008). PCR product was purified with shrimp



Figure 2. Morphology of the whale stranded in Ceará State, northeastern Brazil. (A) General view of the carcass; (B) Small and slightly falcate dorsal fin; (C) Non-U shape rostrum with a central prominent ridge (black arrow); (D) Ventral view of the carcass, showing the grooves extending to the umbilicus and slightly beyond (white arrow).

alkaline phosphatase and exonuclease I (New England Biolabs) following the manufacturer's recommended protocol. Sequencing was performed with the DYEnamic ET Dye Terminator Sequencing kit (GE Healthcare), cleaned through precipitation using 7.5 M ammonium acetate, and sequenced in both directions on a MegaBACE 1000 system (GE Healthcare) at PUCRS, Brazil.

A 658 bp fragment of the mtDNA cytochrome *c* oxidase subunit 1 (*cox1*) was amplified (primers LCO1490 and HCO2198, Folmer *et al.* 1994) as described in Hebert *et al.* (2003). Part of the mtDNA cytochrome *b* (*cytb*) (~450 bp) gene was amplified (primers GLUDG-L and CB2-H, Palumbi 1996) in reactions containing 3.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μM of each primer, 1 U of Platinum Taq DNA Polymerase (Invitrogen), 1 X PCR buffer (Invitrogen), and 50 ng of DNA in a 20 μL reaction volume. The PCR profile consisted of an initial denaturation at 94°C for 1.5 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 45 s, and extension at 72°C for 70 s, and concluded with a final extension at 72°C for 10 min. PCR products were purified as above. Sequencing reactions were performed using a BigDye Dye Terminator Chemistry v3.1 (Applied Biosystems) cycle sequencing kit, cleaned using CleanSEQ (Agencourt) and sequenced in both directions on an ABI 3730XL (Applied Biosystems) by Macrogen Inc. (Seoul, South Korea). SEQUENCER 5.0 (Gene Codes Corporation) was used to check the electropherograms for all three genes, and to create and edit consensus sequences for forward and reverse reads. The PCR products from the two DNA extracts generated the same sequences for the mtDNA control region.

Genetic Species Identification

For species identification, sequence similarity searches were done for all three regions using blastn with BLAST (Johnson *et al.* 2008) searches in GenBank. Control region and *cytb* sequences were also submitted to the Web-based DNA Surveillance system (Ross *et al.* 2003) using the Witness for the Whale Vs4.3 reference database. DNA Surveillance establishes species identity using a phylogenetic reconstruction based on the alignment of the query sequence with references representing all recognized species of cetaceans. A neighbor-joining (NJ) tree is created using the F84 model of evolution with transition/transversion ratio of 2 (Felsenstein 1984), 1,000 bootstrap replicates and rooted using the sperm whale (*Physeter macrocephalus*) as out-group for the mysticetes. *Cox1* sequence was also submitted to the Barcode of Life Data (BOLD) (Ratnasingham and Hebert 2007) specimen identification system, where the query sequence is aligned to their global database of *cox1* sequences, providing the best match and a NJ tree (Kimura 2 parameter model of evolution) showing the relationship of the queried specimen to its 100 nearest neighbors.

For phylogenetic analyses, we selected GenBank sequences (accession numbers are presented in Table S1) of the three mitochondrial regions from Bryde's (*B. e. edeni* and *B. e. brydei*) and Omura's whales (*B. omurai*) from previous studies (Yang *et al.* 2002; Wada *et al.* 2003; Sasaki *et al.* 2005, 2006; Jayasankar *et al.* 2009; Luksenburg *et al.* 2015; Jung *et al.* 2016). We also included sequences from the new Bryde's complex clade from the northern Gulf of Mexico (*B. e. GOMx*) (Rosel and Wilcox 2014) and the sei whale (*B. borealis*) since this species is also considered a member of this complex (Sasaki *et al.* 2006). Following Kershaw *et al.* (2013), fin whale (*B. physalus*) sequences were selected to root the phylogenetic analyses (obtained from Arnason *et al.* 1991 for control region and Archer *et al.* 2013 for *cytb* and *cox1*). The sequences were aligned using MUSCLE (Edgar 2004) under default parameters as implemented in MEGA 6.0 (Tamura *et al.* 2013).

Bayesian phylogenetic trees were estimated with the control region and *cytb+cox1* alignments with the program BEAST v.1.8 (Drummond and Rambaut 2007) using the HKY substitution model with a discrete gamma distribution with four rate categories, a strict molecular clock and the Yule model as tree prior. Ten thousand trees were saved in each analysis, with a chain length of 20,000,000 for the control region and 10,000,000 for the *cytb+cox1* analyses, and convergence and mixing were inspected in Tracer v.1.5 (Rambaut and Drummond 2007). Trees were summarized in the form of a maximum clade credibility tree (the first 10% being discarded as burn-in) and their 95% highest posterior densities (HPD) estimated using TreeAnnotator from the BEAST package, and the final trees were visualized and edited using FigTree v.1.4.2 (Rambaut 2009).

Phylogenetic analyses with the maximum likelihood (ML) approach were also performed in PhyML (Guindon *et al.* 2010) using the General Time Reversible model with a gamma distribution with six rate categories plus invariable sites (GTR+G6+I) and the Tamura-Nei model (T93+G6) for the control region and the *cytb+cox1* analyses, respectively. These were estimated as the best models by Smart Model Selection (<http://www.atgc-montpellier.fr/phyml-smss/>). The nearest-neighbor interchange was used as the heuristic method and branch support was evaluated using 1,000 bootstrap replicates.

Molecular diversity parameters such as the number of haplotypes and polymorphic sites were estimated for the three mtDNA regions in all *B. omurai* sequences available in GenBank using DnaSP v.5.10.1 (Librado and Rozas 2009). Additionally, we

applied a characteristic attributes (CA) diagnosis (Sarkar *et al.* 2002, Lowenstein *et al.* 2009) to control region sequences of *B. e. edeni*, *B. e. brydei*, *B. e. GOMx*, *B. omurai*, and the Brazilian specimen, as described in previous studies (Kershaw *et al.* 2013, Rosel and Wilcox 2014). First, we compared the sequence of the Brazilian specimen to the 25 diagnostic sites for the four taxa as described in Table 3 from Rosel and Wilcox (2014) for a 305 bp alignment. Second, we identified the variable sites that were unique to each species to a longer sequence length (433 bp), corresponding to the nucleotide positions 15505–15934 in the mtDNA genome of *B. e. brydei* (ACCN: AB201259).

RESULTS

Molecular Identification

DNA was successfully extracted, amplified and sequenced for the three mitochondrial regions from the Brazilian whale specimen and no amplification was found in the negative (no DNA) controls. We obtained consensus sequences of 428 bp for the partial control region (GenBank accession number KX254408), 444 bp for the partial *cytb* (GenBank accession number KX254409) and 655 bp for the partial *cox1* (GenBank accession number KX254410), totaling 1,527 bp and representing about 10% of the mitochondrial genome.

The BLAST searches with each of the three regions showed 99%–100% sequence identity with *B. omurai* sequences (Table 1). The DNA Surveillance analyses showed high bootstrap support (100%) grouping the Brazilian sequences with the reference sequences of *B. omurai* for both the control region (Fig. S1a) and the *cytb* (Fig. S1b). Also, the *cox1* query sequence submitted to the BOLD Systems matched to *B. omurai* (100% of sequence similarity, Fig. S2).

The alignments for the phylogenetic analyses, including inferred gaps, were 433 bp for control region, 444 bp for *cytb* and 655 bp for *cox1*. The Bayesian and ML phylogenetic trees (Fig. 3) showed the same basic branching topology, for both the control region and the *cytb* and *cox1* sequences concatenated, clustering with high support (100% bootstrap) the Brazilian whale specimen and all previously published reference sequences of *B. omurai* (Table S1). The *B. omurai* clade formed a sister group to the *B. e. brydei*, *B. borealis*, *B. e. edeni* and *B. e. GOMx* clade, reciprocally monophyletic groups previously recognized (Wada *et al.* 2003, Sasaki *et al.* 2006, Rosel and Wilcox 2014). As BLAST searches with the GenBank database, DNA Surveillance analysis, BOLD Systems identification and phylogenetic trees all generated the same result, we are confident that this Brazilian whale specimen represents the first record of Omura's whale in the South Atlantic Ocean.

The control region alignment (428 bp) from seven Omura's whale samples available so far (including the Brazilian specimen) presented three variable sites that define three haplotypes. The Brazilian sequence matched a haplotype found in four specimens (two in Japan [AB201256 and AB201257], one in China [AF398372] and one in Mauritania [KM233837]), but differed from the two other haplotypes by one (specimen from Cocos Islands [AB116097]) and two (specimen from Solomon Islands [AB116096]) variable sites, respectively. The alignment was trimmed to a shorter length (385 bp) to include the 20 Madagascar samples represented by a single haplotype (Cerchio *et al.* 2015) and here the Brazilian sequence collapsed into this haplotype. Finally, to include the shorter sequence of another specimen from Solomon

Table 1. Results from GenBank BLAST searches of the three mitochondrial sequences obtained from the stranded Brazilian specimen. Closest matches showed 99%–100% sequence identity with *B. omurai* (except two control region sequences misidentified on GenBank as *B. e. edeni*, AF146389 and AF398372, submitted in 1999 and 2001 (before the *B. omurai* description), respectively).

Region (length in bp)	Species	Closest matches GenBank reference	Identity in bp (percentages)	References
Control region (428)	<i>B. omurai</i>	(KM233838, KT582064)	428/428 (100%)	Jung <i>et al.</i> 2016, Cerchio <i>et al.</i> 2015
		(AB201256, AB201257, AB116095)	428/428 (100%)	Sasaki <i>et al.</i> 2006
	<i>(B. edeni)</i>	(AB116096, AB116097)	427/428 (99%)	Wada <i>et al.</i> 2003
		AF398372 AF146389	428/428 (100%) 334/335 (99%)	Yang <i>et al.</i> 2002 Yoshida and Kato 1999
<i>cytb</i> (440)	<i>B. omurai</i>	(AB201257, EF103940)	440/440 (100%)	Sasaki <i>et al.</i> 2006, Ma <i>et al.</i> 2007
		(KM233837)	440/440 (100%)	Jung <i>et al.</i> 2016
		(AB201256)	439/440 (99%)	Sasaki <i>et al.</i> 2006
<i>cox1</i> (655)	<i>B. omurai</i>	(KM233839, AB201256)	655/655 (100%)	Jung <i>et al.</i> 2016, Sasaki <i>et al.</i> 2006
		(AB201257)	654/655 (99%)	Sasaki <i>et al.</i> 2006

Islands (ACCN: AF146389, Yoshida and Kato 1999), the alignment was trimmed to 335 bp. This sequence defined a new haplotype that differed by one site from the most common haplotype, totaling four haplotypes defined by four variable sites in 28 samples. For the *cytb* alignment (444 bp) from four samples, three haplotypes were found so far, defined by three variable sites, the Brazilian being a singleton with two differences from the others. For the *cox1* alignment (655 bp) from four Omura's whale samples, two haplotypes were defined by one variable site. The Brazilian sequence matched a haplotype found in two specimens (one in Japan [AB201256], and one in Mauritania [KM233839]) and differed (by the only variable site) from the other haplotype found in one specimen from Japan (AB201257).

For the 305 bp alignment of the control region, the sequence of the Brazilian specimen presented all 13 diagnostic characters proposed for *B. omurai* (Table 2). We also found 24 of the 25 diagnostic sites described by Rosel and Wilcox (2014) for the four taxa. However, in the nucleotide position 15682 a haplotype of *B. e. brydei* (ACCN: JX090150, Kershaw *et al.* 2013) has a C instead of T (considered diagnostic site for *B. e. brydei*). In addition, we found five more diagnostic sites (positions 15573, 15605 and 15621 for *B. omurai*; 15816 and 15818 for *B. e. GOMx*), totaling 29 diagnostic characters for the four taxa and 16 for *B. omurai* over 305 bp. For a longer alignment (433 bp) we found 32 diagnostic characters for the four taxa and 17 for *B. omurai* (see Table 2).

Morphological Description

The animal was an immature female (4.16 m) with a thin body and the caudal peduncle laterally flattened (Fig. 2A). The dorsal fin was small and slightly falcate,

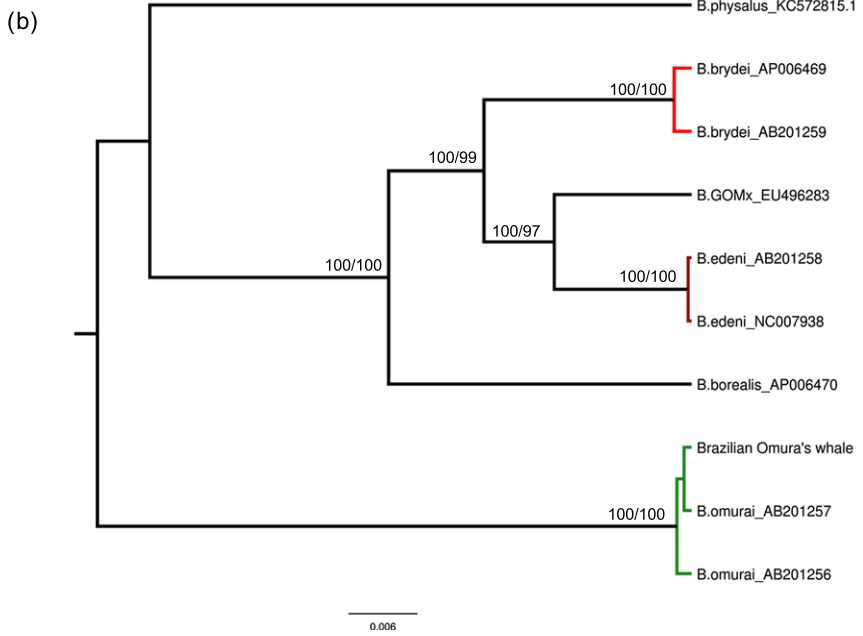
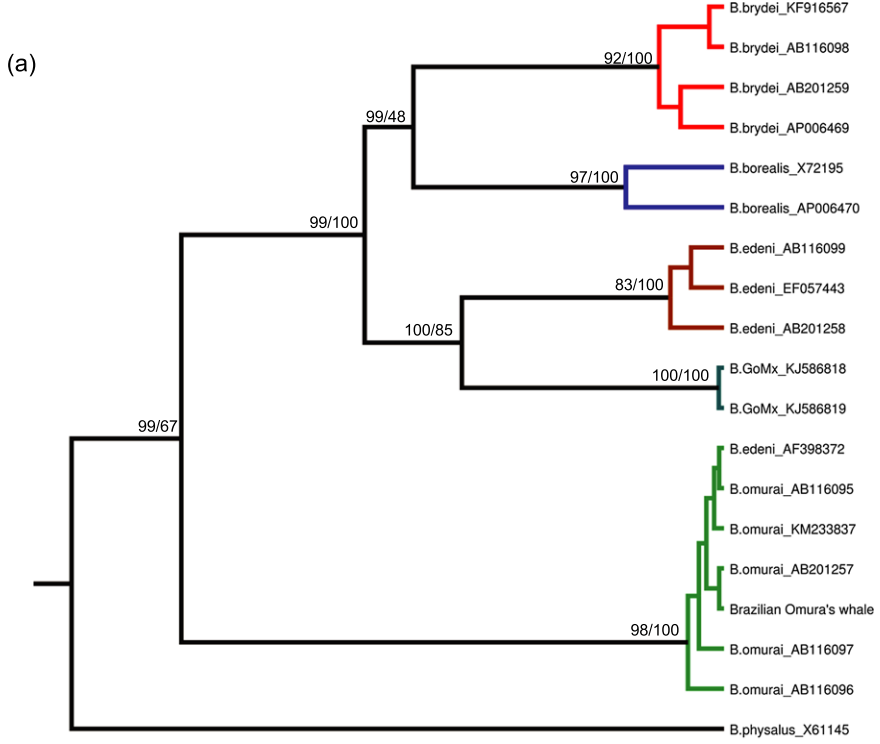


Figure 3. Bayesian phylogenetic trees of the Brazilian specimen and representative Balaenoptera species. Numbers in the nodes refer to ML bootstrap support and Bayesian posterior probabilities values, respectively. (a) Phylogeny based on control region sequences. (b) Phylogeny based on the concatenated *cytb+cox1* sequences. The taxonomic groups are indicated by colored branches. GenBank species names and accession numbers are given as branch tip labels (see Tables S1a and S1b for the reference sequences used in the analysis).

located in the posterior third of the body (Fig. 2B). A single, well-developed central ridge was observed in the rostrum (Fig. 2C). From above, lateral margins of the rostrum were slightly convex, giving it a non-U shape (Fig. 2C). The pectoral fins were small, about 10% of body size. Sixty-five ventral grooves were identified in the postaxillary region and only a few extended beyond the umbilicus (Fig. 2D). The morphometric measures are presented in Table 3. Because most of the skull was absent, the body total length and consequently the body proportions may be inaccurate within a few centimeters. Exterior borders of the maxilla were convex, and the ascending process of the maxilla widened slightly toward the posterior end, which was rounded in the right maxilla and was broken in the left. The angle of the mandible projected posteriorly, with the angular portion ending after the posterior edge of the condyle (articular portion). The osteological measures are also presented in the Table 3. The skin color pattern and the skull were not available.

The morphometric characters of the specimen studied here were compared with those of other Balaenopteridae (Table S2). The small body size excluded blue whale (*Balaenoptera musculus*) and fin whale (*B. physalus*), since newborn of these species are bigger than 6 m at birth (Deméré 2014). The presence of a single central ridge in the rostrum excluded *B. e. edeni* and *B. e. brydei*, the only rorquals that possess a ridge complemented by laterally auxiliary ridges. The rostrum format also excluded minke whale species (*B. acutorostrata* and *B. bonaerensis*) that have a sharply pointed rostrum. These species also have ventral grooves that terminate anterior to the umbilicus, as does the sei whale (*B. borealis*) (Deméré 2014). At the time of the stranding the number of ventral grooves and the shape of dorsal fin did not match the description available for Omura's whale (Wada *et al.* 2003), but with the new data (Cerchio *et al.* 2015, Jung *et al.* 2016) these characteristics are now within the species' range. The mandible shape was also different from the characters described for blue and sei whales, in which the angular portion ends before the hind edge of the condyle (Miller 1924, Omura *et al.* 1970); and for Bryde's whales, in which the posterior extension of the angular portion is at the same level or projects behind the condyle (Junge 1950, Omura *et al.* 1981). Summing up, through a process of elimination, the morphological characteristics of the specimen are compatible with the Brazilian stranding being an Omura's whale.

DISCUSSION

Our results based on three mtDNA markers and morphology clearly indicate that the whale specimen found stranded in the Ceará coast, northeastern Brazil, was an Omura's whale, providing the first report of the species in the South Atlantic Ocean and the second in the Atlantic Ocean. The contamination by exogenous sources could be ruled out since no sample from any Bryde's or Omura's whale was previously stocked or manipulated in the genetics

Table 3. Morphometric and osteological (based on Omura 1975) measures of the specimen of Omura's whale from Brazil. Skull was missing and consequently the measurements may be inaccurate within a few centimeters.

	Measurement (cm)
Morphometric parameter	
Total length	416
Tip of maxilla to dorsal fin base	278
Tip of maxilla to pectoral fin base	135
Tip of maxilla to anus center	302
Caudal fin maximum width	83
Pectoral fin length from anterior insertion to end	48
Pectoral fin length from axilla to end	43
Pectoral fin maximum width	15
Dorsal fin base	26
Dorsal fin height	12
Number of the plates	65
Osteological parameter	
Premaxilla length	56
Rostrum length	51
Rostrum breadth at the middle	19.5
Rostrum breadth at the antorbital notch	27.5
Mandible length along outer surface (curvilinearly)	73
Mandible length (straight)	84
Mandible breadth at 1/2 length of the mandible (curvilinearly)	5
Mandible height at 1/2 length of the mandible (curvilinearly)	6
Coronoid process height	12

laboratory at PUCRS. In addition, the hypothesis that the individual is a hybrid with maternal Omura's whale parent is very unlikely as argued by Jung *et al.* (2016), but to test this it would be necessary to have information on biparental markers on all the species of the group.

The recent description and the difficulty of identifying Omura's whale in the field result in a poorly defined distribution and, until recently, the species was found with confidence only in the western Pacific Ocean and the eastern Indian Ocean (see Fig. 1) (Wada *et al.* 2003; Sasaki *et al.* 2006; Yamada *et al.* 2006a, b, 2008, 2009; Adulyanukosol *et al.* 2012; Garrigue and Poupon 2013). However, when this manuscript was in review, publication of reports of a population off northwest Madagascar (Cerchio *et al.* 2015) and a specimen stranded on the Mauritania coast, in West Africa, in 2013 (Jung *et al.* 2016) occurred, extending the known range of the Omura's whale to the southwestern Indian Ocean and the northeastern Atlantic Ocean. Our finding is the first record of this species in the South Atlantic, and the specimen found stranded on the Mauritania is the first record in the North Atlantic, reinforcing the hypothesis of the occurrence of Omura's whale in the Atlantic Ocean. Bryde's whales have been regularly recorded along the Brazilian coast, mainly in southeastern and southern regions during the austral summer and autumn (Zerbini *et al.* 1997, Siciliano *et al.* 2004, Andriolo *et al.* 2010, Sholl *et al.* 2013, Gonçalves *et al.* 2015, Lodi *et al.* 2015, Pastene *et al.* 2015). The only genetic study of South American Bryde's whales identified whales from Peru, Chile, and Brazil as *B. brydei* (Pastene *et al.* 2015). However, despite the genetic identification of

eight samples from Brazil as *B. brydei*, it is possible that some previously reported Bryde's whales along the Brazilian coast may actually correspond to unrecognized sightings of Omura's whale.

With the new data presented recently and here, it may be possible to begin to answer the question raised by Jung *et al.* (2016): Is there an autochthonous Omura's whale population in the Atlantic or are these vagrant individuals? Jung *et al.* (2016) already presented arguments against the vagrant hypothesis, particularly because the individual they found was a juvenile. The presence of a second juvenile, this time in the South points to the Atlantic Ocean as an area of birth and/or reproduction (breeding ground) for the Omura's whale and argues for a revision of the global range of the species.

With regard to the question we pose about the species being rare or cryptic, we could say it is (or was) certainly a cryptic species, since until the results from Cerchio *et al.* (2015) there has been no detailed information of its external appearance and the species was most likely mistaken for the small form of Bryde's whale, and therefore not regularly identified in the field. The identification of a population off Madagascar and another possible population (or populations) in the Atlantic suggest that Omura's whale may not be as rare as the previously known restricted range would imply, although presently it is not possible to estimate its abundance with any accuracy. Cerchio *et al.*'s (2015) detailed description of the external appearance of the species will be very useful for identification of future sightings, but it is very important that whenever a conclusive morphological identification is not possible, all putative Bryde's whale specimen (stranded, including previously obtained samples and museum material) should be identified by molecular data.

Although the data are yet insufficient for robust estimates, the Omura's whale seems to have low genetic diversity, with only four mtDNA control region haplotypes and four variable sites found in 28 specimens studied so far in all three oceans. This low genetic diversity is similar to that reported in populations of *B. e. edeni* in the Indian Ocean and of *B. e. GOMx* in the Gulf of Mexico (Kershaw *et al.* 2013, Rosel and Wilcox 2014), which are also tropical and nonmigratory species (Rosel and Wilcox 2014, Cerchio *et al.* 2015). On the other hand, there are shared haplotypes between individuals from different oceans, such as between the Brazilian Omura's whale and specimens from Japan, China, and Mauritania. Scenarios that could explain the Omura's whale genetic structure include high gene flow among the populations and/or that the populations were recently established from a small source population. To distinguish between such hypotheses requires further information, including data on biparental genetic markers.

ACKNOWLEDGMENTS

We would like to thank the AQUASIS's *Projeto Manatí*, sponsored by PETROBRAS, and its rescue team, especially to Cristine Negrão. We also thank colleagues from the Laboratório de Biologia Genômica e Molecular/PUCRS, especially Tiago Ferraz da Silva for his help in lab activities. Special thanks to C. Scott Baker, to the editors Dr. Patricia Rosel and Dr. Daryl J. Boness, and three anonymous reviewers for comments and suggestions on earlier versions of the manuscript. S. L. Bonatto received a research grant from the Brazilian Research Bureau (Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq) and from Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). The collecting of specimens was carried out under permit ICMBio n° 14104-3.

LITERATURE CITED

- Adulyanukosol, K., S. Thongsukdee, S. Passada, T. Prempre and T. Wannarangsee. 2012. Bryde's whales in Thailand. Aksornthai Printing Co, Bangkok, Thailand.
- Andriolo, A., P. G. Kinas, M. H. Engel and C. C. A. Martins. 2010. Humpback whale population estimates and distribution along the Brazilian breeding ground. *Endangered Species Research* 11:233–243.
- Archer, F. I., P. A. Morin, B. L. Hancock-Hanser, K. M. Robertson, M. S. Leslie, M. Bérubé and B. L. Taylor. 2013. Mitogenomic phylogenetics of fin whales (*Balaenoptera physalus* spp.): Genetic evidence for revision of subspecies. *PLOS ONE* 8(5):e63396.
- Árnason, U., A. Gullberg and B. Widegren. 1991. The complete nucleotide sequence of the mitochondrial DNA of the fin whale, *Balaenoptera physalus*. *Journal of Molecular Evolution* 33:556–568.
- Baker, C. S., A. Perry, J. L. Bannister, et al. 1993. Abundant mitochondrial DNA variation and worldwide population structure in humpback whales. *Proceedings of the National Academy of Sciences of the United States of America* 90:8239–8243.
- Clapham, P. J., A. Agular and L. T. Hatch. 2008. Determining spatial and temporal scales for management: Lessons from whaling. *Marine Mammal Science* 24:183–201.
- Cerchio, S., B. Andrianantenaina, A. Lindsay, M. Rekdahl, N. Andrianarivelo and T. Rasoloarijao. 2015. Omura's whales (*Balaenoptera omurai*) off northwest Madagascar: Ecology, behaviour and conservation needs. *Royal Society Open Science* 2:150301.
- Committee on Taxonomy. 2014. List of marine mammal species and subspecies. Society for Marine Mammalogy. Available at <http://www.marinemammalscience.org> (accessed 9 September 2014).
- Deméré, T. 2014. Family Balaenopteridae (Rorquals). Pages 242–299 in D. E. Wilson and R. A. Mittermeier, eds. *Handbook of the mammals of the world. Volume 4. Sea mammals*. Lynx Edicions, Barcelona, Spain.
- Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7(1):214.
- Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32:1792–1797.
- Engel, M. H., N. J. R. Fagundes, H. C. Rosenbaum, et al. 2008. Mitochondrial DNA diversity of the southwestern Atlantic humpback whale (*Megaptera novaeangliae*) breeding area off Brazil, and the potential connections to Antarctic feeding areas. *Conservation Genetics* 9:1253–1262.
- Felsenstein, J. 1984. Distance methods for inferring phylogenies: A justification. *Evolution* 38:16–24.
- Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3:294–299.
- Garrigue, C., and M. Poupon. 2013. Guide d'identification: Mammifères Marins de Nouvelle-Calédonie [Identification guide: Marine mammals of New Caladonia]. Artypo, Nouméa, New Caledonia.
- Geraci, J. R., and V. J. Lounsbury. 2005. *Marine mammals ashore: A field guide for strandings*. 2nd edition. National Aquarium in Baltimore, Baltimore, MD.
- Gonçalves, L. R., M. Augustowski and A. Andriolo. 2015. Occurrence, distribution and behaviour of Bryde's whales (Cetacea: Mysticeti) off south-east Brazil. *Journal of the Marine Biological Association of the United Kingdom* 96:943–954.
- Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk and O. Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* 59:307–321.
- Hebert, P. D. N., A. Cywinska, S. L. Ball and J. R. deWaard. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270:313–321.

- Herath, D. R. 2007. Identification of a stranded whale by mitochondrial DNA analysis - www.DNA-Surveillance program in action. *Asian Fisheries Science* 20:319–324.
- Jayasankar, P., B. Anoop, M. Rajagopalan, *et al.* 2009. Indian efforts on the inventorization of marine mammal species for their conservation and management. *Asian Fisheries Science* 22:143–155.
- Johnson, M., I. Zaretskaya, Y. Raytselis, Y. Merezhuik, S. McGinnis and T. L. Madden. 2008. NCBI BLAST: A better web interface. *Nucleic Acids Research* 36(Web Server issue): W5–W9.
- Jung, J. L., W. C. Mullié, K. Van Waerebeek, *et al.* 2016. Omura's whale off West Africa: Autochthonous population or inter-oceanic vagrant in the Atlantic Ocean? *Marine Biology Research* 12:66–75.
- Junge, G. C. A. 1950. On a specimen of the rare fin whale, *Balaenoptera edeni* Anderson, stranded on Puli Sugi near Singapore. *Zoologische Verhandelingen* 9:1–26.
- Kanda, N., M. Goto, H. Kato, M. V. McPhee and L. A. Pastene. 2007. Population genetic structure of Bryde's whales (*Balaenoptera brydei*) at the inter-oceanic and trans-equatorial levels. *Conservation Genetics* 8:853–864.
- Kershaw, F., M. S. Leslie, T. Collins, *et al.* 2013. Population differentiation of 2 forms of Bryde's whales in the Indian and Pacific Oceans. *Journal of Heredity* 104:755–764.
- Librado, P., and J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452.
- Lodi, L., R. H. Tardin, B. Hetzel, I. S. Maciel, L. D. Figueiredo and S. M. Simão. 2015. Bryde's whale (Cetartiodactyla: Balaenopteridae) occurrence and movements in coastal areas of southeastern Brazil. *Zoologia* 32:171–175.
- Lowenstein, J. H., G. Amato and S. O. Kolokotronis. 2009. The real maccoyii: Identifying tuna sushi with DNA barcodes-contrasting characteristic attributes and genetic distances. *PLOS ONE* 4(11):e7866.
- Luksenburg, J. A., A. Henriquez and G. Sangster. 2015. Molecular and morphological evidence for the subspecific identity of Bryde's whales in the southern Caribbean. *Marine Mammal Science* 31:1568–1579.
- Ma, M., Q. Zhu, X. Li, Y. Sun, Y. Liu, Y. Liu and C. Tao. 2007. Identification of *Balaenoptera omurai* from an unknown whale vertebra based on Cyt b gene sequence [in Chinese]. *Acta Theriologica Sinica* 27:288–292.
- Meirelles, A. C. O., C. Monteiro-Neto, A. M. A. Martins, A. F. Costa, H. M. D. R. Barros and M. D. O. Alvez. 2009. Cetacean strandings on the coast of Ceará, north-eastern Brazil (1992–2005). *Journal of the Marine Biological Association of the United Kingdom* 89:1083–1090.
- Meirelles, A. C. O., A. C. Ribeiro, C. P. N. Silva and A. A. Soares-Filho. 2010. Records of Guiana dolphin, *Sotalia guianensis*, in the state of Ceará, northeastern Brazil. *Latin American Journal of Aquatic Mammals* 8:97–102.
- Miller, G. S. 1924. A pollack whale from Florida presented to the National Museum by the Miami Aquarium Association. *Proceedings of the United States National Museum* 66:1–15.
- Nakamura, G., and H. Kato. 2014. Development changes in the skull morphology of common minke whales, *Balaenoptera acutorostrata*. *Journal of Morphology* 275:113–1121.
- Omura, H. 1975. Osteological study of the minke whale from the Antarctic. *Scientific Reports of the Whales Research Institute, Tokyo* 27:1–36.
- Omura, H., T. Ichihara and T. Kasuya. 1970. Osteology of pygmy blue whale with additional information on external and other characteristics. *Scientific Reports of the Whales Research Institute, Tokyo* 22:1–27.
- Omura, H., T. Kasuya, H. Kato and S. Wada. 1981. Osteological study of the Bryde's whale from the central South Pacific and eastern Indian Ocean. *Scientific Reports of the Whales Research Institute, Tokyo* 33:1–26.

- Palumbi, S. R. 1996. Nucleic acids II: The polymerase chain reaction. Pages 205–247 in D. M. Hillis, C. Moritz and B. K. Mable, eds. *Molecular systematics*. Sinauer Associates, Sunderland, MA.
- Pastene, L., J. Acevedo, S. Siciliano, T. G. C. Sholl, J. F. de Moura, P. H. Ott and A. Aguayo-Lobo. 2015. Population genetic structure of the South American Bryde's whale. *Revista de Biología Marina y Oceanografía* 50:453–464.
- Penry, G. S. 2010. The biology of South African Bryde's whales. Ph.D. dissertation, University of St. Andrews, St. Andrews, U.K. 173 pp.
- Ponnampalam, L. S. 2012. Opportunistic observations on the distribution of cetaceans in the Malaysian South China, Sulu and Sulawesi Seas and an updated checklist of marine mammals in Malaysia. *The Raffles Bulletin of Zoology* 60:221–231.
- Rambaut, A. 2009. FigTree, a graphical viewer of phylogenetic trees. Available at <http://tree.bio.ed.ac.uk/software/figtree/>.
- Rambaut, A., and A. J. Drummond. 2007. Tracer v1.4: MCMC trace analyses tool. Available at <http://tree.bio.ed.ac.uk/software/tracer/>.
- Ratnasingham, S., and P. D. Hebert. 2007. BOLD: The barcode of life data systems (www.barcodinglife.org). *Molecular Ecology Notes* 7:355–364.
- Reilly, S. B., J. L. Bannister and P. B. Best, *et al.* 2008. *Balaenoptera omurai*. IUCN Red List of Threatened Species. Version 2015.2. Available at <http://www.iucnredlist.org>.
- Rosel, P. E., and L. A. Wilcox. 2014. Genetic evidence reveals a unique lineage of Bryde's whales in the northern Gulf of Mexico. *Endangered Species Research* 25:19–34.
- Ross, H. A., G. M. Lento, M. L. Dalebout, *et al.* 2003. DNA Surveillance: Web-based molecular identification of whales, dolphins, and porpoises. *Journal of Heredity* 94:111–114.
- Sarkar, I. N., J. W. Thornton, P. J. Planet, D. H. Figurski, B. Schierwater and R. DeSalle. 2002. An automated phylogenetic key for classifying homeoboxes. *Molecular Phylogenetics and Evolution* 24:388–399.
- Sasaki, T., M. Nikaido, H. Hamilton, *et al.* 2005. Mitochondrial phylogenetics and evolution of mysticete whales. *Systematic Biology* 54:77–90.
- Sasaki, T., M. Nikaido, S. Wada, T. K. Yamada, Y. Cao, M. Hasegawa and N. Okada. 2006. *Balaenoptera omurai* is a newly discovered baleen whale that represents an ancient evolutionary lineage. *Molecular Phylogenetics and Evolution* 41:40–52.
- Sholl, T. G. C., J. F. de Moura, P. H. Ott, C. R. Bonvicino, E. C. Reis, D. C. Tavares and S. Siciliano. 2013. Cytochrome b sequencing for the species identification of whale carcasses washed ashore in Brazil. *Marine Biodiversity Records* 6:e30.
- Siciliano, S., M. C. Oliveira Santos, A. F. Vicente, F. S. Alvarenga, É. Zampiroli, J. L. Brito and J. L. A. Pizzorno. 2004. Strandings and feeding records of Bryde's whales (*Balaenoptera edeni*) in south-eastern Brazil. *Journal of the Marine Biological Association of the UK* 84:857–859.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725–2729.
- Vine, S. J., M. S. Crowther, S. J. Lapidge, C. R. Dickman, N. Mooney, M. P. Piggott and A. W. English. 2009. Comparison of methods to detect rare and cryptic species: A case study using the red fox (*Vulpes vulpes*). *Wildlife Research* 36:436–446.
- Wada, S., M. Oishi and T. K. Yamada. 2003. A newly discovered species of living baleen whale. *Nature* 426:278–281.
- Yamada, T. 2009. Omura's whale, *Balaenoptera omurai*. Pages 330–331 in S. D. Ohdachi, Y. Ishibashi, M. A. Iwasa and T. Saitoh, eds. *The wild mammals of Japan*. Shoukahoh Book Sellers and Mammalogical Society of Japan, Kyoto, Japan.
- Yamada, T. K., L.-S. Chou, S. Chantrapornsy, *et al.* 2006a. Middle sized balaenopterid whales specimens (Cetacea: Balaenopteridae) preserved at several institutions in Taiwan, Thailand, and India. *Memoir of National Science Museum, Tokyo* 44:1–10.

- Yamada, T. K., C. Kemper, Y. Tajima, A. Umetani, H. Janetzki and D. Pemberton. 2006*b*. Marine mammals collections in Australia. National Science Museum Monographs 34:116–126.
- Yamada, T. K., T. Kakuda and Y. Tajima. 2008. Middle sized balaenopterid whale specimens in the Philippines and Indonesia. Memoir of National Science Museum, Tokyo 45:75–83.
- Yang, G., H. Liu, K. Zhou and J. Guo Qing. 2002. Identification of a *Balaenoptera edeni* specimen by using mitochondrial DNA sequences. Chinese Journal of Zoology 37:35–38.
- Yoshida, H., and H. Kato. 1999. Phylogenetic relationships of Bryde's whales in the western North Pacific and adjacent waters inferred from mitochondrial DNA sequences. Marine Mammal Science 15:1–10.
- Zerbini, A. N., E. R. Secchi, S. Siciliano and P. C. Simões-Lopes. 1997. A review of the occurrence and distribution of whales of the genus *Balaenoptera* along the Brazilian coast. Report of the International Whaling Commission 47:407–417.

Received: 18 June 2015

Accepted: 19 May 2016

SUPPORTING INFORMATION

The following supporting information is available for this article online at <http://onlinelibrary.wiley.com/doi/10.1111/mms.12348/supinfo>.

Table S1a. Balaenopteridae sequences from GenBank used in phylogenetic analysis for the control region (433 bp). Species names are listed as provided in GenBank. AF398372 (red) is misidentified on GenBank as *B. e. edeni* since this sequence was submitted by Yang *et al.* (2002) before the *B. omurai* description.

Table S1b. Balaenopteridae *cytb* (444 bp) and *cox1* (655 bp) sequences from GenBank used in phylogenetic analysis. Species names are listed as provided in GenBank.

Table S2. External appearance of the whale stranded in Brazil compared with information available on Balaenopteridae species. Gray cells indicate characters that are not observed in the studied specimen.

Figure S1. DNA surveillance trees showing high bootstrap support (100%) grouping Brazilian Omura's whale with the reference sequences of *Balaenoptera omurai* (Bryde's [omurai] or [Solomon Islands]). (a) NJ tree based on control region query and reference sequences of all recognized mysticetes. (b) NJ tree based on cytochrome *b* query and reference sequences of all recognized mysticetes. Bootstrap values based on 1,000 replicates.

Figure S2. BOLD Systems result with the NJ tree for *cox1* sequences showing 100% of sequence similarity between Brazilian Omura's whale ("unknown specimen") and the *B. omurai* reference sequences.