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First specimen record of *Balaenoptera omurai* off West Africa: interoceanic straggler or indication of a possible Atlantic population?

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Abstract

Surveillance of marine mammal strandings within the framework of a biomonitoring scheme of the Mauritanian coast led to the finding in 2013 of a 3.98m long, juvenile rorqual *Balaenoptera* sp. in advanced decomposition near Chott Boul (16°32.488' N, 016°27.0317'W). Photographs and skin samples were taken but the specimen could not be collected. Based on limited morphological evidence only *B. edeni* and *B. omurai* were plausible. Sequences of three mtDNA regions, *i.e.* parts of the *cox1* and the *cytb* genes as well as the D-loop, for a total of 2,636 bp (>16% of the mitogenome) unambiguously identified the specimen as an Omura's whale *Balaenoptera omurai,* the first record in the Atlantic Ocean and at least 18,000 km away from its closest known range in the (sub)tropical Indo-Pacific. The question whether the specimen is a vagrant or belongs to an unrecognized Atlantic population is discussed. Long-distance advection by currents or transport on a ship's bow bulb following collision are discarded. Circumstances (calf/juvenile status, great distance from Indo-Pacific, necessary inter-oceanic passage through cold temperate waters) may slightly favour the hypothesis that *B. omurai*, if rare, could be autochthonous in the Atlantic. Beach surveys remain a useful tool to assess trends in cetacean species composition, to detect unusual mortality events and to help assess impacts of anthropogenic activities. This is particularly applicable to remote areas where the marine mammal fauna is poorly known and where fisheries effort is high, such as the Mauritanian coasts.

Introduction

The Canary Current is recognized as one of the four major eastern boundary upwelling areas of the world, located along the coasts of the Canary Islands, Morocco, Western Sahara, Mauritania, Senegal and The Gambia (Sherman, 1995). Upwelled waters, rich in nutrients, lead to high primary productivity and consequently large stocks of fishes and cephalopods, attracting top predators. With cool coastal upwelling and offshore subtropical habitats juxtaposed, the cetacean fauna of Mauritania is highly diverse, consisting of at least 26 cetacean species, conservatively estimated from confirmed sightings and strandings (Maigret, 1980; Maigret et al. 1976; Perrin & Van Waerebeek, 2012). This places Mauritania among West African coasts with the highest cetacean biodiversity immediately after Senegal and the Canary Islands (both 29 species). The biogeographical origin of this species assemblage ranges from tropical to north temperate to cosmopolitan (Perrin & Van Waerebeek, 2012).

As a key component of the programme "Biodiversité, Gaz, Pétrole" (BGP) spearheaded by the Mauritanian Ministry of Environment, in close cooperation with the Ministry of Fisheries and the Ministry of Oil, an integrated marine and coastal biomonitoring project was launched in November 2012. Beach surveys were conducted every three months to monitor stranded cetaceans, turtles and birds along 390 km of the southern coast between the Senegalese border (16°6.4316'N, 016°30.6183'W) and Mamghar (19°21.2233'N, 016°30.8883W) with less frequent visits to parts of the coast of the Banc d'Arguin National Park and with limited transects along the Baie de Lévrier and the Atlantic coast of Cap Blanc, near Nouadhibou (Mullié et al. 2013). Remains of stranded cetaceans were most frequently in an advanced state of decomposition and/or very incomplete or damaged, making species identification often difficult. Since August 2013 tissue or bone samples were also taken for molecular identification, via a DNA barcoding approach (Alfonsi et al. 2013).

This paper describes a balaenopterid whale stranding on a South Mauritanian beach in 2013, which, following mtDNA sequencing, was identified as an Omura's whale *Balaenoptera omurai* Wada, Oishi, and Yamada, 2003. Although Omura's whale was only recently described (Wada et al. 2003), its separate species status is now well established phylogenetically (Sasaki et al. 2006). Remarkably, the known distribution of Omura's whale is limited to the eastern Indian Ocean and the western Pacific Ocean (Reilly et al. 2008; Wada et al. 2003). The key question is discussed whether the specimen is a vagrant or whether *B. omurai* is autochthonous also in the Atlantic Ocean.

Materials and methods

Beach surveys

Beach surveys were conducted in November 2012, February, April, August and November 2013 by two or three 4x4 wheel drive vehicles, from two hours past high tide till low tide while driving on the lower part of the beach. During each mission, the coast between Nouakchott and the Senegalese border (223 km) was surveyed during three successive days and between Nouakchott and Mamghar (165 km) usually in two-three days. Because the higher parts of the beach beyond the most recent high tide marks often could not properly be scouted from the car, since August 2013, 30-45 km of transects were done on foot, usually in stretches of about 6-8 km either side of each camp site.

DNA-Based analysis

Genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen), following the instructions of the manufacturer. *Cox1, CytB and* D-Loop regions were amplified as described (Alfonsi et al. 2012; Garrigue et al. 2004; LeDuc et al. 1999; Viricel & Rosel, 2012). DNA sequencing was performed by a provider (GATC, Germany), using one of the PCR primers. Electropherograms were edited manually using the Sequence scanner software (Applied Biosystems). Similarity searches with databases of known sequences were performed for *cox1* using the Barcode of Life Data Systems (BOLD) interface (accessible at http://www.boldsystems.org), and for all 3 markers to the whole GenBank using BLAST search (Johnson et al. 2008). A maximum-likelihood (ML) approach was used to construct phylogenetic trees on an online phylogeny pipeline (Dereeper et al. 2008).

Results

Beach surveys, specimen analysis and sampling

On 3 November 2013 an unidentified juvenile baleen whale *Balaenoptera* sp. with a total length of 3.98 m was found by WCM and ZAOS some 7 km south of Chott Boul adjacent to the Diawling National Park (16° 32.4883' N, 016° 27.0317' W). Due to logistical limitations the specimen (referred to as MauBs) could neither be necropsied nor collected, hence no osteological study was possible. The following *a posteriori* exclusion analysis of candidate rorqual species was based on photographs (Fig.1) and field notes.

With the carcass in advanced decomposition (Code 4, Geraci & Lounsbury, 1993), no colour pattern was discernible. Also, all baleen plates had detached from the palate and none were found at the stranding site, strongly suggesting that the whale had washed ashore dead after floating some time at sea. The anogenital area was damaged and sex could not be determined. The head showed a relatively narrow, pointed rostrum, but less triangular than in minke whales (Fig.1). No head ridges were examined as the animal laid on its back.

A minimum of 70 ventral pleats was estimated from the photos. The small body size, dorsal fin shape and the non U-shaped rostrum excluded *B. musculus* (neonatal SL 7-8m), small size and shape of the dorsal fin excluded *B. physalus* (neonatal SL 6-6.5m), while size was also incongruent with *B. borealis* (neonatal SL 4.5-4.8m) (Jefferson et al. 2008). A broad-based and almost triangular dorsal fin was reminiscent of fins seen in some Bryde's and Omura's whales. There was no indication of a conspicuously backwards-pointed dorsal fin, a feature Jefferson et al. (2008) had suggested as potentially characteristic for Omura's whale.

The mandibles of MauBs appeared uncharacteristically slender for *B. brydei.* In addition, despite a body length compatible with *B. brydei* at a neonatal stage (4 m, Jefferson et al. 2008) the lack of a prominent umbilicus, suggesting post-neonatal status, also tended towards excluding this species. The ventral pleats extended far posteriad, beyond the tip of the flippers and probably beyond the umbilicus, in contrast with the very short pleats anteriad of the umbilicus in *B. borealis* (Jefferson et al. 2008) and longer also than the moderately short pleats in *B. acutorostrata* and *B. bonaerensis.* While *B. acutorostrata* occurs at least as far south as Hann, Senegal (14°21'N, 17°27'W (Van Waerebeek et al. 1999)), the most boreal specimen record of Antarctic minke whale *B. bonaerensis* in the eastern Atlantic is from Togo, at 06°12.34'N, 01°29.64'E (Segniagbeto et al. 2014). Despite the limited morphological evidence, only two rorqual species (*B. edeni* and *B. omurai*) remained plausible candidates, both without known Atlantic distribution, thus pointing to a potentially unusual specimen.

Figure 1: External morphology of rorqual specimen MauBs, general view of carcass.

© Image: Wim C. Mullié

A sample of the ventral skin was collected and stored in a cryo-vial with 90^{o} ethanol for DNA analysis. Skin samples of *B. omurai* were shipped to France under CITES export permit no. 2014-001 of 27/03/2014 and CITES import permit no. FR1402900008-1 of 28/01/2014 provided by the designated authorities in respectively

Mauritania and France.

Molecular identification

We amplified and sequenced 3 different mitochondrial regions from MauBs DNA: the 5' end of the *cox1* (cytochrome C Oxidase subunit 1) gene (732 bp, Genbank accession number KM233839), part of the *cytb* (cytochrome b) gene (1106 bp, Genbank accession number KM233837) and 798 bp corresponding to the end of the tRNA-pro gene and to the D-Loop (Genbank accession number KM233838). These 3 sequences represented a total of 2,636 bp (more than 16% of the mitochondrial genome) and targeted the 3 most used markers in population genetics and species identification. The sequences were compared to the Genbank nucleotide database using a Blast search (Johnson et al. 2008), and matched baleen whale sequences. For the 3 markers, results were unambiguous and gave a 99-100% match to *B. omurai* sequences (Table 1). All other tested mysticetes sequences presented strong similarities, but clearly lower than with the *B. omurai* ones.

Sequences determined from Mauritanian specimen		**Closest matches**		**Nearest neighbours, except the closest matches**	
Marker	**Length (bp)**	**Species (Genbank reference)**	**Identities (percentages)**	**Species (Genbank reference)**	**Average identity percentages**
Cox1	732	*B. omurai* (AB201257.1, AB201256.1)	732/732 and 731/732 **(100%-99%)**	*B. edeni* (AB201258.1) *B. physalus* (KC572859.1) *B. musculus* (X72204.1) B. borealis (AP006470.1) *B. bonaerensis* (AP006466.1) *M. novaeangliae* (GQ353285.1)	**93%**
Cytb	1106	*B. omurai* (AB201257.1, AB201256.1)	1106/1106 and 1103/1106 **(100%-99%)**	*B. borealis* (X75582.1) *B. edeni* (AB201258.1) *B. musculus* (X72204.1) *B physalus* (KC572827.1)	**91%-92%**
D-Loop	798	*B. omurai* (AB201257.1, AB201256.1, AB116095, AB116097.1, AB116096.1) *“B. edeni” (*AF398372)	Between 798/798 and 706/710, **(100%-99%)**	*B. edeni* (AB201258.1) *B. borealis (*AP006470.1) *B. brydei* (AP006469.1) *B. physalus* (AY582748.1)	**91%-92%**

Table 1: The sequences of the 3 markers determined from MauBs specimen were blasted against the whole Genbank. For the three markers, sequences of the complete mitochondrial genome of *B. omurai* specimens gave the closest matches, with more than 99% of identities. The only exception was a D-Loop sequence mislabelled as "*Balaenoptera edeni*" (AF398372), which was submitted in august 2001, before *B. omurai* was recognized, and not corrected since. Except these closest matches, the nearest neighbours (between 90% and 93% of identities) comprise the other members of the rorqual family (Balaenopteridae). One genbank reference is given as example for each species.

We also used the identification engine of the Barcoding of Life Database System (www.boldsystem.org), which made a species level identification with a 100% probability of placement in the *B. omurai* species. We then reconstructed 4 maximum likelihood (ML) trees, based on *cox1*, *cytb*, D-Loop and on the concatenation (see Fig. 2) of the three regions, using Genbank sequences of one representative for each species of the rorqual family (Balaenopteridae, genera *Balaenoptera* and *Megaptera*). The four trees all formed a specific clade grouping MauBs and the *B. omurai* sequence, all supported by a 100% bootstrap. BOLD identification engine, Blast search against Genbank and ML trees all produced very robust identifications of MauBs as a member of *B. omurai*.

Discussion

Convincing comparative studies of a clade grouping the rorquals (Balaenopteridae), including the recently described *B. omurai* (Wada et al. 2003), and the Eschrichtiidae families, using mtDNA coding sequences and SINE (short interspersed repetitive element) insertions in nuclear DNA, led to the hypothesis of a separation in four lineages of the clade around 20Mya (Nikaido et al. 2006; Sasaki et al. 2005; 2006). One of these lineages would comprise *B. omurai, B. edeni*, *B. brydei* (*i.e.* the former "Bryde's whale complex", Rice, 1998), *B. musculus* and *B. borealis*.

B. omurai diverged very early from the other species of the clade (around 17 Mya), making the history of the species ancient (Sasaki et al. 2006). All sequences of *B. omurai* contained in Genbank (holotype and all other positively identified specimen (Wada et al. 2003), and see (Sasaki et al. 2006) for a deciphering of samples before 2003) are almost identical while at the same time markedly different from all other rorqual species sequences found in Genbank. This leads to a relatively clear phylogenetic tree of the lineage 3 (defined by Nikaido et al. 2006 and by Sasaki et al. 2005) in the rorqual family.

The *sensu stricto* DNA barcoding approach (Hebert et al. 2003; Valentini et al. 2009) has been successfully applied to the identification of Balaenopteridae species (Alfonsi et al. 2013; Viricel & Rosel, 2012). A clear gap exists between interspecific and intraspecific variations of the *cox1* sequences in the Balaenopteridae (*i.e.* the barcoding gap Wiemers & Fiedler, 2007). One of the consequences is that undoubtful molecular identifications of *B. omurai* samples among all other species can be performed relatively easily.

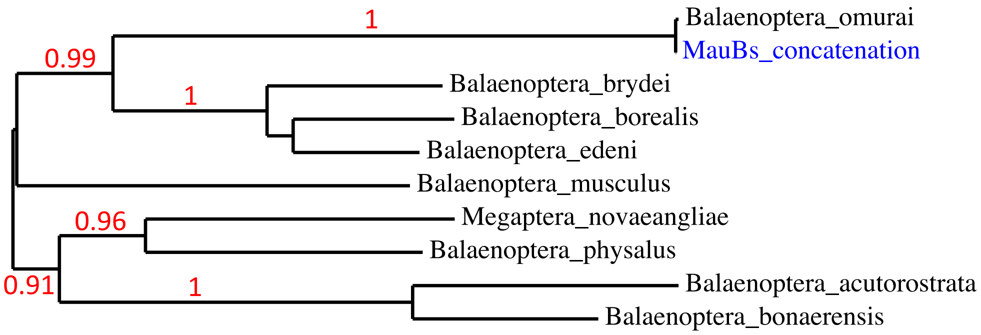

Figure 2: ML tree-based identification of specimen MauBs. Reference sequences were extracted from the complete mitochondrial genome sequences of *B. omurai* (Genbank ref. AB201257.1), *B. borealis* (AP006470.1), *B. edeni* (AB201258.1), *B. brydei* (AP006469.1), *B. musculus* (X72204.1), *B. physalus* (KC572839.1), *B. acutorostrata* (AP006468.1), *B. bonaerensis* (AP006466.1) and *M. novaeangliae* (AP006467.1). The sequences of 3 different markers (two partial coding sequences, *cox1* gene, 732bp and the *cytb* gene, 1106 bp; and 798 bp comprising the D-Loop) were concatenated, aligned with concatenated reference sequences, and ML tree constructed. The sequences of the specimen MauBs and of *B. omurai* group in a monophyletic clade supported by a maximum bootstrap of 1.

In contrast, *B. omurai* specimens are still difficult to identify morphologically as the current small sample of documented individuals does not allow a proper assessment of intraspecific variation. Small body size and long ventral pleats (Fig.1) helped to narrow down identification to *B. edeni* and *B. omurai*, both unknown in the Atlantic Ocean. Besides *B. edeni* and both minke whale species, *B. omurai* is the rorqual with the smaller adult body length (less than 12 m, Wada et al. 2003). Its general morphology and colouration resembles that of a small *B. physalus*, presenting an asymmetrical white pigmentation on the right side of the throat and ventrum. The inner surface of both flippers and the ventral surface of the flukes are white (Wada et al. 2003). Low in number (ca. 200) the baleen plates are short and broad, also with an asymmetrical colouration. A single rostrum ridge distinguishes it from Bryde's and Eden's whales which have three head ridges. It is less clear whether a very falciform dorsal fin can help identify *B. omurai* specimens (Reilly et al. 2008; Wada et al. 2003). Thus, external diagnostic characters exist but the apparent scarcity of the species may complicate identification at sea or when stranded. The skull and postcranial skeleton offer additional clues, in particular the morphology of the synvertex, nasal area, maxillaries and mandibles (Wada et al. 2003).

The sequencing results were absolutely unambiguous as for the three mitochondrial markers analysed the MauBs sequences matched *B. omurai* sequences with more than 99% similarity. The closer sequences found in other species presented more than 7% of divergence with MauBs, whatever the region considered (at least 7% of divergence for the 5' part of *cox1* with the closest Balaenopteridae species, 8% for *cytb* and 9% for the D-loop), which led to clear phylogenetic trees. The use of the BOLD identification engine (Ratnasingham & Hebert, 2007) also led to the unequivocal identification of MauBs as *B. omurai*. The use of the three targeted regions, provided a phylogenetic resolution that could be compared to the whole mitogenome (Duchêne et al. 2011). No *B. omurai* DNA has ever been manipulated or conserved in the BioGeMME laboratory in Brest

(France) before the arrival of the MauBs sample, and no other labs in the building work with marine mammal DNA. This eliminates any possibility of contamination of the PCRs by a previous *B. omurai* sample.

The known geographic range of *B. omurai,* based on limited data, is restricted to the tropical and subtropical waters between the eastern Indian Ocean and the western North Pacific Ocean (Reilly et al. 2008; Sasaki et al. 2006; Wada et al. 2003; Yamada et al. 2006). The species was described from specimens caught in the Sea of Japan (Wada et al. 2003), and molecular identification of archived specimens proved its presence in the Solomon Sea and around the Philippines (see Sasaki et al. 2006). *B. omurai* specimens have also recently been observed with more or less confidence off the Cook Islands[1], New Caledonia (Garrigue & Poupon, 2013) and off North Australia[2].

Specimen MauBs is the first *B. omurai* detected in the Atlantic Ocean and only two hypotheses can explain its presence. Either the whale was a long-distance vagrant from the Indo-Pacific, or the species is autochthonous also in the Atlantic Ocean. The coasts of Mauritania are at least 18,000 km away from the previously determined geographical range. Long-range advection of a carcass carried by ocean currents can be confidently discarded, as well as a 'bow bulb draping' event following a ship strike. A global review showed that only larger cetaceans including blue, fin, sei, Bryde's, and very rarely humpback whales, occasionally become wedged across a ship's bow bulb following a direct collision and may thus be transported up to a few hundred km from where they originated (Van Waerebeek et al. 2008). Significantly, no cases of bow bulb draping have been documented for minke whales nor for any other smaller cetacean (cf. 4 m of MauBs), presumably because short body length causes a carcass to slide off (Van Waerebeek et al. 2008). However, MauBs may still have died from a ship strike, but then in Mauritanian waters. Deep transversal incisions with smooth borders on the tail stock, evidently anthropogenic in origin, do not exclude that specimen MauBs may have been a bycatch victim either.

Most balaenopterids seasonally migrate. *B. brydei* and *B. edeni,* although capable of moving over considerable distances, do not leave (sub)tropical waters. Whether *B. omurai* migrates is unknown. Travels of more than 8,000 km are for instance demonstrated for humpback whales (Stone et al. 1990). But these spectacular seasonal migrations occur most often along a north-south axis within the same ocean basin, although examples of interoceanic travels exist (Pomilla & Rosenbaum, 2005).

While there are hardly any data about reproductive and growth parameters in *B. omurai,* such as size and age at weaning, the 3.98 m MauBs specimen was a calf or a juvenile which may or may not have been weaned, and a maternal female may have been present in the vicinity at the time of death. This age class or a mother-calf pair would seem unlikely candidates as extremely long distance vagrants. Moreover an interoceanic migration would have required a cold-water passage from the Indian Ocean (via Cape Agulhas) or South Pacific (via Cape Horn) towards the North Atlantic Ocean. After an Arctic passage, a Cape Horn route is considered the least plausible, as it would assume crossing both the eastern Pacific and Atlantic Oceans. However, the sighting of a gray whale *Eschrichtius robustus* in the Mediterranean in 2010, for instance, suggests that long-distance Pacific-to-North Atlantic movements are possible (Scheinin et al. 2011). The alternate hypothesis, that specimen MauBs might belong to an undescribed Northeast Atlantic population of *B. omurai,* may be as plausible as the vagrant explanation. Other individuals may have been encountered before at sea or stranded, but possibly were not recognized. The apparent scarcity of the species, its recent description, great morphological similarity to other small rorquals and its supposed Indo-Pacific range could all have contributed to making a positive identification difficult. An additional record would necessitate a profound revision of *B. omurai* global distribution and migratory behaviour. The Canary Current in general and the highly productive coasts of Mauritania in particular represent a suitable year-round or occasional feeding and breeding ground.

The monitoring of marine mammal strandings continues to provide information of great value in regards to biodiversity. Local and global oceanographic and climatic events influence marine mammal stranding patterns, but accurate data can be obtained with appropriate protocols (Camphuysen & Siemensma, 2011; Evans et al. 2005; Jung et al. 2009; Leeney et al. 2008; Mullié et al. 2013; Peltier et al. 2013). As suggested by several authors (Alfonsi et al. 2013; Baker, 2008), DNA-based identifications of decomposed carcasses are highly relevant for species that infrequently strand in remote locations and which are difficult to identify by field correspondents. Explorations of Mauritanian waters by oceanographic research vessels during the past decade have not only added many marine species new to science (e.g. De Matos-Pita & Ramil, 2014) but even a completely unknown 400 km long cold water coral ecosystem at a depth of 600 m (Colman et al. 2005). Although a national stranding network does not exist in Mauritania, the trimestrial beach surveys of the

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[1] http://www.whaleresearch.org/ourwork/mystery_whale.php

[2] http://www.pbase.com/wildlifeimages/omuras_whale

Programme BGP have already shown their use in cetacean biodiversity assessment and in documenting unusual mortality events (Mullié et al. 2013; Programme BGP, unpublished data). Beach surveys, thanks to relatively simple logistics as compared to either aerial or ship based surveys, will be maintained as a means to assess temporal trends in the cetacean species composition, to detect unusually high mortality and to estimate the impacts of anthropogenic activities in Mauritanian waters.

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