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**Short Note**

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# Genetic monitoring of pilot whales, *Globicephala* spp. (Cetacea: Delphinidae), stranded on French coasts

**Abstract:** We used mitochondrial DNA control region sequences to genetically identify to the species level 23 pilot whales (genus *Globicephala*) that stranded on the French coasts between 1996 and 2011. Genetic analysis complemented morphological diagnoses, often hampered by an overlap in morphological characters between the two species or incomplete measurements. Mitochondrial DNA data allowed identification of 21 long-finned pilot whales (*Globicephala melas*) and two unusual stranding events of the more tropical species (*Globicephala macrorhynchus*). In pilot whales as in most cetaceans, shifts in species range are expected to occur due to global climate change. In this context, our study contributes to the long-term monitoring of pilot whale stranding events, providing indirect information on species occurrence.

**Keywords:** forensics; genetic monitoring; marine mammal; mitochondrial DNA; stranding.

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Every year, several hundreds to 1000 marine mammals strand on French coasts (Van Canneyt et al. 2012). Each stranding is recorded by the Joint Service Unit PELAGIS (La Rochelle, France). The French marine mammal stranding network (coordinated by PELAGIS) performs species identifications and necropsies of stranded animals in an effort to monitor the marine mammal biodiversity and to report evidence of potential anthropogenic impacts (e.g., marks from incidental catch from fisheries). In cases of badly decomposed carcasses, or whenever there is ambiguity in morphological identification, genetic data can aid in the species identification process (Alfonsi et al. 2013). In this short communication, we complemented morphological species diagnoses with genetic data to assist with the monitoring of pilot whale (genus *Globicephala*, Lesson 1828) stranding events.

The genus *Globicephala* is comprised of two species that have opposite distribution patterns: the long-finned pilot whale [LFPW: *Globicephala melas*, (Traill 1809)] and the short-finned pilot whale (SFPW: *Globicephala macrorhynchus*, Gray 1846) (Wilson and Reeder 2005). The LFPW has an antitropical distribution and is present in cold temperate waters of the Atlantic Ocean, the Mediterranean Sea, and the southern hemisphere (Fullard et al. 2000), while the SFPW inhabits tropical and warm temperate waters all around the globe (Kasuya and Marsh 1984). The two species co-occur in a region of overlap in the North Atlantic Ocean, and the Bay of Biscay represents the northern limit of the SFPW's distribution range (Nores and Pérez 1988, Gonzalez et al. 2000). Furthermore, two extant subspecies of LFPW are recognized: *G. melas melas* (Traill 1809) (North Atlantic LFPW) and *G. melas edwardii*, Smith 1834 (Southern LFPW) (Wilson and Reeder 2005). Stranding events of LFPW on French coasts are a common phenomenon with 10–30 cases reported every year. Comparatively, stranding events of SFPW are rare (Van Canneyt et al. 2012). Indeed, the first stranding event of a SFPW in France was reported in 1966 (Duguy 1968), followed by another one in 1988 (Van Canneyt et al. 2012). More recently, two stranding events have been reported on the French Atlantic coast: one in 2008, and one (pending species confirmation) in 2011 (Van Canneyt et al. 2012).

In the current context of global climate change, shifts in distribution ranges are expected to occur for most cetacean species (MacLeod 2009). As pilot whales' distribution is correlated with water temperature (e.g., Hoydal and Lastein 1993), their species range may indeed be

indirectly affected by ongoing changes in sea-surface temperature. In this context, long-term monitoring of pilot whale stranding events can be used as a proxy to detect potential shifts in their distribution, especially to follow the evolution of the northern limit of the SFPW in the Atlantic Ocean.

Morphological features used to identify pilot whales to the species level are the number of teeth per half jaw (LFPW: 9–12; SFPW: 7–9), and the ratio of the length of the pectoral fin to the total length of the body (LFPW: 18–27%; SFPW: 14–19%) (Van Bree 1971, Robineau 2005). However, the range overlap in these morphological features hampers species diagnosis. Thus, genetic analysis appears to be a good complementary tool to the morphological approach. We reexamined species identification of past pilot whale stranding events using genetics to complement morphological diagnoses and confirm some of the recent unusual stranding events of SFPW that occurred on French coasts.

The mitochondrial DNA (mtDNA) control region can be used to reliably distinguish between the two species of pilot whales thanks to six diagnostic substitutions (Oremus et al. 2009). Thus, we sequenced a portion of the mtDNA control region to genetically identify the species of 23 pilot whales that stranded on French coasts between 1996 and 2011 and for which tissue samples had been collected (Table 1: voucher information provided by PELAGIS). DNA extractions were performed from approximately 25 mg of skin, muscle, or kidney tissue preserved in ethanol using a Nucleospin Tissue kit (Macherey-Nagel EURL, Hoerdt, France) following the manufacturer's protocol. PCR amplifications were performed using primers L15824 (5′-CCTCACTCCTCCCTAAGACT-3′) (Rosel et al. 1999) and H16498 (5′-CCTGAAGTAAGAACCAGATG-3′) (Rosel et al. 1994), and the PCR profile described in Vollmer et al. (2011). The 50-µl reactions included 50 ng genomic DNA, 0.3 µM for each primer, 0.15 mM dNTPs (Euromedex, Mundolsheim, France), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, and 2 U Taq polymerase (VWR, Fontenay sous Bois, France). PCR products were sent to Genoscreen (Lilles, France) for purification and Sanger sequencing (in both directions). Sequences were edited and aligned by eye using the BioEdit Sequence Alignment

**Table 1** Voucher information for 23 pilot whales genetically analyzed in this study.

| Voucher | Date | Lat. | Long. | State | M. | M. ID | G. ID | Hap |
|---|---|---|---|---|---|---|---|---|
| 9601009 | 30-Jan-96 | 44.9855 | -1.2052 | 2 | A | LFPW | LFPW | S |
| 9706105 | 22-Jun-97 | 45.9696 | -1.3899 | 2 | NA | | LFPW | S |
| 9904034 | 19-Apr-99 | 45.8777 | -1.2666 | 3 | NA | | LFPW | S |
| 9912065 | 17-Dec-99 | 46.2049 | -1.5359 | 2 | NA | | LFPW | S |
| 10104070 | 13-Apr-01 | 46.6406 | -1.8977 | 2 | NA | | LFPW | S |
| 10206217 | 20-Jun-02 | 44.5894 | -1.2398 | 3 | I | | LFPW | S |
| 10404055 | 21-Apr-04 | 44.8933 | -1.2171 | 2 | I | | LFPW | S |
| 10505023 | 01-May-05 | 46.4940 | -1.8127 | NA | NA | | LFPW | S |
| 10512100 | 28-Feb-05 | 43.8181 | -1.4047 | NA | O | | LFPW | S |
| 10602023 | 21-Feb-06 | 44.2144 | -1.2987 | 3 | I | | LFPW | S |
| 10706033 | 27-Jun-07 | 46.5400 | -1.8273 | 3 | NA | | LFPW | S |
| 10712119 | 13-Mar-07 | 44.0395 | -1.3407 | 4 | * | | LFPW | R |
| 10803050 | 16-Mar-08 | 44.6463 | -1.1999 | 2 | O | | LFPW | S |
| 10804078 | 25-Apr-08 | 44.8933 | -1.2171 | 2 | I | | LFPW | R |
| 10809120 | 16-Sep-08 | 43.3895 | -1.6643 | 1 | A | SFPW | SFPW | A |
| 10902053 | 11-Feb-09 | 44.0395 | -1.3407 | 3 | I | | LFPW | R |
| 10903073 | 11-Mar-09 | 43.5562 | -1.5028 | 1 | I | | LFPW | S |
| 10903078 | 11-Mar-09 | 44.0921 | -1.3257 | 1 | I | | LFPW | S |
| 10907116 | 09-Jul-09 | 47.8111 | -3.9489 | 3 | NA | | LFPW | S |
| 11007041 | 19-Jul-10 | 46.0020 | -1.3203 | 2 | NA | | LFPW | S |
| 11108074 | 02-Aug-11 | 45.4126 | -1.1604 | 1 | I | | SFPW | D |
| 11111087 | 24-Jun-11 | 43.4405 | -1.5939 | 2 | NA | | LFPW | S |
| 11112093 | 21-Dec-11 | 47.0204 | -2.2461 | 1 | NA | | LFPW | S |

For each individual, the voucher number, stranding date and location (latitude and longitude), and body state of decomposition are shown (State: 1, stranded alive and died within 48 h; 2, freshly dead; 3, dead and decomposed; 4, dead and highly decomposed; NA, not available.). Morphological data (M.) were categorized as available and unambiguous (A), incomplete (I), available but characters were in the zone of overlap (O), ambiguous due to characters in contradiction (*), not available (NA). The last columns report the species identification made using morphology (M. ID) when possible, the genetic species identification (G. ID), and the individual's mtDNA control region haplotype (Hap: names according to Oremus et al. 2009). LFPW: *Globicephala melas*; SFPW: *Globicephala macrorhynchus*.

Editor v. 7.1.11 (Hall 1999). Mitochondrial DNA control region sequences from Oremus et al. (2009) published on Genbank were used as a reference dataset to identify the species and haplotype of each sample. Thus, the final sequence alignment was reduced to 345 base pairs (bp) to match the portion analyzed in Oremus et al. (2009).

Among these 23 individuals, morphological information was either not available or incomplete for 18 individuals. For the remaining specimens, the species could not be determined using morphology for three individuals, as their measurements lay in the range of overlap between the two species, or the ratio and number of teeth used in the key were in contradiction yielding ambiguity in species diagnosis (Table 1). For the latter specimen, however, we considered measurements as unreliable due to the advanced state of decomposition of the body (Table 1), which prevented accurate morphometric analysis. A map of stranding locations for the 23 specimens was constructed using the marmap package v. 0.5 (Pante and Simon-Bouhet 2013) in R v. 3.0.1 (R Development Core Team 2013).

A complete haplotype of the mtDNA control region (345 bp) was successfully obtained for all individuals. The 23 individuals were characterized by four different haplotypes: two LFPWs haplotypes referenced by Oremus et al. (2009) as haplotypes R (GenBank: FJ513345) and S (GenBank: FJ513346), and two SFPWs haplotypes referenced as haplotypes A (GenBank: FJ513328) and D (GenBank: FJ513331). Species identification made using genetic data matched morphological identification (when applicable) suggesting that the morphological key is reliable when measurements are not within the range of overlap between the two species. Sequences allowed us to genetically identify or to confirm the species of all individuals, resulting in 21 LFPWs and 2 SFPWs (Table 1 and Figure 1). Thus, genetic analysis appears to be a complementary tool to the morphological key, and this highlights the importance of collecting tissue samples after every stranding event to allow genetic analysis whenever morphological identification is ambiguous or impossible. It is important to note, however, that unless nuclear loci are analyzed, one cannot rule out hybrid ancestry for individuals presenting morphological characters in the zone of overlap or of ambiguous phenotype, as illustrated by a recent study reporting an interspecific hybrid between the two *Globicephala* species (Miralles et al. 2013).

To place our sequences in a worldwide phylogeographic context, we compared mtDNA control region haplotype frequencies observed in this study with those obtained by Oremus et al. (2009). For LFPWs, we sequenced two different haplotypes, R (n=3) and S (n=18),

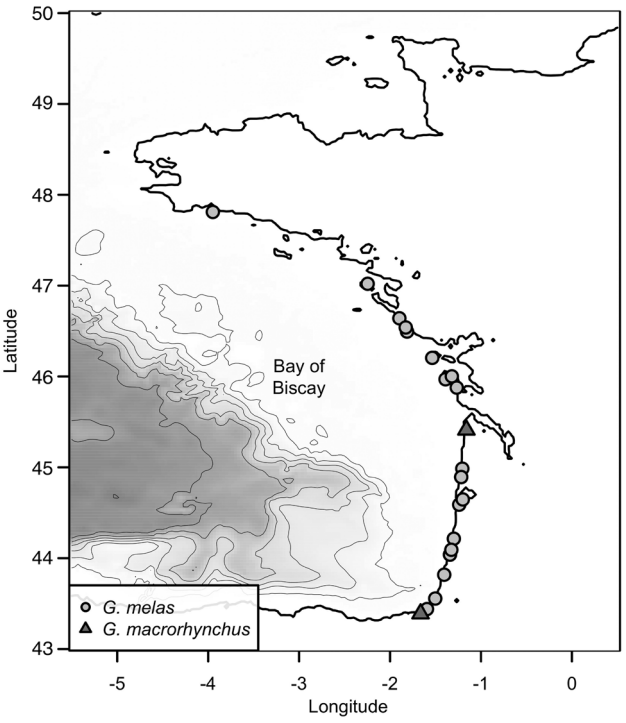


**Figure 1** Stranding locations of 23 pilot whales (*Globicephala* spp.) genetically identified using mtDNA control region sequences.

while Oremus et al. (2009) reported three haplotypes for LFPWs in the North Atlantic Ocean: S (n=56), X (n=3), and P (n=11). Thus, haplotype S was the most common haplotype for North Atlantic LFPWs in both studies. Interestingly, however, haplotype R had only been reported in LFPWs from the southern hemisphere (i.e., *Globicephala melas edwardii*). Thus, we report an additional shared haplotype between the two *G. melas* subspecies compared to Oremus et al. (2009), and our study elevates to four the number of haplotypes found in LFPWs from the North Atlantic. The two haplotypes sequenced in our study for SFPWs (haplotype A: n=1; haplotype D: n=1) had been previously reported in Oremus et al. (2009) for individuals sampled in the North Atlantic. However, haplotype A had been mostly sequenced in individuals from the Pacific Ocean (n=21) and was found for a single individual from the Atlantic Ocean (Oremus et al. 2009). Comparatively, a recent study conducted on SFPWs biopsy sampled from 14 groups around Madeira reported that all 29 individuals sequenced shared haplotype A (Alves et al. 2013).

In conclusion, our study constitutes a first step toward genetic monitoring of the two species of pilot whales along the French coasts, a region of particular importance as it constitutes the northern limit of the SFPW's species range.

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