MOLECULAR IDENTIFICATION OF SEABIRD REMAINS FOUND IN HUMPBACK WHALE FECES

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SUMMARY

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Cetaceans and seabirds frequently come into close contact when they target the same prey. Seabirds are generally thought to benefit from these associations; however, here we report a negative consequence for seabirds from associating with Humpback Whales *Megaptera novaeangliae*. We examined the remains of three seabirds found in a Humpback Whale fecal sample from Glacier Bay, Alaska. Genetic identification of the carcasses revealed one to be an Ancient Murrelet *Synthliboramphus antiquus* and two to be Marbled Murrelets *Brachyramphus marmoratus*. Presumably, these birds were accidentally ingested while the whale was foraging.

Key words: cytochrome oxidase I, DNA barcoding, forage flock, murrelets, seabird mortality, southeast Alaska

INTRODUCTION

Cetaceans and seabirds have long been known to associate at sea (Harrison 1979, Evans 1982). Associations include seabirds obtaining foraging opportunities from the feeding behavior of cetaceans (e.g., Harrison 1979, Obst & Hunt 1990, Verheyden 1993), and seabirds and cetaceans overlapping in spatial distribution when targeting the same productive foraging habitats or the same prey (e.g., Skov 1995, Torres 2009). Although seabirds may often benefit from the foraging efforts of cetaceans, they also face the possibility of being accidentally ingested by cetaceans when feeding on the same prey patch. Thus, close spatial associations can have negative consequences for seabirds, but these consequences have rarely been reported.

It is unclear how often and under what conditions accidental ingestion might occur. When dense concentrations of prey are trapped at the surface by diving birds such as alcids, which make the prey available to surface feeders such as gulls (Laridae), the result is a multi-species feeding frenzy (hereafter "multi-species feeding associations"; e.g., Haynes *et al*. 2011a). When these associations occur, baleen whales can target prey trapped by the foraging seabirds (Anderwald *et al*. 2011, Haynes *et al*. 2011a). Rorquals such as Humpback *Megaptera novaeangliae* and Minke *Balaenoptera acutorostrata* whales feed in the midst of the bird activity, which generally breaks up the feeding association (Anderwald *et al*. 2011, Haynes *et al*. 2011a) and creates an increased potential for accidental ingestion of seabirds by the whales.

Southeast Alaska is a productive summering area for Humpback Whales (Hendrix *et al*. 2012) and seabirds (e.g., Agler *et al*.1998, Haynes *et al*. 2011b). In fact, Humpback Whales are abundant and increasing in this area, with a population estimate of 1 585 animals in 2008 and a 5.1% annual rate of increase (Hendrix *et al*. 2012). Humpback Whales and seabirds in this region often target the same prey, including Pacific Herring *Clupea pallasi*, Capelin *Mallotus villosus*, juvenile Walleye Pollock *Theragra chalcogramma*, Pacific Sand Lance *Ammodytes hexapterus*, and euphausiids (*Thysanoessa* or *Euphausia* spp.; Krieger & Wing 1984, Haynes *et al.* 2011a). The whales and birds often forage at the same patch of prey (Haynes *et al*. 2011a). Humpback Whales capture their prey by lunge feeding, during which they 1) accelerate toward prey, 2) open their mouth, which generates drag and expands the buccal cavity, 3) close their mouth around a large volume of prey-laden seawater, and 4) expel the volume through baleen plates located on their upper mandibles, while retaining the prey inside the buccal cavity (Goldbogen *et al*. 2007). Lunge feeding occurs both at the sea surface and at depth, and Humpback Whales have been documented performing as many as 15 underwater lunges per dive (Goldbogen *et al*. 2008). During lunge feeding events, Humpback Whales may engulf seabirds associated with the target prey. Here, we report an instance where seabirds were ingested by a foraging Humpback Whale and passed through the whale's digestive tract. We used forensic genetics to determine the identification of these partially digested birds.

SAMPLE ORIGINS

In 2005, the National Park Service conducted Humpback Whale monitoring surveys from 27 May to 19 October in Glacier Bay and Icy Strait, Alaska. During a survey on 3 October, three partially digested birds coated with whale feces were observed floating in the immediate vicinity of two adult female Humpback Whales (southeast Alaska identification #1302 and #1486 of ages 13 and 6 years, respectively) near Flapjack Island in Glacier Bay (58°37′N, 136°00′W). Positive species identification of the birds was not possible during collection because of the advanced state

of decomposition of the carcasses (Fig. 1); however, the general characteristics of the specimens indicated that they were alcids. Specimens were retained and frozen for identification through genetic analysis. We obtained four reference tissues of two alcid species, *Brachyramphus marmoratus* (UAM 28521 and UAM 9885) and *B. brevirostris* (UAM 27040 and UAM 11764), from the University of Alaska Museum. We used these reference tissues as a positive control for the molecular work, as they provided reference sequences from unambiguously identified and vouchered representatives of *Brachyramphus*.

METHODS

DNA extraction and sequencing

We removed tissue samples from remains of the three birds and rinsed them in 100% ethanol to reduce fecal contamination. Tendon and flesh were removed from bones of two samples, and tissue from an intact webbed foot was removed from the third sample. Tissue samples were stored in 100% ethanol at -20°C. We extracted DNA from each sample and from the reference tissues using a Qiagen QIAmp DNA extraction kit following the manufacturer's instructions. We sequenced a segment of the mitochondrial cytochrome oxidase I (COI) gene for molecular identification of birds because of the high copy number of mtDNA and the many publicly available COI sequences to reference (Kerr *et al*. 2007). We initially amplified target gene fragments from extracted DNA by polymerase chain reaction (PCR) with barcoding primers designed for fishes (FishF1 and FishR1; Ward *et al*. 2005). After evaluating this first round of sequencing results, we applied bird-specific primers (AWCF1 and AWCR6; Patel *et al*. 2010) for amplification of poorly performing samples. With FishF1 and FishR1, we used a temperature cycling profile with an initial denaturing step at 94°C for 2 min, with 35 cycles of denaturing at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 45 s. The profile ended with a final extension step at 72°C for 5 min. With AWCF1 and AWCR6 we altered the temperature profile by performing annealing at 58°C. Reagent concentrations for both primer sets were 0.025 U/µL GoTaq Flexi Taq polymerase, 0.25 mmol/L dNTPs, 2.0 mmol/L $MgCl_2$, 0.4 mol/L forward primer, 0.4 µmol/L reverse primer, and 1 µL template DNA at variable concentrations. Unpurified PCR products were sent to a commercial institution for enzymatic purification and Sanger sequencing in both directions. Sequencing output was examined and assembled into contigs using the chromatogram viewing and manipulation tools implemented in CodonCode Aligner (ver. 3.0.3).

Identification of sequencing products

We used Basic Local Alignment Search Tool (BLAST) queries to compare COI sequences from our samples to all the publicly available sequences that make up the nucleotide database maintained by the National Center for Biotechnology (Altschul *et al*. 1997). We searched the nucleotide collection database without taxonomic limits with megablast ver. 2.2.7 (Zhang *et al*. 2000, Morgulis *et al*. 2008) to both validate our initial assumption of family level assignment

Fig. 1. (A) One of the three birds found floating in Glacier Bay, Alaska, and (B) the three birds analyzed in this study. US National Park Service photos.

(Alcidae) of the specimens and to identify the specimens. From the BLAST search we retained the top five matches and associated GenBank accession numbers, the total score for the top five matches as calculated by the BLAST algorithm, the query coverage in terms of the length of alignment between found and query sequence in terms of percent of query sequence length, and maximum identity (the percent similarity between the query and found sequence over the length of their alignment).

We performed a phylogenetic analysis of the sequences reported here and publicly available alcid COI sequences. We downloaded a mitochondrial COI sequence cluster for Alcidae using the Phylota Browser ver. 1.5 (www.phylota.net). The COI alcid sequence cluster consists of 143 sequences assigned to 29 different taxa representing 11 genera. The newly determined sequences and those from the alcid cluster were aligned using MUSCLE v 3.8.31 (Edgar 2004a, b) and a phylogenetic tree was generated from the resulting alignment by neighbor-joining (Saitou & Nei 1987) based on pairwise sequence distances corrected with the Kimura two-parameter model (Kimura 1980). The phylogenetic analysis was performed in the program PAUP* ver. 4.0b10 (Swofford 2003) and was rooted at the two major divisions of Alcidae as found in Pereira & Baker (2008).

RESULTS

Sequences from unknown samples one and two were obtained using AWCF1 and AWCR6. Amplification and sequencing using Fish F1 and Fish R2 of these samples produced amplifications from mixed templates. Identifiable fragments from these mixed amplifications were identified as Humpback Whale and *Brachyramphus* sp., respectively. Unknown sample three and the four museum vouchered samples were sequenced using Fish F1 and Fish R2. Sequences generated in this study have GenBank accession numbers KC812719-KC812725. BLAST results from the unknown samples indicated that they were alcids. Two of the unknown samples matched Marbled Murrelet *B. marmoratus* sequences in the database with 99%–100% identity. The third unknown sample was identified as an Ancient Murrelet (*Synthliboramphus antiquus*; Table 1) with 99% identity to existing sequences. Accordingly, phylogenetic inference placed the unknown samples as representing *B. marmoratus* and *S. antiquus* (Figure 2). Two of the sequences from the phylota cluster used as references were not placed within the species identified by the phylogenetic analysis: Gene Identifier (GI) 148466799 *Cepphus carbo* and GI 148466803 *Cerorhinca monocerata*.

DISCUSSION

The rarity of incidental engulfment or ingestion of seabirds by Humpback Whales is evidenced by the infrequent appearances of such anecdotes in the literature. In a study of multi-species feeding associations near Port Snettisham, Southeast Alaska, Haynes *et al*. (2011a) observed that Humpback Whales targeted prey trapped at the surface by foraging seabirds. They noted that Humpback Whales appeared to engulf murrelets at two of the 14 observed surface lunge feeding events. Dolphin & McSweeney (1983) reported two Cassin's Auklets *Ptychoramphus aleuticus* in Humpback Whale feces in an area where the whales were participating in multi-species feeding associations in Frederick Sound, southeast Alaska. Beyond these published accounts, we are aware of numerous unpublished anecdotes of seabirds being engulfed or ingested by Humpback Whales in Alaska. On 28 August 2008, three juvenile gulls (likely Glaucous-winged Gull *Larus glaucenscens*) were engulfed by a

TABLE 1
Summary of megablast queries of the National Center for Biotechnology Information nucleotide collection database

Query	Match	Rank	Total score	Query coverage, %	Maximum identity, %	Accession number
Unknown Sample 1: 771 bp	*B. marmoratus* COI	1	1373	97	99	EU525328.1
	B. marmoratus COI	2	1352	94	100	EF380322.1
	B. marmoratus COI	3	1280	90	99	DQ433360.1
	B. marmoratus COI	4	1273	90	99	DQ433361.1
	B. brevirostris COI	5	1181	100	94	EU525325.1
Unknown Sample 2: 789 bp	*B. marmoratus* COI	1	1389	96	99	EU525328.1
	B. marmoratus COI	2	1369	93	100	EF380322.1
	B. marmoratus COI	3	1280	88	99	DQ433360.1
	B. marmoratus COI	4	1273	88	99	DQ433361.1
	B. brevirostris COI	5	1208	100	94	EU525325.1
Unknown Sample 3: 678 bp	*S. antiquus* COI	1	1247	100	99	AP009042.1
	S. antiquus COI	2	1229	98	99	EF380331.1
	S. antiquus COI	3	1229	98	99	DQ434183.1
	S. antiquus COI	4	1225	98	99	AY666374.1
	S. antiquus COI	5	1194	96	99	GQ482737.1

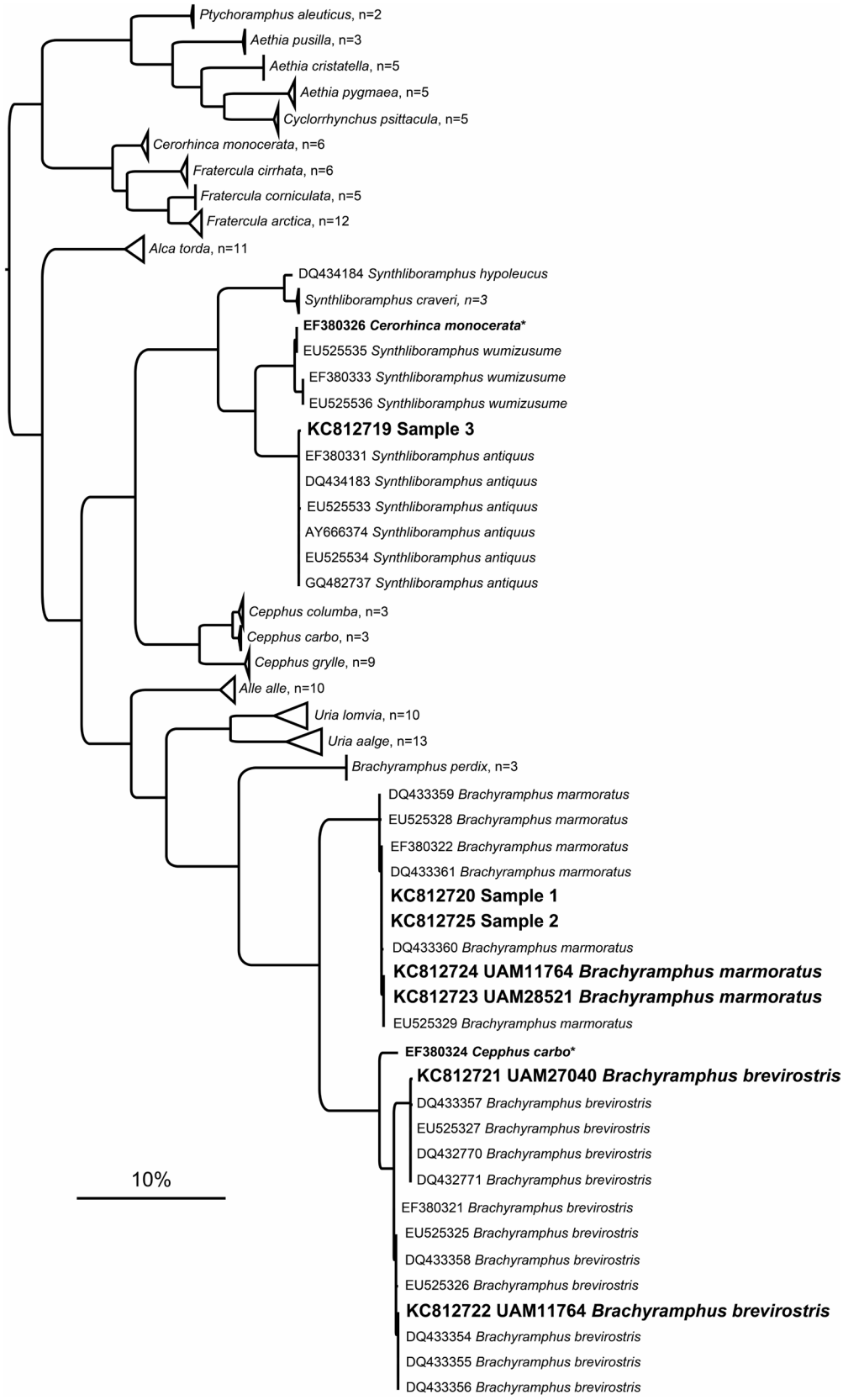

Fig. 2. Neighbor-joining phylogenetic tree of alcid cytochrome oxidase I sequences generated from a Kimura two-parameter distance matrix. The tree contains 150 sequences, 143 from GenBank and seven determined for this study. The sequences reported here are highlighted in bold and larger typeface. The tree is rooted at division between the two clades of Alcidae (Pereira & Baker 2007). Two sequences from GenBank marked with an asterisk (*) appear to be misassigned in the GenBank record. Horizontal distance on the tree is proportional to the number of changes inferred between sequences.

surface lunge feeding adult female Humpback Whale (southeast Alaska identification #1428, age 11) in Icy Strait, southeast Alaska (C.M. Gabriele, pers. comm.). After being expelled from the whale's mouth, their plumage was saturated with water, they were unable to fly, and they likely did not survive. In July 2004, two murrelets (species not identified) were found in Humpback Whale feces in upper Lynn Canal, southeast Alaska (S. Lewis, pers. comm.). Both carcasses were identified as murrelets because they were in good condition, with the feathers and appendages still intact. In Unimak Pass, Alaska, Humpback Whales have been reported to take dozens of Short-tailed Shearwaters *Puffinus tenuirostris* every summer during lunge feeding events (R. Pitman, pers. comm.), and they have been frequently observed engulfing shearwaters near Unalaska (D. Weber, pers. comm.).

The extent to which our specimens were intact suggest that Humpback Whales are poorly suited to digesting seabirds and likely do not target them as prey, which was also noted by Dolphin & McSweeney (1983). Although observations of incidental ingestion are scarce in the literature, suggesting it is likely a rare occurrence, the lack of reports may also be related to the difficulty of observing these events in the field. Diving seabirds may be ingested as whales feed underwater, which precludes direct observations at the surface. In addition, when a whale lunge feeds at the surface, it is often difficult to observe whether birds were engulfed because lunge feeding is a quick event that generates considerable commotion. Although it is unclear how frequently seabirds suffer mortality in this manner, it is likely only a minor source of mortality. However, there is potential for it to be a more substantial source of mortality in local areas under specific conditions, such as when the densities of multi-species foraging associations are high and whales are targeting the prey trapped by seabirds.

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