MOLECULAR DATA CONFIRM THE FIRST RECORD OF THE LONG-BILLED MURRELET FOR NEW MEXICO

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ABSTRACT: A small alcid of uncertain identity was salvaged from a brine pool associated with a potash mine in Eddy County, New Mexico, on 12 July 2009. The carcass was brought to the Museum of Southwestern Biology, prepared as a specimen, and tentatively identified as a Long-billed Murrelet (*Brachyramphus perdix*), but identification based on measurements and plumage characteristics was not conclusive. DNA sequence from the mitochondrial gene cytochrome-*b* confirmed the specific identity but revealed a previously unrecognized mitochondrial variant of the Long-billed Murrelet. This specimen provides the first documentation of the Long-billed Murrelet in New Mexico, a record that was anticipated from the species' established pattern of vagrancy across North America. This vagrant's novel mitochondrial DNA haplotype reveals previously undescribed population genetic structure within the Long-billed Murrelet.

The Long-billed Murrelet (*Brachyramphus perdix*), of northeastern Asia, has a well-established pattern of intercontinental vagrancy and a tendency to wander far from coastlines. As of 2003, there were over 50 documented records for inland areas of the United States and Canada (Thompson et al. 2003). Remarkably, there were no conclusive records of the species in North America before 1979 (Sealy 1982). It continues to occur in North America regularly (e.g. Barnes 2009, Svingen 2009), and the first record for Europe was in 1997 (Maumary and Knaus 2000). By contrast, there are no records of inland vagrancy of the Marbled Murrelet (*B. marmoratus*), which breeds in northwestern North America. The reason for the recent pulse of long-distance vagrancy by the Long-billed Murrelet is unknown, but various authors have proposed that cyclical regional weather patterns, erratic food supplies, and long-distance dispersal behavior might be contributing to this unique pattern (Sealy et al. 1982, 1991, Mlodinow 1997, Thompson et al. 2003). A better understanding of patterns of geographic variation, dispersal, and migratory behavior in the Long-billed Murrelet will be critical to explaining this phenomenon.

The Long-billed Murrelet was considered to be an Asiatic subspecies of the Marbled Murrelet until genetic studies revealed it to be highly divergent (Friesen et al. 1996). Phylogenies of the genus based on nuclear and mitochondrial DNA demonstrate that the Long-billed Murrelet is sister to a clade containing Kittlitz's Murrelet (*B. brevirostris*) and the Marbled Murrelet (Friesen et al. 1996, Pereira and Baker 2008). Estimates of the time of divergence between the Long-billed Murrelet and its congeners range from 35 to 48 million years, which is surprisingly ancient given the overall phenotypic similarity of the three species of *Brachyramphus* (Pereira and Baker 2008). Previously published DNA sequences from the Long-billed

Murrelet are based on birds collected near Magadan, Russia, and archived at the Burke Museum (University of Washington, Seattle).

On 12 July 2009, staff of Mosaic Corporation found a small alcid dead in a pool of brine associated with a potash mine southeast of Carlsbad in southern Eddy County, New Mexico. The finders did not provide a more exact location, but we estimate it at approximately 23 km east-southeast of Carlsbad. They took the carcass to Desert Willow Veterinary Services where it underwent necropsy, at which time the gonads were discarded without being noted. The carcass was subsequently brought to the Museum of Southwestern Biology (University of New Mexico, Albuquerque), where we tentatively identified it to the genus *Brachyramphus*, pending further investigation. Andrew B. Johnson prepared the specimen as a traditional study skin, partial skeleton, spread wing, and frozen tissue sample (catalog number MSB 29200, tissue number NK170062, preparator number ABJ2319; record accessible at http://arctos.database.museum/guid/MSB:Bird:29200). Its feathers were heavily encrusted with salt, only a portion of which could be removed during specimen preparation. Although not weighed, the bird was not emaciated, and Johnson noted its fat as "light." No evidence of molt was noted. The tip of the maxilla was broken but remained attached.

PLUMAGE

Feathers of the underparts were white, with dark tips forming short bars or scallops that were sparse on the throat and upper breast, becoming dense on the abdomen and flanks (Figure 1A). The plumage of the upperparts, wings, and tail was dark grayish brown. A narrow stripe was formed by white feathers that extended from the side of the neck around to the nape. White scapulars formed a prominent V pattern on the back (Figure 1B, C). The wing, including the underwing coverts, was uniform dark grayish brown with light gray tips on the outer secondaries and on all primaries except the outermost (Figure 1D, E). The Long-billed Murrelet's underwing coverts have been widely reported as being extensively white, but Thompson et al. (2003) showed that white in the wing linings is associated with immature plumages of the Long-billed and, to a lesser extent, Marbled Murrelet. Thus, the color of this bird's wing lining is consistent with that of an adult, even though the breast plumage is much more extensively white than in specimens of adult or subadult Long-billed Murrelets taken during August in California (Sealy et al. 1991) and Washington (Thompson et al. 2003), respectively.

Another characteristic that has been used to diagnose the Long-billed Murrelet is whitish marbling in the outer vanes of the rectrices. Thompson et al. (2003) showed that this characteristic is present in some Long-billed Murrelets but never present in the Marbled Murrelet. Curiously, this specimen has whitish marbling in the outer vanes of the rectrices on the left but not the right half of the tail. Both left and right rectrices have fine pale edging on the tips.

MEASUREMENTS

The measurements were as follows: flattened wing 135.0 mm, tarsus 16.2 mm, exposed culmen 17.1 mm, bill depth at anterior end of nares

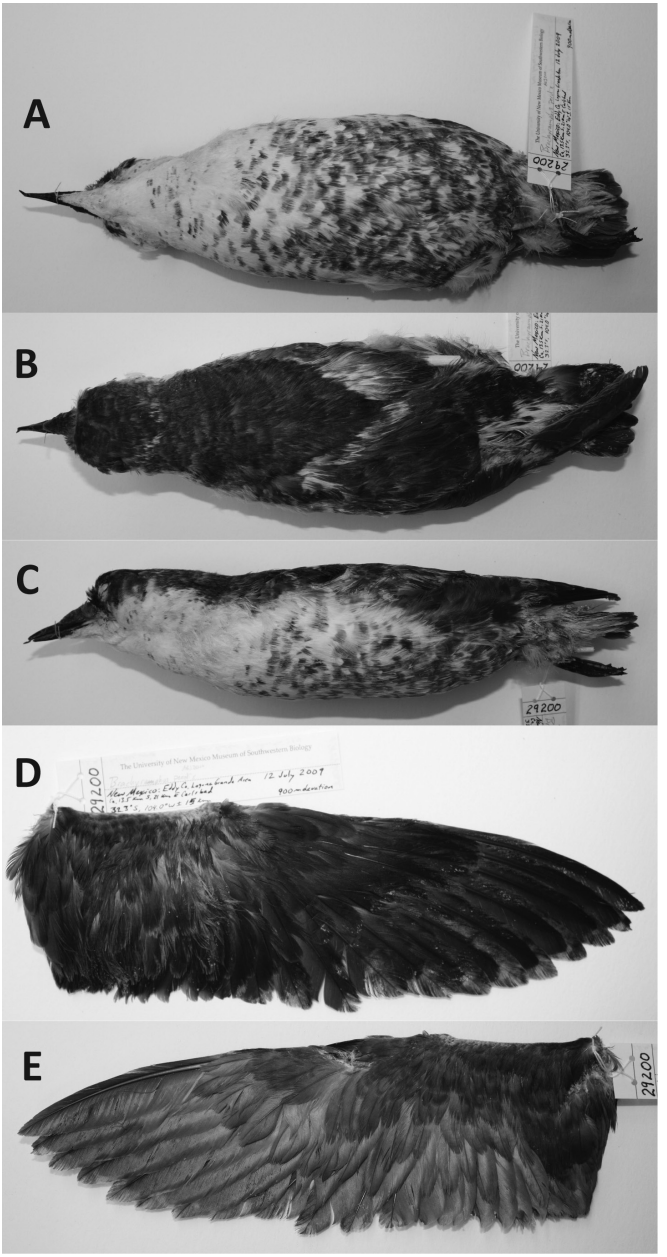

Figure 1. *Brachyramphus perdix*, MSB 29200. Ventral view (A), dorsal view (B), lateral view (C), dorsal surface of spread wing (D), and ventral surface of spread wing (E).

6.2 mm, and tail 36 mm. None of the measurements was diagnostic for identification with the possible exception of the tail length, which fits that reported for the Long-billed Murrelet (mean adult tail length 35.9 ± 1.5 mm, n = 5) and would be slightly long for a Marbled Murrelet (mean adult tail length 32.8 ± 2.2 mm, n = 35; Sealy et al. 1982, 1991, Thompson et al. 2003). By contrast, the exposed culmen was shorter than previously measured for an adult Long-billed Murrelet (18.0–23.5 mm, n = 23; Nelson 1997) and fell within the normal range for the Marbled Murrelet (mean for after-hatching-year males 15.5 ± 0.8 mm, n = 36, range 13.7–17.6 mm; mean for "adult" females 17.4 ± 0.9 mm, n = 108; Sealy 1975, Sealy et al. 1982, 1991). Although the tip of the culmen was partially broken off, we believe the measured length to approximate the intact length closely.

GENETIC METHODS

Following the manufacturer's protocol, we used a Qiagen DNEasy kit to extract DNA from pectoral muscle and a contour feather separately. The feather was used because the muscle tissue appeared to be heavily degraded and impregnated with salt. For the feather extraction, we removed the barbs with a razor blade and added 30 µL of 0.1 M dithiothreitol to the initial tissue incubation and digestion to reduce the disulfide bonds of the keratinous rachis and calamus. Using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA), we assayed each extraction for DNA content. The mitochondrial gene cytochrome-*b* was amplified in a 25-µL reaction with 1 µL of the DNA extract and the following reagents: 2.5 units of Taq polymerase (ExTaq, Takara, Shiga, Japan), 200 µM of each dNTP, 1.5 mM $MgCl_2$, and 1 µM of each primer. Primers used for amplification and sequencing are primers universal for avian cytochrome-*b*, L14841 (Kocher et al. 1989) and H4a (Harshman 1996). We used Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) thermal cyclers to carry out the polymerase chain reaction as follows: 95 °C for 8 min, (95 °C for 45 sec, 50 °C for 30 sec, 72 °C for 45 sec) × 35 cycles, 72 °C for 10 min. We visualized the reaction's products on a 1% agarose gel and cleaned them with Exo-Sap-It (USB, Cleveland, Ohio). For sequencing reactions, with external primers, we used BigDye 3.1 chemistry (ABI, Mountain View, CA), and we read the sequences with an ABI 3130 automated sequencer. Using Sequencher 4.7 (GeneCodes, Ann Arbor, MI), we assembled sequence contigs and inspected chromatograms manually. We used the software package MUSCLE (Edgar 2004) for alignment with all cytochrome-*b* sequences previously published for the genus *Brachyramphus*, as well as with sequences of representatives of the related genera *Synthliboramphus*, *Cepphus*, and *Fratercula* (Pereira and Baker 2008). We used the program MEGA (Kumar et al. 2008) to calculate pairwise distances and for distance-based phylogenetic analysis. We used the program Phyml (Guindon and Gascuel 2003) to analyze phylogeny by maximum likelihood. Using the HKY85 model of molecular evolution and simultaneous estimation of the model's parameters, we ran 500 bootstrap replicates of the maximum-likelihood analysis.

GENETIC RESULTS

According to the Nanodrop, the extraction from muscle tissue contained no detectable levels of DNA (0.0 ng/μL). By contrast, the extraction from the feather contained 4.1 ng/μL of DNA. The cytochrome-*b* gene was successfully amplified and sequenced only from the feather extraction, and reactions using the external primers produced sequences comprising 1036 base pairs, with an overlap between the two strands of 429 base pairs. The chromatograms of the sequences were clean and unambiguous, with no double peaks or conflicts between readings that would suggest the possibility that we had mistakenly sequenced a nuclear DNA pseudogene. The complete sequence is available on Genbank (accession HM072000).

Comparison of the sequence with published sequences for species of the Alcidae revealed uncorrected divergence levels of 0.5% with the Long-billed Murrelet but 8.4% and 9.8% with Kittlitz's and Marbled murrelets, respectively (Table 1). Divergences between the New Mexico specimen and representatives of the related genera *Cepphus* (10.1%), *Fratercula* (11.5%), and *Synthliboramphus* (11.8%) were higher than those within the genus *Brachyramphus*. The five DNA substitutions observed between the New Mexico specimen and published sequences of the Long-billed Murrelet were all transitions, and four out of the five were synonymous changes. The one nonsynonymous difference (codon 23) was at a site for which the New Mexico specimen shares the same amino acid (leucine) with all other alcids examined, but published Long-billed Murrelet specimens uniquely represent a different amino acid (phenylalanine). Phylogenetic analysis based on maximum likelihood corroborated the distance results. The New Mexico specimen's sequence was grouped with previously published sequences of the Long-billed Murrelet with strong bootstrap support (Figure 2).

Table 1 Pairwise Levels of Percent Sequence Divergence (*p* Distances) between the New Mexico Specimen[a] and Representatives of Related Species in the Family Alcidae.[b]

	(1)	(2)	(3)	(4)	(5)	(6)
(1) *Fratercula arctica*						
(2) *Synthliboramphus hypoleucus*	0.107					
(3) *Cepphus columba*	0.096	0.103				
(4) *Brachyramphus marmoratus*	0.109	0.113	0.101			
(5) *Brachyramphus brevirostris*	0.104	0.099	0.093	0.058		
(6) *Brachyramphus perdix*	0.116	0.117	0.100	0.096	0.081	
(7) New Mexico specimen	0.115	0.118	0.101	0.098	0.084	0.005

[a]MSB 29200; tissue NK170062; Genbank accession no. HM072000.

[b]Based on 1036 base pairs of the sequence of the cytochrome-*b* gene. Genbank accession numbers of the previously published sequences used for this analysis: *Fratercula arctica*, DQ385228; *Synthliboramphus hypoleucus*, U37305; *Cepphus columba*, U37293; *Brachyramphus marmoratus*, U63050; *B. brevirostris*, U63058; *B. perdix*, U63057.

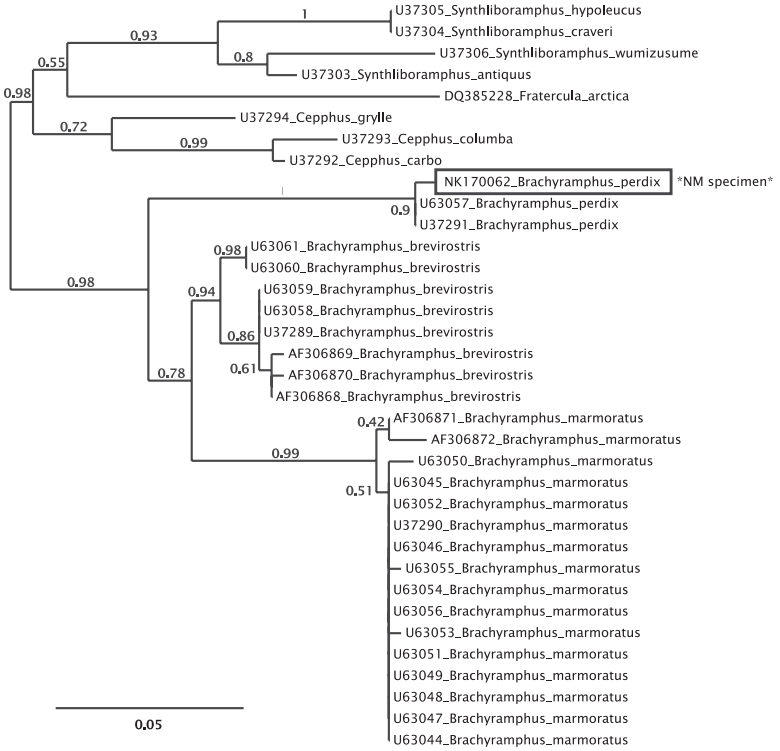

Figure 2. Phylogeny of *Brachyramphus* and related genera based on the cytochrome-*b* gene, estimated by maximum likelihood (HKY85 model). The values at the nodes represent the proportion of 500 bootstrap replicate analyses in which that node appeared. Numbers that are preceded by "U," "DQ," or "AF" correspond to Genbank accession numbers. The New Mexico specimen is grouped with *Brachyramphus perdix*, with strong support.

DISCUSSION

Mitochondrial DNA data confirm that the New Mexico specimen can be identified as a Long-billed Murrelet and therefore represents a transoceanic vagrant and a first state record. We caution that the date of collection, 12 July 2009, should be taken as only an approximation of the bird's date of death. We cannot be certain how long the bird was dead before being discovered because of the unusual circumstance of the carcass being preserved by the salt in the brine pool. A long interval between death and salvage might explain the bird's unusual plumage (for July), the lack of molt, and the absence of DNA of high molecular weight in the muscle tissue.

The mitochondrial DNA of the specimen was not identical to sequences published for the Long-billed Murrelet, diverging by 0.5%. This difference

suggests that significant population structure exists within the Long-billed Murrelet and that this vagrant is unlikely to have originated from the same population represented by sequences already recorded in Genbank. The high ratio of transition to transversions and the absence of stop codons in the DNA sequence indicate that the sequence was mitochondrial in origin and did not represent a nuclear DNA pseudogene. The previously published sequences all represent birds collected in the northern Sea of Okhotsk, in the vicinity of Magadan, Russia (Friesen et al. 1996, Pereira and Baker 2008). The breeding distribution of the Long-billed Murrelet extends from Kamchatka, in the Russian Far East, south to Hokkaido, Japan, and is highly fragmented, reflecting the complexity of coastlines in the species' range (Friesen et al.1996, Dickinson 2003). A phylogeographic study with population-level sampling from throughout this range is clearly warranted. Such a study is likely to reveal that the mtDNA variation we observed has a geographic basis and might shed light on the geographic origins of these transoceanic vagrants. Furthermore, this specimen's anomalous variation in plumage and measurements may reflect previously undescribed geographic variation in the Long-billed Murrelet.

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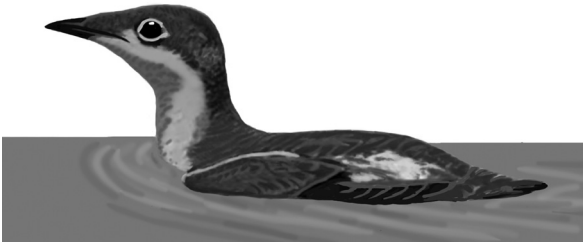

Long-billed Murrelet

Computer painting by George C. West