

# MITOCHONDRIAL DNA DEMONSTRATES THAT A HEN HARRIER (*CIRCUS CYANEUS*) REACHED ATTU ISLAND, ALASKA

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**ABSTRACT:** A partial wing of a juvenile male harrier salvaged at Attu Island, Aleutian Islands, Alaska in June 1999 has long been discussed as an example of the Old World taxon *Circus (cyaneus) cyaneus* on the basis of morphological features and a geographic deduction. This identification took on additional weight after many taxonomic authorities decided to treat the Old and New World forms as separate species—*C. cyaneus* and *C. hudsonius*, respectively. Mitochondrial DNA (1887 base pairs) clearly group the specimen with *C. cyaneus*, and thus the specimen represents the first record for North America of the Hen Harrier.

Gibson and Byrd (2007) inferred that at least eight observations of the Northern Harrier in the western Aleutian Islands, Alaska, likely represented the Old World *Circus cyaneus cyaneus*, at the time considered the nominate subspecies of a polytypic, Holarctic harrier (see also Gibson and Withrow 2015). The only physical evidence of such an occurrence was a distal right wing and associated feathers picked up at Attu Island in early June 1999 (the bird presumably having died the previous fall/winter, but phenology uncertain; Figure 1). The specimen was “identified to species [i.e., *C. cyaneus, sensu lato*] by C. J. Dove” at the U.S. National Museum of Natural History (Gibson and Byrd 2007:284), and “its length (chord 318 mm) points to this subspecies [i.e., nominate *cyaneus*]” (Gibson et al. 2013:185).

In 2017 the American Ornithological Society’s North American Classification Committee split the Northern Harrier (*Circus cyaneus, sensu lato*) into two species, the Hen Harrier (*C. cyaneus*) occurring in the Old World and the Northern Harrier (*C. hudsonius*) in North America (Chesser et al. 2017), but the committee preferred to wait for additional confirming evidence regarding the Attu Island wing. Thus *Circus cyaneus*—as a now purely extralimital species—was removed from the North American list. A report of a “probable” Hen Harrier from New Jersey (Duffy et al. 2012) had not, as of early 2023, been submitted to the New Jersey Bird Records Committee (K. Duffy, B. Clark, and J. Hough in litt. 2023).

The Attu Island wing’s plumage color, pattern, size, and shape, assessed in direct comparison with specimens, point to its belonging to the genus *Circus*. Details of wing formula, primary emargination, and size rule out all other migratory species of Palearctic harriers other than *cyaneus* (i.e., *spilonotus*, *aeruginosus*, *pygargus*, *macrourus*, and *melanoleucos*; see Dement’ev and Gladkov 1966, Nieboer 1973, Cramp 1980, Baker 1993). Phenotypic differentiation of nominate *cyaneus* and *hudsonius* is more difficult (e.g., Grant 1983, Wallace 1998, Martin 2008, Pyle 2008, Mullarney and Forsman 2010, Duffy et al. 2012, Etherington and Mobley 2016), but several criteria have

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FIGURE 1. A, lower surface; B, upper surface. Right distal wing of a harrier salvaged at Attu Island, Aleutian Islands, Alaska in June 1999 and subsequently confirmed to belong to a Hen Harrier (*Circus cyaneus*) by comparison of mtDNA sequences. Note that most of the primary coverts are missing, and counting the bars on undersides of the primaries from the photo is complicated by this and the disheveled condition of the feathers (see text), but the underwing barring strongly suggests *cyaneus*.

been advanced that involve characteristics of the outer wing, namely, wing length and the number of bars on the underside of the outer primaries. We did not find the relative darkness of the trailing edge to the inner primaries, the width of the inner primary bars (both Martin 2008), or the ground color of the primaries (Cramp 1980) to be useful criteria.

The wing measured 318 mm (chord; Gibson et al. 2013) and 327 mm (flat; it no longer has any chord left, as a result probably of both its decomposed state and being bagged and stored flat for 24 years). This is shorter than any female *cyaneus* or *hudsonius* (Nieboer 1973, Scharf and Hamerstrom 1975, Schultz 1996, Etherington and Mobley 2016), and the barred (not black) primaries indicate a juvenile male. North American *hudsonius* is often described as longer winged than nominate *cyaneus*; some of the best comparisons come from Scharf and Hamerstrom (1975), who reported flat wing length for breeding (adult) male *cyaneus* as 322–340 mm (mean = 332.3) and for *hudsonius* as 334–370 mm (mean = 351.3), measurements used in the past to suggest the Attu wing belonged to *cyaneus* (Gibson et al. 2013). However, Cramp (1980:126) described nominate *cyaneus* as “clinally larger from western Europe to east Asia.” Measurements given by Nieboer (1973:51, 89) demonstrate this cline in wing length and that east Asian birds have wings just as long as North American birds. Furthermore, some juvenile males of *hudsonius* can apparently have wings as short as the Attu wing (Schultz 1996, Duffy et al. 2012), although this appears to be rare (as it might be for east Asian *cyaneus* as well). Thus wing length is of little value in distinguishing the two species in this instance.

The number of dark bars on the undersides of the outer primaries in the two species differs. Nominate *cyaneus* generally has three or four (sometimes five) bars on the longest primaries (P7 and/or 8), as opposed to five to seven in *hudsonius*. Nominate *cyaneus* usually has three on P10 in contrast to normally four on that primary in *hudsonius* (e.g., Martin 2008, Pyle 2008, Mullarney and Forsman 2010, Duffy et al. 2012). In the Attu wing, the number of bars (not including the dark tip) on the underside of primaries 6 through 10 is as follows: 5, 5, 6, 5, and 3. However, the innermost bar on P6–9 is usually very faint, and probably invisible in the field in all but exceptional views. It may be concealed by the primary coverts (as many inner bars are on specimens and presumably live birds as well). Most of the primary coverts on the Attu wing are missing, and the generally disheveled nature of the feathers—several detached—impairs reconstruction of the look of a live bird. Nevertheless, in life (from which most work on this character has been based), the bar count would probably have been seen as 4, 4, 5, 4, and 3. A fair amount of individual variation exists in this trait, and there has been no rigorous assessment of its use in diagnosing species or in its potential geographic variation across the Holarctic, although the taxa certainly appear to differ on average. The number of bars in the Attu wing strongly suggests nominate *cyaneus*.

Thus identification of this partial wing specimen rested on a geographic deduction (see Discussion) and identification criteria whose limits of diagnosability have not been quantified and/or are inadequately validated across the Bering Strait. Because it would represent the sole incontrovertible evidence of *C. cyaneus* in North America, we sequenced a combined total of 1887 base pairs (bp) of mitochondrial DNA (mtDNA) from three gene regions to

verify the identity of the specimen (or at least the clade to which its mother belonged). We selected these three mtDNA markers—NADH dehydrogenase subunit 1 (ND1), NADH dehydrogenase subunit 2 (ND2), and cytochrome oxidase I (COI)—because of their ability to distinguish between *C. circeus* and *C. hudsonius* and their successful use in avian “barcode” identifications (Hebert et al. 2004, Kerr et al. 2007, Gaber et al. 2020, Luttrell et al. 2020).

## METHODS

For amplification and sequencing of the mitochondrial genes ND1, ND2, and COI, we isolated DNA from the Attu specimen and seven additional reference specimens. The resulting DNA sequences were compared and evaluated to identify the maternal clade of origin for the Attu specimen. We also included in the comparisons sequences publicly available via GenBank (National Center for Biotechnology Information; Table 1).

To prevent cross-contamination, we completed all sampling, extraction, amplification, and sequencing steps for the quill of the Attu specimen prior to work on reference specimens. All reagents and consumables were new, and we included three negative controls—an extraction blank, a reagent blank, and an amplification blank—for all mtDNA-sequencing steps to confirm the absence of contamination.

We obtained vouchered reference-tissue specimens from two representatives of *cyaneus* and four representatives of *hudsonius* from museum collections (Table 1). One additional sample of *hudsonius* tissue specimen was obtained post-necropsy from the California Department of Fish and Wildlife following a mortality investigation.

We used two different extraction protocols, one for a quill tip from the Attu specimen, the other for the reference-tissue specimens. From the Attu specimen, we removed the proximal tip of a single quill (~0.5 cm) with a sterile razor and with clean forceps placed it into a 2.0-mL EZ1 sample tube. The quill tip was digested in 400  $\mu$ L of a dithiothreitol-based digestion buffer (78 mM dithiothreitol; 100 mM NaCl; 0.5 mg/mL proteinase K; 2 g/mL sodium dodecyl sulfate; 3 mM CaCl<sub>2</sub>; and Tris-EDTA buffer, pH 8.0) and incubated for 24 hours at 56 °C at 1000 revolutions per minute on an Eppendorf Thermomixer C (Eppendorf AG). The DNA was extracted with a Qiagen EZ1 Advanced XL DNA extraction robot (Qiagen, Inc.) according to the manufacturer’s protocol for trace samples and eluted in 50  $\mu$ L of water. Reference-tissue specimens were incubated in 200  $\mu$ L (190  $\mu$ L G2 buffer and 10  $\mu$ L proteinase K) of manufacturer-supplied reagents for EZ1 DNA Investigator trace samples and followed the same incubation and extraction conditions as the quill tip, with the exception that they were eluted in 100  $\mu$ L of water.

We amplified the resulting genetic material by using a combination of primer sequences shown in Table 2. All sequences were amplified in 25- $\mu$ L reaction volumes containing 2.5  $\mu$ L 10 $\times$  PCR buffer (Applied Biosystems), 1.3  $\mu$ L deoxynucleotide triphosphate mix (2.5 mM each), 0.5  $\mu$ L forward and reverse primers (10  $\mu$ M each) tagged with M13F or M13R, respectively, 2.0  $\mu$ L MgCl<sub>2</sub> (25 mM; Applied Biosystems), 0.4  $\mu$ L bovine serum albumen (10 mg/mL), 0.26  $\mu$ L AmpliTaq Gold (Applied Biosystems), 2.5  $\mu$ L template DNA,

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**TABLE 1** Specimens of Harriers Sampled for Comparison of Sequences of Mitochondrial DNA

Species and mtDNA gene region	Location	Source	Institution <sup>a</sup> and voucher number	GenBank accession(s)
<i>Circus cyaneus</i>				
ND1, ND2, COI	Alaska: Attu Island	this study	UAM 9062	PQ508332, PQ508340, PQ499575
ND1, ND2, COI	France	this study	AMNH DOT 7157	PQ508333, PQ508341, PQ499576
ND1, ND2, COI	Japan: Suruga	this study	AMNH SKIN 535911	PQ508334, PQ508342, PQ499577
ND1, ND2, COI	South Korea	Choi et al. (2021)	NIBR GEIBGR0000289530	KU237286
ND1, ND2, COI	China: Inner Mongolia	Gao et al. (2018)	unknown	KX925606
ND1	Sweden: Norrbotten	Oatley et al. (2015)	NRM 966543	KP857894
ND1	France: Languedoc-Roussillon	Oatley et al. (2015)	MNHN 6365	KP857866
<i>Circus hudsonius</i>				
ND1, ND2, COI	Alaska: Paxson area	this study	UAM 15027	PQ508335, PQ508343, PQ499578
ND1, ND2, COI	Alaska: Livengood	this study	UAM 38477	PQ508336, PQ508344, PQ499579
ND1, ND2, COI	Alaska: Seward Peninsula	this study	UAM 43070	PQ508337, PQ508345, PQ499580
ND1, ND2, COI	Alaska: Seward Peninsula	this study	UAM 49053	PQ508338, PQ508346, PQ499581
ND1, ND2, COI	California: San Diego Co.	this study	CDFW NOHA001	PQ508339, PQ508347, PQ499582
ND1	California, Kern Co.	Oatley et al. (2015)	CAS 90674	KP857846
ND1	New Jersey	Oatley et al. (2015)	AMNH DOT 7204	KP857851
ND1	New York	Oatley et al. (2015)	AMNH DOT 5970	KP857852
ND1	California: Alameda Co.	Oatley et al. (2015)	MVZ 181753	KP857870
COI	Canada: Ontario	Kerr et al. (2007)	ROM CWSL95-71859-02	DQ433513
COI	Mississippi	Kerr et al. (2007)	LSU 0496	DQ432854
COI	Canada: Ontario	Hebert et al. (2004)	ROM 1B-2778	AY666437
COI	Canada: Ontario	Hebert et al. (2004)	ROM 1B-2912	AY666427

<sup>a</sup>AMNH, American Museum of Natural History, New York; CAS, California Academy of Sciences, San Francisco; CDFW, California Department of Fish and Wildlife, Sacramento; LSU, Louisiana State University, Baton Rouge; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; NIBR, National Institute of Biological Resources, Incheon, South Korea; NRM, Swedish Museum of Natural History, Stockholm; ROM, Royal Ontario Museum, Toronto; UAM, University of Alaska Museum, Fairbanks.

**TABLE 2** Primers Used to Amplify and Sequence the Mitochondrial Genes ND1, ND2, and COI of Harriers

mtDNA gene region and primer <sup>a</sup>	5' to 3' primer sequence	Reference
ND1		
ND1-L3827	GCAATCCAGGTCGGTTTCTATC	Sorenson et al. (1999)
ND1-H5201	CCATCATTTTCGGGTATGG	Oatley et al. (2015)
ND1 degraded		
ND1-JF-Cir16sL	GTAAGGCCAATGCIYACATGAC	Oatley et al. (2015)
ND1-JF-Circus140L	ATTGCGGTAGCTTTCCTCACAT	Oatley et al. (2015)
ND1-JF-Cir400L	CTCCTAGCCATATCAAGCCTA	Oatley et al. (2015)
ND1-JF-Cir600L	ACCATCTCCTACGAAGTCAC	Oatley et al. (2015)
ND1-JF-Cir900L	GGATTCTTATGARTCCGCGCC	Oatley et al. (2015)
ND1-JF-Circus340H	GGATTCAAATGGTTAGTGCTAG	Oatley et al. (2015)
ND1-JF-Cir450H	TTGAGTTTGAGGCYCATCCAGA	Oatley et al. (2015)
ND1-JF-Cir640H	ATTATTATAAGGGGTCAGGAG	Oatley et al. (2015)
ND1-JF-Cir960H	GTGYATYAGYTGRTCATAGCG	Oatley et al. (2015)
ND1-JF-Cir1250H	GYGTAGGTTTCGATYCCACTT	Oatley et al. (2015)
ND2		
ND2-L5216	GGCCCATACCCCGRAAATG	McCracken and Sorenson (2005)
ND2-H5766	RGAKGAGAARGCYAGGATYTTKCG	McCracken and Sorenson (2005)
COI		
CirCOIF1	GCTTCGGAAACTGACTAGTC	Martin Collinson (pers. comm.)
CirCOIR2	GGTGTTTGGTATTGAGAGAGG	Martin Collinson (pers. comm.)
Not applicable		
M13F	TGTA AACGACGGCCAGT	Messing (1983)
M13R	CAGGAAACAGCTATGAC	Messing 1983

<sup>a</sup>M13F tags were added to the 5' end of L-strand (L) and forward (F) primer sequences; M13R tags were added to the 5' end of H-strand (H) and reverse (R) primer sequences. PCR-amplified products were subsequently sequenced with M13F and M13R primers.

and PCR-grade water to volume. The thermocycling regimen for COI and ND1 was as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 sec, 55 °C (COI)/56 °C (ND1) for 30 sec, 72 °C for 60 sec, and a 5 min final extension at 72 °C. The regimen for ND2 was 95 °C for 10 min, followed by 40 cycles of 95 °C for 60 sec, 54 °C for 60 sec, 72 °C for 60 sec, and a 5 min final extension at 72 °C.

Next we purified the products with ExoSAP-IT PCR product-cleanup reagent (Applied Biosystems), following a low-volume protocol in a 5- $\mu$ L reaction volume containing 0.40  $\mu$ L ExoSAP-IT, 4.60  $\mu$ L Tris buffer, pH 8.0 (50 mM), and 1–2  $\mu$ L of PCR amplification product with thermocycling conditions of 37 °C for 30 min, followed by 85 °C for 15 min. Using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems), we sequenced the purified amplification products with M13F and M13R primers and purified them with the BigDye XTerminator Purification Kit (Applied Biosystems). For electrophoresis we used an ABI 3500xL Genetic Analyzer with POP-7 polymer, 50-cm capillary array, and default instrument settings (Applied Biosystems). We aligned the forward and reverse sequences, checked them for quality, and trimmed primer sequences with Sequencher

version 5.4.6 (Gene Codes Corporation). For specimens that failed to yield sufficiently long sequences with the primer pair ND1-L3827 and ND1-H5201, we assembled a contiguous sequence from overlapping sequences obtained from the degraded ND1 primer pairs. We aligned and compared the resulting sequences of the Attu specimen and vouchered reference specimens and identified variations between them in Sequencher version 5.4.6 (Gene Codes Corporation). We deposited our original sequences in GenBank with accession numbers PQ508332–PQ499582 (Table 1).

To assess interspecific and intraspecific variation, we evaluated differences among the sequences of ND1, ND2, and COI. To show the differences between species graphically, we used PopART version 1.7 (Leigh and Bryant 2015) to construct a median-joining network.

## RESULTS

We successfully sequenced 1234 bp of ND1, 549 bp of ND2, and 275 bp of COI from all specimens sampled, with one exception: American Museum of Natural History (AMNH) 535911 yielded an incomplete ND2 sequence of 455 bp (Table 1). For simplicity's sake, we trimmed all other ND2 sequences to 455 bp in subsequent comparisons. Similarly, we trimmed ND1 sequences to 1157 bp (*cyaneus*) or to 1156 bp (*hudsonius*) to align with the sequences available through GenBank. A deletion at consensus bp position 1145 in reference sequences of ND1 of *hudsonius* resulted in a 1156-bp trimmed sequence when it was aligned with that of *cyaneus*.

With the sequences trimmed, the numbers of parsimony-informative sites between the *Circus* species were 21 for ND1, 13 for ND2, and 4 for COI. For all three mtDNA regions interspecific variation was greater than intraspecific. In *C. hudsonius*, sequences in all three mtDNA gene regions were identical. In *C. cyaneus* ND1 had the greatest number of haplotypes, with KX925606 from the Inner Mongolia region of China diverging the furthest from its conspecifics. The Attu specimen shared an ND1 haplotype with KP857894 from Sweden. The ND2 and COI haplotypes of *C. cyaneus* were invariant, aside from a unique haplotype in KX925606 from Inner Mongolia.

To compare the sequences of ND1 and COI of *cyaneus* and *hudsonius* with those of the Attu specimen, we used a combination of sequences in publicly available databases and those that we generated for this study (Table 1). Because publicly available sequences of ND2 of *hudsonius* were lacking, comparisons for this marker relied on specimens sequenced for this study. In both ND1 and ND2 genetic divergence between *cyaneus* and *hudsonius* was sufficient to identify the Attu specimen's maternal species of origin as *cyaneus*, and to exclude *hudsonius*. The shorter COI fragment had the fewest informative differences between the two *Circus* species, but it still corroborated the grouping of the Attu wing with *cyaneus*. Of these three mtDNA markers, ND1 was the most variable with each of the five reference specimens of *C. cyaneus* representing a unique haplotype. Based on a concatenated dataset of 1887 bp from the ten individuals for which we had all three genes, Figure 2 represents the relationships of the sequenced haplotypes and demonstrates the affinities of the Attu wing.

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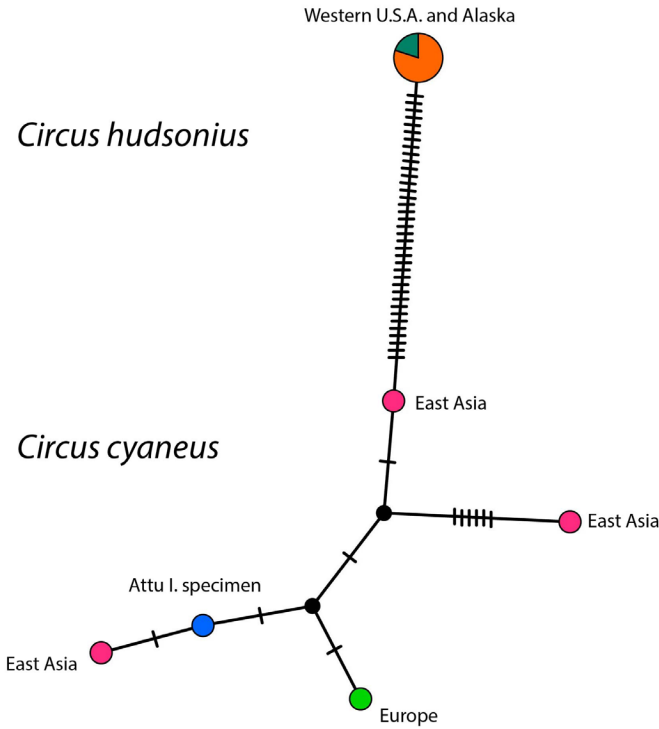


FIGURE 2. Median-joining haplotype network created in PopART software representing the relationship of haplotypes in a concatenated data set of 1887 bp of ND1, ND2, and COI from *Circus cyaneus* and *Circus hudsonius*. For *C. cyaneus*, each colored circle represents one specimen; for *C. hudsonius*, it represents the entire set of that taxon, which comprised only one haplotype. Each crossbar represents a difference in one base pair; black dots represent unsampled haplotypes. The Attu specimen clusters with the reference specimens of *C. cyaneus*.

DISCUSSION

Northern Harriers nest widely in mainland western Alaska from the Alaska Peninsula (Gill et al. 1981, Savage 2018) north through the Yukon-Kuskokwim delta (e.g., Petersen et al. 1991) and Seward Peninsula (Kessel 1989) to the Point Hope region (see Williamson et al. 1966). Despite this wide distribution and the highly migratory nature of Alaska populations, there are no records of any *Circus* from St. Lawrence Island (Lehman 2019), and the sole harrier reported from St. Matthew Island was not identified to (then) subspecies (Winker et al. 2002). Farther south, in the Pribilof Islands, where the Northern Harrier is best known (recently?) as a casual or intermittent fall migrant, all those identified to species are thought to represent *hudsonius* (S. Schuette in litt. 2019). Only slightly better known in the Aleutian Islands

since the publication of Gibson and Byrd (2007)—who assessed both taxa as casual, predominantly fall migrants throughout—there are now photographs of birds from the eastern and central Aleutians that appear to be *hudsonius* (Unalaska, 28 Dec 2014, S. Golodoff, e.g., Macaulay Library [ML] 232648091; Adak, 24 Sep 2017, F. Hass, e.g., ML 69903381; Adak, 22 Nov 2024, M. Kramer, e.g., ML 626610800). A photo of an apparent female on 10 Apr 2012 at Shemya Island in the western Aleutians (B. Trotter in litt. 2023) is not identifiable to species, as was another harrier there on 18 Apr 2002 (M. Schwitters, Tobish 2002). However, both would have been seasonally early for an Alaska migrant (see Swem 1982, Mindell and Mindell 1984), particularly female-plumaged birds, which tend to migrate later than adult males (Swem 1982, Palmer 1988, MacWhirter and Bildstein 1996). Whether this implies an origin in Asia or interisland movements of a bird that wintered in the Aleutians is unknown (Gibson and Byrd 2007). Harriers have reached the Hawaiian and Leeward islands numerous times, and those well documented have been reported as *hudsonius* (Clapp and Woodward 1968, Pyle and Pyle 2017, Rutt 2017). In Japan, *hudsonius* has been recorded at least three times (Morioka 1999, Brazil 2009, Tanuma et al. 2018, Y. Odaya in litt. 2023). A collection of feathers salvaged from near Meinypil'gyno, Chukotka, Russia, in 2021 represented *hudsonius* (Zinevich and Tomkovich 2023). This pattern of extralimital occurrence of North American birds vitiates inference of the origin of unidentified harriers in the western Aleutians, particularly in fall.

The Hen Harrier breeds across Eurasia east to the northern and western coasts of the Sea of Okhotsk, generally migrating south for the winter (e.g., Vaurie 1965, Cramp 1980). In extreme northeast Asia, it apparently nests as far east as the Kolyma River (Dement'ev and Gladkov 1966, Brazil 2009). Vaurie's (1965:203) statement that “the range becom[es] uncertain farther east [from the Kolyma] where this bird has been seen, but not found breeding, on the middle Anadyr [River] and in Kamchatka” remains largely true today, as we could still find no evidence for nesting east of the Kolyma River. For example, Tomkovich (2008) did not mention it from the middle Anadyr River, nor has he seen it in many years of field work in eastern Chukotka. In all likelihood the only specimen from Chukotka is a hatching-year bird taken on 7 Sep 1905 (Gregorian) on the middle Anadyr River (Zoological Museum of Moscow University [ZMMU] R-42416, photos examined; P. Tomkovich in litt. 2023). Lobokov (1986) did not mention *Circus cyaneus* as a breeding bird in Kamchatka, and Artyukhin et al. (2000) discussed it as a migrant there, including on Karaginsky Island. At least one specimen from western Kamchatka (Ust-Kamchatsk) is *cyaneus* as expected (Zoological Institute of Russian Academy of Sciences, St. Petersburg [ZIN] 118286; photos examined). Syroechkovsky et al. (2019) listed *Circus cyaneus* as a stray in the Koryak Highlands, outlining the first records in that region. There is apparently only one record from the Commander Islands (Gibson and Byrd 2007 and citations therein); the specimen from there mentioned by Shul'pin (1936) is *cyaneus* (ZIN 118287; photos examined). Migrants/winter visitors are known from the southern Kurile Islands (OSJ 2012) and (mostly northern) Japan (Brazil 1991).

On the other side of the continent, the lone *Circus* report from Greenland is thought to represent *hudsonius* (D. Boertmann in litt. 2023). In Iceland,

there are three records of *hudsonius*, including two specimens, and roughly five times that many records of nominate *cyaneus* (Y. Kolbeinsson in litt. 2023). Still farther east, *hudsonius* has reached the British Isles (Mullarney and Forsman 2010, McInerney et al. 2022) and the Azores (Alfry et al. 2018). As far as we are aware Duffy et al. (2012) is the only suggestion that Hen Harrier has reached North America from the east.

In a comparison of 1887 bp of mtDNA from three gene regions (ND1, ND2, and COI), the Attu specimen unequivocally groups with *cyaneus*. But mitochondrial DNA alone cannot exclude the possibility the bird was a hybrid. However, there were no morphological features of the Attu specimen suggest a hybrid origin. Therefore, our comparisons of mtDNA sequences identify the Attu specimen as the first confirmed Hen Harrier in North America, presumably originating somewhere in east Asia.

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