



# Independent lines of evidence document the identification of bird–aircraft collision sample of Western Marsh Harrier (*Circus aeruginosus*) in continental USA

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## ABSTRACT

Bird–aircraft collisions (birdstrikes) occur daily and are global in scale. In the USA, the Federal Aviation Administration (FAA) tracks information regarding birdstrikes in the National Wildlife Strike Database to improve aviation safety. Here, we describe the use of independent lines of evidence for final species identification of an unusual birdstrike sample submitted for identification from Newark Liberty International Airport, New Jersey, in November 2022. Using DNA barcoding, molecular sexing, feather pattern matching, feather ultrastructure, and online community science reports, we surmise that the birdstrike sample originated from the same individual Western Marsh Harrier (*Circus aeruginosus*) that was photographed and reported to eBird at a location proximate to where the birdstrike occurred. The identification of this sample suggests an unusual end to a rare bird in the USA with the feather sample representing the first specimen record (USNM 665437) of Western Marsh Harrier in the continental United States. This case study underscores the importance of linking independent lines of evidence to confirm complex bird identifications, help document bird records, and track unusual bird movements.

## KEYWORDS

Birdstrike; feather; molecular; morphology; online databases

## PALABRAS CLAVE

bases de datos en línea; colisión ave-avión; molecular; morfología; pluma

## Líneas independientes de evidencia documentan la identificación de muestras de colisiones ave-avión de aguilucho *Circus aeruginosus* en EEUU continental

## RESUMEN

Colisiones entre aves y aviones (colisiones ave-avión) ocurren diariamente y son a escala global. En EEUU, la Administración de aviación federal (FAA) rastrea información acerca de las colisiones entre aviones y aves en la Base de datos de colisiones de fauna silvestre para mejorar la seguridad aeronáutica. Aquí describimos el uso de líneas independientes de evidencia para hacer una identificación final de una muestra de colisión ave-avión inusual sometida por el el aeropuerto Newark Liberty International, New Jersey, en noviembre del 2022. Usando código de barras de ADN, sexado molecular, correspondencia de patrón de plumas, ultraestructura de plumas y reportes de la

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comunidad científica en línea, supusimos que la muestra de colisión ave-avión se originó del mismo individuo de aguilucho *Circus aeruginosus* que fue fotografiado y reportado en eBird en una localidad cercana al sitio donde ocurrió la colisión ave-avión. La identificación de esta muestra sugiere un final inusual para un ave rara en los EEUU cuya muestra de plumas representa el primer registro (USNM 665437) de aguilucho *Circus aeruginosus* en los Estados Unidos continentales. Este caso de estudio subraya la importancia de unir diferentes líneas de evidencia para confirmar identificaciones complejas de aves, ayudar a documentar registros de aves y rastrear movimientos inusuales de aves.

Bird–aircraft collisions (birdstrikes) are a known common occurrence globally and are reported to civil aviation over 17,000 times per year in the USA (Dolbeer et al. 2023). Knowing the species of bird involved in birdstrikes is a fundamental step in preventing these human–wildlife conflicts by providing species information necessary to manage airport environments to deter birds (DeVault et al. 2013). The species identification techniques (molecular and morphological) used for birdstrike samples are conducted in specialized laboratories, such as the one located at the Smithsonian Institution’s National Museum of Natural History (NMNH) in Washington, DC, where thousands of samples are processed and identified annually (Dove et al. 2009). Here, we present how we use molecular and morphological evidence to narrow a bird strike sample received from New Jersey (USA) to a subset of harrier species (*Circus*) not known to occur in the USA. Then, we describe how we use independent lines of evidence to surmise that, remarkably, the bird struck by an aircraft on 19 November 2022 was the same individual Western Marsh Harrier (*Circus aeruginosus*) that was observed and photographed by birdwatchers on 25–27 August 2022 in Maine and 8–12 November 2022 in New Jersey.

On 19 November 2022, a birdstrike was reported to the Federal Aviation Administration (FAA) (National Wildlife Strike Database 2024; Dolbeer et al. 2023) from Newark Liberty International Airport, New Jersey. The birdstrike occurred at 1545 h local time (NJ) to a United Airlines Flight (UA224) Boeing 737–900 aircraft (aircraft registration N75436) inbound from Chicago O’Hare International Airport (Illinois, USA). Based on the FAA Wildlife Strike Report (2022-11-19-213703), the flight crew indicated the birdstrike occurred at 3,000 feet (914 m) above ground level (AGL), 9 nautical miles (NM) (16 km) northeast of the airport on approach to runway 22L. No damage was reported to the fuselage of the aircraft from where the bird sample was removed. Following FAA guidelines, the bird samples were submitted to the NMNH Feather Identification Lab for identification; the samples were received on 30 November 2022.

The sample evidence consisted of a single damaged contour feather (Fig. 1) approximately 6 cm × 4 cm with attached afterfeather, and an alcohol pad (3 cm × 3 cm) with bird tissue (snarge) collected from the aircraft fuselage. The morphology of the feather was initially diagnostic to us as a bird of prey but because of the amount of variation within the plumages in this group of birds, we conducted molecular analysis for possible species-level identification. The tissue sample was analyzed using DNA barcoding (Hebert et al. 2003; Kerr et al. 2007) following general methods in Dove et al. (2008) but modified with a phenol-chloroform extraction followed by a 10 µL PCR reaction with degenerate CO1 primers (dgLCO-1490 and dgHCO-



**Figure 1.** USNM 665437 contour feather sample recovered from birdstrike in New Jersey and ultimately identified as Western Marsh Harrier, the first specimen record for the continental USA.

2198; Meyer 2003) at 48°C annealing temperature, and sequence on an ABI 3730x sequencer (Applied Biosystems Inc., Foster City, California, USA). DNA identification results, based on 98% or better match (Kerr et al. 2007), narrowed the possibilities to four harrier (*Circus* spp.) species (none of which occur in USA) included in online DNA libraries (GenBank and Barcode of Life Database). The *cytochrome c oxidase subunit 1* (CO1) DNA sequence (AGTCGGCA CCGCCCTTAGCCTACTTATTCGCGCAGAACTTGGCCAACCAGGCACACTACTAGG-TGATGATCAAATCTATAATGTAATCGTTACCGCACATGCCTTCGTAATAATCTTCT-TCATAGTCATACCAATCATAATCGGAGGCTTCGGGAACTGACTAGTCCCCTCAT-AATCGGCGCCCCGACATAGCCTTCCCACGCATAAAACAACATGAGCTTCTGACTA-CCTCCCCCATCTTTCCTCCTCCTATTAGCCTCCTCAACAGTAGAAGCAGGGGCTGG-TACCGGATGAACTGTCTACCCCCACTAGCCGGCAACATAGCCCATGCTGGCGCC-TCAGTAGATTTAGCTATCTTCTCCCTACACCTAGCTGGAATCTCATCTATCCTAGG-GGCAATTAACCTTCATCACAACCGCTATTAACATAAAACCCCCAGCCCTCTCCCAAT-ACCAAACACCCCTATTCGTATGATCTGTCTCATTACTGCTGTCTACTATTACTC-

TCTCTCCCAGTCCTTGCTGCTGGCATCACCATACTACTAACGGACCGAAACCTTAA-TACAACATTCTTTGACCCTGCCGGCGGAGGCGACCCTATCCTATATCAACACCTCT-TCTGATTCTTT) of the birdstrike sample matched 99.69–100% to *Circus aeruginosus* (Western Marsh Harrier); 98.72–98.89% to *Circus spilonotus* (Eastern Marsh Harrier); 98.9–99.21% to *Circus approximans* (Swamp Harrier); 98.27–98.28% to *Circus melanoleucos* (Pied Harrier). The CO1 sequence of the birdstrike sample only matched 93.55% to *Circus hudsonius* (Northern Harrier), the only harrier known in the USA, and is far below acceptable species-level identification consideration using this molecular marker. Because of the close molecular similarity of the CO1 sequence of the sample to other closely related harrier species, and because Western Marsh Harrier is known to hybridize with Eastern Marsh Harrier (Fefelov 2001), these molecular results presented a problematic conclusion.

Next, we conducted a thorough morphological assessment of the whole feather by comparison of the feather size, pattern, texture, and color, with museum specimens (*Circus* spp.) at NMNH. We also examined the microscopic plumage characters of the downy feather barbs using a compound light microscope at 100–400× (Leica DM750; Leica Microsystems) and determined that the microstructure was consistent with *Circus* spp. by having very long barbules, stippled internodal pigmentation, and numerous nodes per barbule (Coddington et al. 2022). For the whole feather examination, similar plumaged specimens (i.e., female, juvenile) to the unknown sample were selected for comparison. The Munsell (1998) soil color chart was used for reference of feather color descriptions. The unknown feather sample has a wide brown tip (Hue 10YR, 4/3) that narrows to a V-shaped point where the brown color meets the white feather base (Fig. 1). The white area surrounding the V-shape extends halfway up the feather. A very narrow brownish yellow transition color (Hue 10YR, 6/8) is visible between the brown and white areas.

Feather morphology and geographical range indicated that two of the potential species, Pied and Swamp harriers, were poor matches for our sample. Contour feathers of Pied Harrier (the lowest genetic match) were inconsistent with the feather sample by overall smaller size and most have a narrower area of dark brown (Hue 7.5, 3/2) at the tip with a greater amount of white at the base (as opposed to the brown V-shaped stripe of the unknown feather sample). Pied Harrier is a medium-distance migrant that breeds in S Siberia, E Mongolia, and NW China and spends the nonbreeding season across S and SE Asia to N Borneo and the Philippines (Orta et al. 2020). Swamp Harrier was eliminated as a close morphological match to the unknown sample because the overall plumage of specimens was much darker in hue with the brown tip (Hue 7.5, 4/3) not forming the distinct V-shaped pattern in most feathers. Swamp Harrier is a short-distance migrant mainly distributed across New Guinea, Melanesia, Australia, New Zealand, and Polynesia east to Tonga (Debus and Kirwan 2020). Given the ranges of Pied and Swamp harriers, these species were considered unlikely to have strayed so far off course to the eastern USA.

Eastern and Western marsh harriers have been considered conspecific (Fefelov 2001) and plumages were more difficult to discern from the unknown feather sample than the other harriers examined. Specimens of Eastern Marsh Harrier differ from the unknown feather by having a smaller and narrower area of brown coloration at the tip, which does not extend as far toward the feather base. Eastern Marsh Harrier also differs in having more overall area of the brownish yellow (Hue 10YR, 6/8) bordering the V-shaped stripe. The unknown sample feather best matched the Western Marsh Harrier in the way the brown color pattern formed a V-shape against a white rather than brownish yellow base. No

distinction could be made between the plumages of the immature male and female specimens of Western Marsh Harrier when compared with the unknown feather sample.

While the physical examination of the feather agreed with the best molecular match (Western Marsh Harrier), we could not explain the presence of this species in the USA. We checked flight records by reviewing FlightAware (2022) and determined that the aircraft had not recently traveled out of the country (possibly striking the bird elsewhere and inadvertently transporting the bird remains back to the USA). With no probable explanation for the origin of the Western Marsh Harrier sample we remained unsure of the results and subsequently consulted eBird (Sullivan et al. 2009) to check for community science records. To our surprise, eBird postings from 25 August 2022 to 12 November 2022 by over 60 birders reported sightings and photographs of Western Marsh Harrier from two states (Maine and New Jersey) within the USA, describing details of appearance (female) and molt enough to validate the sightings as the probable same individual (Bevier et al. 2023). The last observation of Western Marsh Harrier reported to eBird from New Jersey was on 12 November 2022 (Ellerbusch 2022) at Troy Meadows Natural Area. Because no reports of Western Marsh Harrier were reported to eBird after the birdstrike event date (19 November 2022), and the morphological feather examination and DNA analysis were most consistent with Western Marsh Harrier, we strongly suspect that, remarkably, the aircraft struck the same individual bird that had been reported online. The aircraft, coming from the west, likely turned south on its approach to 22L before it lined up at 220 degrees for a final approach to the airport, putting the strike a maximum of 12 NM (22 km) from the last known sighting of the Western Marsh Harrier in Troy Meadows Natural Area (New Jersey), 7 days earlier.

To provide further documentation that the sightings and definitive photographs of a female Western Marsh Harrier (Bevier et al. 2023) were the same individual as the birdstrike sample, we conducted molecular sexing on the sample. In addition, female Western Marsh Harriers are about 25% heavier than males (Ferguson-Lees and Christie 2001) and body mass information is important in aircraft design and engine certification and is included in the FAA Wildlife Strike Database. In birds, females have heterogametic sex chromosomes (Z and W), while males have homogametic sex chromosomes (ZZ; review in Krocak et al. 2021). Fragments of the avian sex chromosomes for the birdstrike sample as well as two known-sex individuals of Western Marsh Harrier (USNM 665054 and USNM 663160) were amplified following protocols from Lee et al. (2010) and Van der Velde et al. (2017) using the primers CHD1F/CHD1R and 2602F/2669R in 30  $\mu$ L PCR reactions at 50°C annealing temperature. The fragments were visualized on a TapeStation 4200 (Agilent, Santa Clara, California, USA) and confirmed that the sample struck by the aircraft had fragments from both the Z and W chromosome, indicative of a female. This result is consistent with visual reports included in Bevier et al. (2023).

The Western Marsh Harrier as currently recognized includes two subspecies. One (*C. a. aeruginosus*) breeds across Eurasia, east to Mongolia, and the other (*C. a. harterti*) is resident coastally in northwest Africa from Morocco to Tunisia (Gill et al. 2024). Northern European and western Asian populations of Western Marsh Harrier spend the nonbreeding season south to tropical Africa as far as northern South Africa. Easternmost populations from central Asia spend the nonbreeding season south to the Indian subcontinent. Northernmost breeding populations are long-distance migrants, leap-frogging relatively sedentary southern European breeders (Orta, Boesman, Marks, Garcia, et al. 2020; Agostini 2021). Western Marsh Harrier is listed as accidental/casual in the American

Ornithological Society Check-list based on observations reported from Guadeloupe (2023), but until the photographic records in August and November 2022, it had not definitively been recorded in the continental USA (Levesque and Malglaive 2004; Merkord et al. 2006; Bevier et al. 2023; Pyle et al. 2023; Chesser et al. 2024). The New Jersey sightings were 565 km southwest of the original sighting in Maine, and analysis of the plumage patterns, molt, and sex of the photographed birds strongly suggested the same bird was at both locations (Bevier et al. 2023). In Europe, Western Marsh Harriers are known to move over large bodies of water during migration (Agostini and Panuccio 2010), and the species has demonstrated a propensity to stray out of range with several records on islands in the eastern Atlantic (e.g., Azores and Cape Verde) and on islands in the Indian Ocean (Ferguson-Lees and Christie 2001). The only harrier that regularly occurs in North America is the Northern Harrier, which mainly breeds in Canada but occurs throughout the USA and spends the nonbreeding season as far south as Colombia (Smith et al. 2020).

This case study underscores the importance of birdstrike reporting and collection of bird remains, detailed physical identification of samples, and using independent lines of evidence to validate species identification conclusions. Moreover, the feather specimen (USNM 665437), cataloged into the bird collections at NMNH, represents the first specimen record of Western Marsh Harrier in the continental USA and provides a museum voucher that is available for future study.

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No potential conflict of interest was reported by the author(s).

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