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Molecular phylogeny, morphology and life-history comparisons within *Circus cyaneus* reveal the presence of two distinct evolutionary lineages

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Abstract

Background: *Circus cyaneus* is a medium-sized bird of prey that is widely distributed across the Northern Hemisphere. There are two currently recognized forms, the Palearctic form *C. c. cyaneus* (Hen Harrier), and the Nearctic form *C. c. hudsonius* (Northern Harrier). The forms have recently been split by the British Ornithologists' Union but the American Ornithologists' Union and some other taxonomic committees have not yet made any change. Here we examine the phylogenetic relationship between the two forms using sequence data from multiple nuclear and mitochondrial genes and examine breeding biology, body size, morphology, dispersal and other behaviors.

Methods: In order to fully compare *cyaneus* and *hudsonius*, we carried out a full literature review, measured museum skins and carried out phylogenetic analysis using a number of different mitochondrial genes and compare our findings to other recent work.

Results: We find that these two allopatric taxa form reciprocally monophyletic groups, show substantial mtDNA sequence divergence, and further differ significantly with respect to body size, plumage characters, breeding biology, dispersal and other behavioral traits.

Conclusions: Based on an array of consistently divergent characteristics, it is suggested that the two forms are best regarded as separate species, Hen Harrier (*Circus cyaneus*) and Northern Harrier (*Circus hudsonius*).

Keywords: *Circus cyaneus*, *Circus hudsonius*, Hen Harrier, Northern Harrier, Phylogenetics, Speciation, Evolution

Background

Circus cyaneus is a medium-sized diurnal raptor found across most of the Northern Hemisphere. Known as "Annoch-kee-naepeek-quaeshew" (Snake Hunter) by the Cree Indians (Swainson and Richardson 1831), it was first described by Edwards in 1750 as "the Ring-tailed Hawk" (Edwards 1750) and subsequently classified as *Falco cyaneus* by Linné in his original 1766 classification of birds (Linné 1766). Edwards also described the bird as "The Blue Hawk" (Edwards 1758) and then "Marsh Hawk" (Edwards 1760). After being associated with a number of different scientific and common names, the Nearctic form was officially designated *Circus hudsonius* when the genus *Circus* was first recognized (Swainson and Richardson 1831). The British Ornithologists' Union (BOU) later adopted the scientific name *Circus cyaneus* in 1883, recognizing "an allied form" in North America (BOU 1915). In 1920 the Palearctic form was recognized as a subspecies, *C. c. cyaneus* (Witherby 1920), again acknowledging the existence of a different, but unspecified, race in North America. The British common name for the form has always been Hen Harrier.

This taxonomy remained so until 1931 when the Nearctic form was relegated to sub-specific status and recognized as *C. cyaneus hudsonius* by the American

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Ornithologists' Union (AOU). The AOU considered the Nearctic and Palearctic forms to be conspecific (Peters 1931), such that the Eurasian form continued to be recognized as *C. c. cyaneus*. The North American common name of Marsh Hawk for the Nearctic form remained so until 1983, when the currently recognized common name became Northern Harrier when it was first recognized by the AOU (1983).

Recently though, the BOU has acknowledged the status of *Circus cyaneus* as representing two species—Hen Harrier (*Circus cyaneus*) and Northern Harrier (*Circus hudsonius*) (Sangster et al. 2016), based on differences in plumage and morphometrics, substantial genetic divergence between *cyaneus* and *hudsonius* in mitochondrial and nuclear DNA (divergence similar to or larger than between several other recognized species of *Circus*) and a closer relationship between *hudsonius* and Cinereous Harrier *C. cinereus* than to *cyaneus*. This move has also been followed by a number of other authors (Ferguson-Lees and Christie 2001; Rasmussen and Anderton 2005; Gill and Donsker 2015). This movement of splitting the forms into two species has not been followed by the AOU (Chesser et al. 2015, 2016) and others.

North Americas' current temperate climate allows access to suitable Northern Harrier breeding areas north of the Arctic Circle. These same areas become completely uninhabitable during the winter and the populations that breed there migrate south in autumn to overwinter in more hospitable regions. Terrestrial bird species that normally undertake an annual long-distance migration typically face constraints that inhibit them from dispersing in an east–west direction between the major landmasses (Boehning-Gaese et al. 1998). Such dispersal constraints, along with the notable tendency to avoid crossing large bodies of water, may account for the lack of dispersal that has been observed between the Nearctic and Palearctic forms of *Circus cyaneus*, although extremely rare occurrences of *C. c. hudsonius* in the Western Palearctic have been documented (Martin 2008; Mullarney and Forsman 2011).

Previous phylogenetic research on harriers has concentrated mainly on the relationship of the genus *Circus* to various other raptors. Both *cyaneus* and *hudsonius* were included in a phylogenetic reconstruction of Mediterranean raptors (Wink and Seibold 1996), showing they were separate, sister taxa but no specific comment regarding their historical relationship was made. An additional study of genetic relationships within Holarctic raptors also included both of the forms and commented that the two forms are considerably genetically divergent and might represent distinct species (Wink et al. 1998). Furthermore, the same author also observed that *cyaneus* and *hudsonius* show 1.7 % divergence in the Cytochrome *b* gene (Wink and Sauer-Gürth 2000). In "Harriers of the World" (Simmons 2000), a molecular phylogeny of the harriers (*Circus* spp.), considers the *cyaneus* and *hudsonius* forms to be separate species and notes that they are treated as such because "their genomes are more strongly divergent than those of species already separated on other grounds".

Historically, there has clearly been some level of disagreement, and perhaps even confusion, regarding the appropriate taxonomic ranking of *cyaneus* and *hudsonius*. Recently however, an in-depth molecular phylogeny of all the harrier species was carried out, using one mitochondrial and three nuclear loci and concluded that *cyaneus* and *hudsonius* were two distinct species, with Cinereous Harrier (*Circus cinereus*) being a sister species of *hudsonius* (Oatley et al. 2015). However, despite the different taxonomies employed by previous workers studying *Circus cyaneus*, no formal systematic treatment of the two forms has been performed. Here, we build on previous phylogenetic work (Oatley et al. 2015) and carry out a comparative analysis of several key characters associated with the life-history and morphology of the two forms *cyaneus* and *hudsonius* and discuss their taxonomic ranking in light of these new perspectives.

Methods

A complete review of the scientific literature for *Circus cyaneus* was carried out, which are covered in the "Discussion" section under "Vocalization", "Habitat", "Distribution, dispersal and migration" and "Breeding behavior (Mate choice, Nest site and Male desertion)". In addition, analyses of phylogeny and morphology (size difference and plumage) were also carried out as follows.

Phylogenetic analyses

Frozen tissue samples for nine representatives of *hudsonius* and five representatives of *cyaneus* were obtained from various museum collections for the molecular phylogenetic analyses of the mitochondrial Cytochrome *b* gene (Additional file 1: S1). A single additional Cytochrome *b* sequence of *C. c. cyaneus* obtained from GenBank (accession number X86745) (Benson et al. 2002) served as an independent verification of sequencing accuracy during alignment, and was included in the final phylogenetic analyses. A Cytochrome *b* sequence for *Circus aeruginosus* (Western Marsh Harrier) from GenBank (accession number AY987305) was designated as the outgroup in the phylogenetic reconstructions. Full details of tissue extraction and DNA isolation can be found in the Additional file 2: S4.

Of the 9 *hudsonius* samples obtained, 8 amplified well enough for sequencing, while of the 5 *cyaneus* samples obtained, only 3 amplified sufficiently to allow

sequencing. Specifically, the toe-pad samples acquired from The University of Copenhagen did not amplify. The *hudsonius* samples were designated EX1.1–1.3, EX2.1 and EX2.3–2.6, while the *cyaneus* samples were identified as EX3.1–3.3.

In addition to our Cytochrome *b* sequence data, we also used publicly available data for various gene sets. To create our second dataset, we examined the Cytochrome *c* oxidase subunit 1 (COI) gene for which a number of barcoding sequences (7 for *cyaneus* and for 4 *hudsonius*) are available in GenBank.

Finally, for our third dataset we downloaded sequences from a previous study (Oatley et al. 2015), where the authors sequenced a 1.2 kb fragment of NADH dehydrogenase (ND1). We extracted all *Circus cyaneus* sp. sequences from ND1 and carried out identical analyses as used in our Cytochrome *b* analyses. We also refer to the authors' findings for three nuclear loci, namely, Myoglobin intron-2 (MB), Beta Fibrinogen intron-5 (FGB) and TGFβ2 intron-5 (TGFB2) (see "Discussion" section).

All datasets were aligned using ClustalW (Thompson et al. 2002) through the graphical interface MEGA6 (Tamura et al. 2013). For each dataset the corresponding gene for *C. aeruginosus* (Western Marsh Harrier) was used as an outgroup. The model test method in MEGA6 was used to identify the best Maximum Likelihood (ML) substitution model for each set of aligned sequences. The best model was noted and the appropriate tree construction algorithm for the data was used to construct a ML phylogenetic tree, with 1000 bootstrap replications. Also, after assigning the sequences to groups ("hudsonius" or "cyaneus"), pairwise, inter-group and intra-group genetic distances (p-distance) were calculated, along with nucleotide content.

Morphology

New measurements of museum specimens, plus data from material referenced in the literature, were used to examine size differences between the two forms. Data collected from the literature (Scharf and Hamerstrom 1975; Watson 1977; Cramp and Simmons 1980; Palmer 1988; Johnsgard 1990; Wheeler and Clarke 1995; MacWhirter and Bildstein 1996; Simmons 2000) was not combined with our new measurements of study skins in the final statistical analyses, due to the confounding effects of individual variation in the biometric measurements taken by different workers. The following measurements were taken: body length (distal tip of central tail feather to proximal tip of bill), wing cord, tail length (distal tip of central tail feather to base of tail), bill-nostril to tip, bill-cere to tip, bill depth (dorsal surface of upper mandible to ventral surface of lower mandible at base of bill) and tarsus length. The measurements taken follow those made by other authors. The biometric data were analyzed according to a multivariate analysis of variance (MANOVA) for joint analysis of males and females between both species and two-way ANOVA for single-sex comparisons, using the Genstat software package (Payne et al. 2008).

Plumage

In order to compare variation in plumage characteristics of the harriers, notes and photographs were taken whilst examining museum specimens and additional reference was made to photographs published in the literature and found online.

Results

Phylogenetic analyses

We aligned *cyaneus* and *hudsonius* gene datasets for Cytochrome b (Cyt*b*), Cytochrome *c* oxidase subunit 1 (COI) and NADH dehydrogenase (ND1), calculated the best ML substitution model for the data and used that model to construct the best phylogenetic tree for the alignment (Fig. 1). For all three datasets, HKY85 was found to be the best model (Hasegawa et al. 1985).

As can be seen from the phylogenies in Fig. 1, all of the sequences from *cyaneus* and all the sequences from *hudsonius* cluster into separate clades supporting the monophyly of each clade.

As a summary, for each dataset, genetic distances (*p*-distance) within and between each form, as well as overall minimum, maximum and mean genetic distances are shown in Table 1.

Cytochrome *b*

After alignment, the sequences were edited manually (gapped regions trimmed at start and end), resulting in a total sequence length of 719 nucleotides for all but one sample (*C. cyaneus* EX3.2, which was 653 nucleotides long). A close examination of the molecular data shows that the Cytochrome *b* sequences for the *hudsonius* samples are identical, except for EX1.2, which has a single transition mutation (C to T). The *cyaneus* sequences, on the other hand, showed a slight degree of variation with two variable sites. No indels were located in any of the sequences. From the above mutations, each of the forms had one non-synonymous change. In *hudsonius* the single transition leads from an Arginine to a Tryptophan and in *cyaneus* the non-synonymous transition leads from a Glycine to an Asparagine.

There are also 11 positions where all samples of one form consistently show a different nucleotide sequence to all samples of the other. All of these differences have occurred through transitions (A-G or T-C).

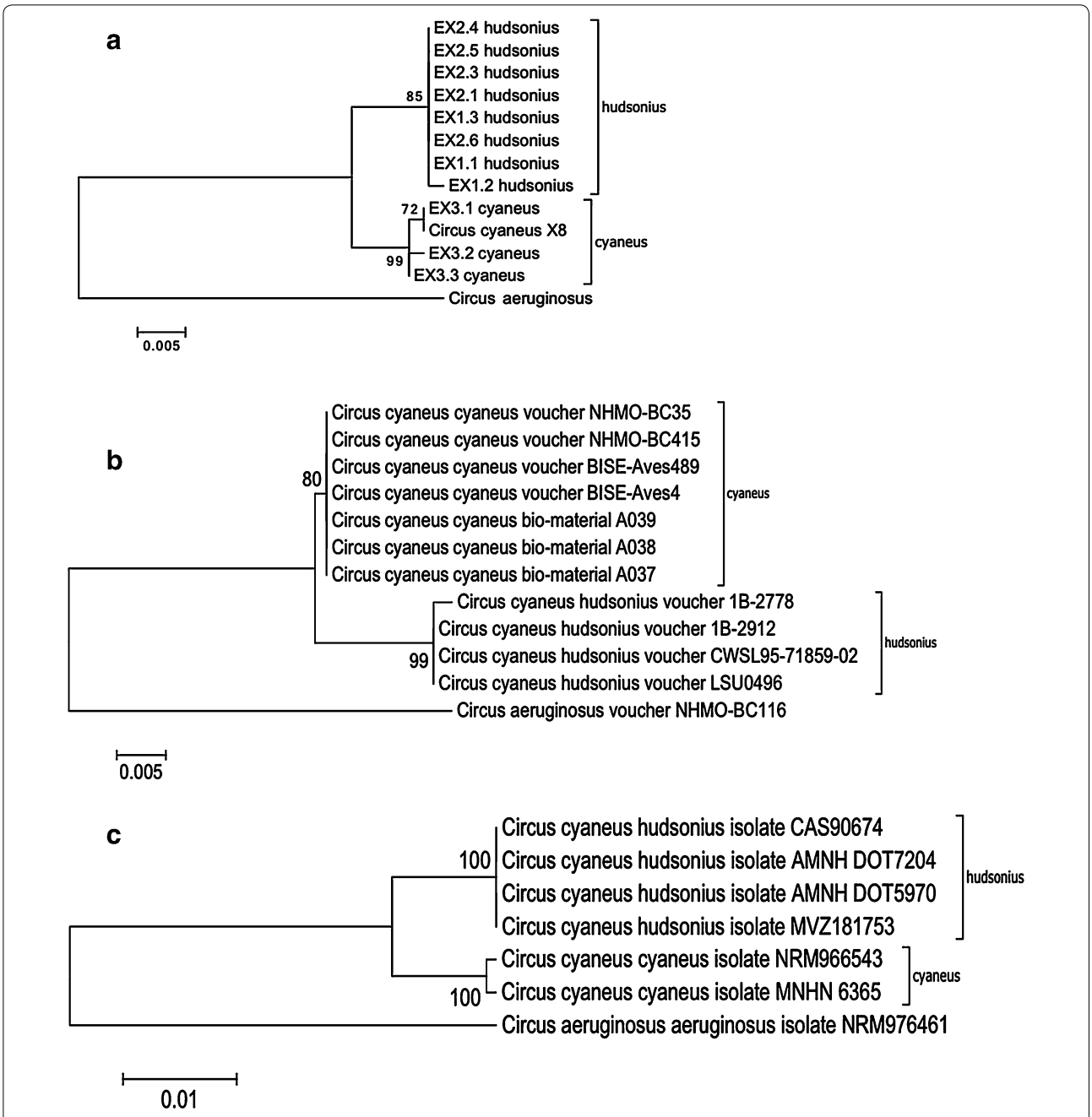

Fig. 1 Phylogenetic trees with bootstrap values for *C. c. cyaneus* and *C.c. hudsonius* for **a** Cytochrome *b*, **b** Cytochrome Oxidase subunit 1 and **c** NADH dehydrogenase 1. *C. aeruginosus* (Western Marsh Harrier) was used as the outgroup for all trees

Nucleotide frequencies were also calculated for each form. The overall nucleotide frequency for both forms was A—0.28, T—0.24, C—0.35 and G—0.13 (GC content = 0.48).

A pairwise genetic distance matrix for Cytochrome *b* is shown in Additional file 3: S5. As can be seen from the summary in Table 1, the overall genetic distance within both forms is very low, while the genetic distance between each form is much higher with the maximum value between the forms (0.01838) being over 13 times greater than the highest value within either form (0.00139 in *cyaneus*). The minimum distance value between the two forms (0.0153) is also five times greater than the maximum distance value calculated within either form (0.00307 in *cyaneus*).

Table 1 Summary of pairwise genetic distances matrix for Cytochrome *b*, ND1 and COI sequences providing minimum, maximum and mean distances (%) for both intra-form and inter-form comparisons

Gene/form	Minimum intra-form distance	Maximum intra-form distance	Mean intra-form distance	Minimum inter-form distance	Maximum inter-form distance	Mean inter-form distance
Cyt*b*/*cyaneus*	0	0.307	0.174	1.53	1.838	1.623
Cyt*b*/*hudsonius*	0	0.139	0.035			
ND1/*cyaneus*	0.164	0.164	0.164	1.802	1.802	1.802
ND1/*hudsonius*	0	0	0			
COI/*cyaneus*	0	0	0	1.292	1.487	1.34
COI/*hudsonius*	0	0.186	0.093			

COI

The aligned COI sequences resulted in an alignment 555 nucleotides in length. Unlike the other datasets, all the *cyaneus* sequences were identical and there was one transversion in *hudsonius*, with one sample having a T to G substitution. There were seven positions in the alignment where *cyaneus* sequences showed constant differences to *hudsonius* sequences of which six were transitions and one a transversion.

Nucleotide frequencies were also calculated for each form. The overall average nucleotide frequency was A—0.26, T—0.27, C—0.31 and G—0.16 (GC content = 0.47).

A pairwise genetic distance matrix is shown in Additional file 4: S7 and summarized in Table 1. As found in the other datasets, the overall genetic distance within both forms is very low, while the genetic distance between each form is much higher with the maximum value between the forms (0.01292) being 14 times greater than the highest value within either form (0.00093 in *hudsonius*).

ND1

For the ND1 sequences, all the *hudsonius* ND1 sequences were identical, with no substitutions across the complete 1223 nucleotide stretch of sequences. The two *cyaneus* sequences, on the other hand, showed a slight degree of variation. At one position, a non-synonymous A to G transition can be found which results in an Isoleucine to Valine amino acid change along with another synonymous transition of a C to T.

One indel was identified between *hudsonius* and *cyaneus*, giving a gap in *hudsonius* and a "T" in *cyaneus*, although this was outside the ND1 coding region. SNPS were identified at a further 21 locations, of which 20 were transitions and one was a transversion. Two of the transitions within the coding regions resulted in non-synonymous changes.

Nucleotide frequencies were also calculated for each form. The overall nucleotide frequency was A—0.29, T—0.26, C—0.32 and G—0.13 (GC content = 0.45).

A pairwise genetic distance matrix is shown in Additional file 5: S6 and summarized in Table 1. As seen in the distance matrix for other datasets, the overall genetic distance within both forms is very low, while the genetic distance between each form is much higher with the maximum value between the forms (0.01802) being 11 times greater than the highest value within either form (0.00164 in *cyaneus*).

Morphology

We carried out a range of measurements from museum specimens of both taxa and provide the mean of those measurements in Table 2. A complete list of all our museum measurement data and measurements from the literature can be found in the Additional file 6: S2 for our museum measurements and Additional file 7: S3 for measurements from the literature.

Males versus females—As in most raptor species, female harriers are significantly larger than males. The MANOVA test indicated that there is a significant size difference between the sexes in both *cyaneus* and *hudsonius* (averaged across taxa, $p \leq 0.001$). Also, all of the mean measurements of male size are smaller than those for female size (Additional file 6: S2; Additional file 7: S3).

Table 2 Mean measurements (with SD) in mms of seven different features for 43 museum specimens

	C. c. hudsonius		*C. c. cyaneus*	
	Male ($n = 18$)	Female ($n = 10$)	Male ($n = 9$)	Female ($n = 6$)
Length	439 (21)	496 (29)	432 (17)	496.8 (34)
Wing (cord)	345 (7)	382 (15)	339 (8)	368 (12)
Tail length	229 (9)	260 (14)	231 (12)	258 (7)
Bill-nostril-tip	16.3 (0.5)	20.3 (3.4)	15.9 (0.75)	17.9 (0.9)
Bill-cere-tip	16.4 (0.9)	19.9 (0.98)	15.8 (0.84)	18 (0.8)
Bill depth	16.6 (0.1)	18.3 (0.98)	16.5 (1.3)	17.6 (0.6)
Tarsus	69.9 (4.1)	76.3 (5.6)	68.1 (3.2)	74.5 (1.5)

A complete list of all the museum measurement data can be found in Additional file 6: S2

Cyaneus versus *hudsonius*—Our data show that with respect to the individual measurements, in all but one case both male and female *hudsonius* are consistently larger than their corresponding sex in *cyaneus*. The only exception to this is male tail length, where *cyaneus* shows a 0.2 cm longer tail. Although this may represent a real difference between the two forms it should be noted that tail length is notoriously difficult to measure accurately and consistently as the tail feathers disappear into the rump of the birds, and finding the actual base of the tail can be difficult. The variation in tail length measurements reported by other authors also seems to echo this inconsistency (Additional file 7: S3). The MANOVA test suggests that the mean of the seven individual size measurements averaged across both sexes is significantly different between *cyaneus* and *hudsonius* ($p = 0.017$). Two-way ANOVA test also suggests a significant difference between only males of the two forms ($p = 0.067$, $p = 0.037$ when tail length not included) and only females of the two forms ($p = 0.012$).

Plumage

Circus cyaneus obtains full adult plumage in its third calendar year, going through various plumages before reaching maturity (Fig. 2).

The differences between each sex and age group are as follows:

Adult males

Adult male *hudsonius* (Fig. 2a) differs from *cyaneus* (Fig. 2b, c) in being a much darker and more streaked bird overall. It has a dark "saddle" across the back, darker wing-coverts and more variably streaked under-parts, including the under-wing. Adult male *hudsonius* also has five black outer primaries (p6–10), p7–10 having roughly half of the primary base coloured white on the dorsal surface, and with only the tip of p6 being black. Adult male *cyaneus* has six black outer primaries, with almost the entire vane of p6–10 being black, with a notably longer black tip to p5. Adult male *cyaneus* also show an even black trailing edge on all of the inner primaries and secondaries, whereas *hudsonius* has less well-marked blackish sub-terminal spots on the inner five primaries, but broader black tips to the secondaries. Adult male *hudsonius* also exhibits black barring on the secondaries and rufous barring on the under-wing coverts and axillaries, while *cyaneus* typically shows only white on the under-wing coverts and axillaries, with very little streaking or barring on the flight feathers or under-wing coverts. The dark appearance of the upper-wing of *hudsonius* is created by the combined effect of the dark tips on the greater and median wing coverts (including the alula), almost completely dark lesser coverts, dark central streaking on the primary coverts and the "background" grey of the wing being of a darker shade than in *cyaneus*. Furthermore, the grey secondaries and primaries are surrounded by darker feathers (primaries, trailing edge, and coverts), which forms a grey "window" in the flight feathers of *hudsonius*. The scapular and mantle feathers of *hudsonius* are all very dark, lending a dark "saddled" effect to the back. In comparison, adult male *cyaneus* has a consistently pale grey back and upper wing, with contrasting black primaries. The back of *cyaneus* sometimes appears darker, but never to the degree that it does in *hudsonius*, and may very well be just a characteristic which varies among younger adults. In *cyaneus* the dark tips to the secondaries appear as a dark grey band along the length of the trailing edge of the open wing and just a few dark-shafted coverts create the only hint of streaking.

The tail of adult male *hudsonius* is also very different from that of *cyaneus*. It is a shade of grey lighter than that of the secondaries, with a thick black sub-terminal band and between four and six thinner, but distinct bars across all but the much paler outer rectrices, where they appear to be fainter and thinner. Also, the sixth (basal) tail bar is often obscured by the upper-tail coverts. Adult male *cyaneus* has an evenly pale-grey tail, usually lacking any noticeable tail bars and only the faintest of any other dark markings.

The under-parts of *hudsonius* and *cyaneus* also differ considerably. Adult male *cyaneus* has a white vent, belly, and lower breast, with a smoky-grey upper breast, throat and chin which extends up onto the side of the head and is very much consistent with the overall greyness of the bird. Adult male *hudsonius*, on the other hand, has a completely white background to the under-parts, with extensive rufous streaking, barring, and spotting. Generally, the thighs, flanks, and under-tail coverts are spotted, the side of the breast is barred, and the central region of the breast, throat and chin have long, vermiculated streaking that creates a rufous-grey breast band on more heavily marked individuals. Furthermore, whereas *cyaneus* has an unmarked grey head, *hudsonius* has a finely streaked head and often shows a whitish forecrown, supercilium and lower-eye crescent.

Sub-adult males

Male *hudsonius* acquire their first adult-type plumage through a protracted moult from April to October during the second calendar year (Wheeler 2003), whilst male *cyaneus* has a similarly timed second-year moult that occurs between May and October (Forsman 1999). Sub-adult male *hudsonius* are much darker and more strongly patterned than fully mature adult males, often retaining signs of their juvenile plumage around the head, neck and breast. They also show extensive streaking on the breast

Fig. 2 The range of plumages found in *hudsonius* and *cyaneus*. **a** *hudsonius* male, **b**, **c** *cyaneus* male, **d** *hudsonius* female, **e** *cyaneus* female, **f** *hudsonius* juvenile and **g** *cyaneus* juvenile. A full account of plumage characteristics is discussed in the text. (Photo credits: **a**—Simon Richards, **b**—Hiyashi Haka, **c**—Dirk-Jan Hoek, **d**, **f**—Julian Hough, **e**—Matti Suopajärvi and **g**—Peter Blanchard)

and throat, with the thighs, vent, axillaries and under-wing-coverts being heavily spotted with rufous, although by this age they do show the adult-type distribution of black on the primaries. While rufous spotting and streaking may be present in sub-adult *cyaneus*, it is restricted to the breast and upper belly, and when present, it tends to be very light on the thighs and vent. Even by this age, the smoky-grey upper breast and throat characteristic of adult male *cyaneus* is present and the streaking does not extend far onto this area. The mantle feathers of *cyaneus* at this age are also browner, as opposed to the dark grey mantle of adult *hudsonius*.

Another notable difference in the plumage of sub-adult males is in the tail markings. In *cyaneus*, the only complete tail bar is also the most terminal and is usually quite subtle. The outer rectrices of *cyaneus* have thin, faint barring while the remainder of the tail feathers exhibit barring that does not reach the outer edges of the feather and is restricted mainly to the inner webs of the feathers. As in the adults, sub-adult *hudsonius* have up to seven tail bars (including sub-terminal) that are all quite thick and extend the whole width of the feather.

Juveniles

Typically-plumaged juvenile *hudsonius* (Fig. 2f) has deep chestnut-orange under-parts with little or no streaking, from the throat to the undertail-coverts. It has a dark hooded appearance, created by dark ear-coverts and dark sides to the neck forming a solid "boa" around the bird's neck. This coloration makes it appear quite similar to juvenile Pallid Harrier (*Circus macrourus*), a Palearctic species. Juvenile *hudsonius* also has dark upper-parts that contrast markedly with their white rumps. Juvenile *cyaneus* (Fig. 2g) typically have a buff background to their under-parts and heavy streaking across the breast, belly and flanks. Although *cyaneus* also has dark ear-coverts, it rarely shows the dark "boa" of *hudsonius* and those *cyaneus* individuals that do exhibit a dark "boa" are usually at the more extreme-dark end of the plumage spectrum and also show heavily marked under-parts (Mullarney and Forsman 2011). Juvenile *cyaneus* also have paler upper-parts than *hudsonius*, rarely reaching the dark sepia-brown that is so characteristic of the latter.

While juvenile *hudsonius* can be distinguished from juvenile *cyaneus* by a range of characters, there are extremely rare occasions where they may appear quite similar overall (Thorpe 1988). Although the amount of streaking present in *cyaneus* is rarely as sparse as that of *hudsonius*, where it is restricted to the flanks and upper breast, the most heavily streaked *hudsonius* individuals may overlap with some of the least-streaked *cyaneus* individuals (Mullarney and Forsman 2011).

With respect to the under-wing of *hudsonius*, p6–8 usually show five or six strong, yet thin, bars plus a dark tip. In *cyaneus*, there are usually only three or four such bars present on these same feathers, and they are thicker in comparison. Also p10 (the shortest, outer primary) in *cyaneus* has three bars, whereas *hudsonius* typically has four bars, but may show only three on occasion (Mullarney and Forsman 2011). Juveniles of both forms exhibit a series of three bars across the under-wing on the secondaries, with the terminal bar (nearest the tip), being the thickest. In *hudsonius*, the central bar is usually noticeably thinner than that of *cyaneus*, although there is substantial variation and overlap in this particular character.

The under-wing pattern of juvenile *hudsonius* is generally very much like that of adult females, but with a buff, not white, background to the flight feathers. The under-wing coverts of *hudsonius* juveniles are streaked, rather than spotted as in females, and the background coloring is fairly rufous. The under-parts of juvenile *cyaneus* are usually streaked and look very similar to adult females, although with comparatively warmer tones to the under-parts and under-wing. Juveniles of both forms can be sexed according to iris color with females having completely dark eyes and males having a light grey-green iris.

Adult females

Adult female *hudsonius* (Fig. 2d) and *cyaneus* (Fig. 2e) superficially resemble juvenile birds in that they have dark brown upper-parts, white rumps and similarly marked under-wings. The under-wing markings previously noted for juveniles also apply generally to females of each form. In adult female *hudsonius* the breast, belly, vent and flanks are light buff, lacking the orange tones characteristic of juveniles, and have strong streaking over the entire under-parts. Female *hudsonius* usually show diamond shaped markings on their flanks, whereas *cyaneus* usually shows streaking in this area, although some of the *cyaneus* museum specimens examined also had diamond shaped markings on their thighs. Furthermore, in the field the bars on the under-wing of *hudsonius* are easier to see than in *cyaneus*, due to the paler background color of the secondaries in *hudsonius*.

Discussion

We have shown how the two forms of *Circus cyaneus*—*C. c. cyaneus* and *C. c. hudsonius* vary in a number of morphological and genetic characters and we discuss this, along with other work further.

Mitochondrial and nuclear phylogeny

A complete phylogenetic analysis has been carried out using three mitochondrial loci which consistently show that the two forms represent divergent linages.

Previous work (Oatley et al. 2015) was carried out using a total of 2032 bp from three nuclear genes [Myoglobin intron-2 (MB), Beta Fibrinogen intron-5 (FGB) and TGFβ2 intron-5 (TGFB2)] as well as the mitochondrial ND1 gene from 39 Accipitriformes, which included 16 different *Circus* taxa. They also showed *cyaneus* and *hudsonius* to be separate species, with *Circus cinereus* (Cinereous Harrier) being a sister species to *hudsonius* and then *cyaneus* sister to those two species. This work subsequently led to the recognition of *hudsonius* as a full species by some authorities (Gill and Donsker 2015; Sangster et al. 2016).

To demonstrate further the substantial degree of divergence between the two forms, examples of differences in overall appearance, behavior and life history are provided. Differences in habitat, dispersal, mate choice, nesting site and other ecological characters are numerous.

The most notable morphological variation between *cyaneus* and *hudsonius* may be observed in the consistent differences in their respective plumages. These differences were also compared between each sex and age group. Morphologically, individuals from each form are diagnosable by a number of qualitative differences. Adult males can be distinguished by approximately 13 different characters, females by about 4 characters and juveniles by at least three. *hudsonius* also averages slightly larger than *cyaneus* over a range of morphological characters. The differences in morphology (both body size and plumage details), vocalization and ecology between the two forms mirrors their existence in separate ecological niches. The expansion of *hudsonius* into North America would have meant that it underwent fundamental ecological changes in order to adapt to the new environment, climate and habitats.

The genetics of the two forms can also be examined from the molecular work. As can be seen from Table 1, the intra-taxa genetic distances are quite low, but the inter-taxa genetic distances are relatively high. The intra-taxa genetic distances are generally higher in *cyaneus* than in the more genetically uniform *hudsonius*. The general mutation rate for Cytochrome *b* in birds has been estimated at around 2 % per million years (Avise et al. 1987; Weir and Schluter 2008) but other estimates suggest as little as 0.64 % (Pereira and Baker 2006). Using these two rates (0.64 and 2.0) as a minimum and maximum mutation rate, we can estimate a divergence time for our sequences. For Cytochrome *b*, using the maximum genetic distance between forms of 0.01838, a divergence time of between 0.765 and 2.39 mya is found. Using the minimum genetic distance between forms of 0.0153, a divergence time of between 0.915 and 2.87 mya is found. For ND1 (in which the min/max are the same), a divergence time off 0.9 and 2.815 mya is found, echoing the estimates calculated for Cytochrome *b*. Oatley et al. (2015) also examined divergence times of the harriers and estimated that the emergence of the *Circus* clade occurred between 2.7 and 6.6 mya during the expansion of the C4 grasses, followed by the diversification of the steppe harrier clade (in which *cyaneus* and *hudsonius* are placed) between 2.2 and 5.5 mya. These estimates overlap and agree well with our estimates of *Circus cyaneus* divergence times. These estimates have been examined further. Generation time and body size were found to be correlated with the rate of mitochondrial genome evolution and caused biases in molecular dating (Nabholz et al. 2016). The authors re-examined the study by Oatley et al. (2015) and calibrated the emergence of the *Circus* clade to between 11 and 13.1 mya, with the subsequent splitting of the *cyaneus*/*hudsonius* complex of 2.1–2.5 mya. The authors conclude that although their estimates are much older, the dates still largely agree with the appearance of C4 grasses during the mid-Miocene.

Much avian phylogenetic work in the Accipitriformes has been carried out using mitochondrial DNA sequences, of which the resulting genetic distances between well-defined species are comparable to those found in our study (approaching 2 %). In a study of Old and New World Vultures (Wink 1995), species within the *Torgos* and *Gyps* genera typically showed genetic divergence of only 2 % with Griffon Vulture (*Gyps fulvus*) and Cape Vulture (*G. coprotheres*) having a genetic divergence as low as 0.9 %. In a study of the Old World Buzzards (*Buteo*) (Kruckenhauser et al. 2004), genetic distances between undisputed species ranged between 1.0 and 1.6 %. In a mitochondrial phylogeny of Sea Eagles (*Haliaeetus*) (Wink et al. 1996) genetic distances between seven species of sea eagles varied between 0.3 and 9.8 %. Also, a Cytochrome *b* sequence divergence of only 1.75 % was found between Greater Spotted Eagle (*Aquila clanga*) and Lesser Spotted Eagle (*A. pomarina*) (Helbig et al. 2005).

There are also numerous differences between *cyaneus* and *hudsonius* when it comes to vocalization, habitat, distribution and movements, mate choice and breeding biology.

Vocalization

Comparisons were made between single-samples of previously published spectrographs of *hudsonius* and *cyaneus* (Cramp and Simmons 1980; MacWhirter and Bildstein 1996). Male harriers sometimes give a distress call when attacking a potential predator. In *cyaneus* eleven "keks" are emitted per second, each of which starts at 2 kHz and finishes at less than 6 kHz (Cramp and Simmons 1980). In *hudsonius* six "kek" calls are

emitted per second, starting at 0 kHz and finishing at 6 kHz (MacWhirter and Bildstein 1996).

Female harriers often give a distress call when they are approached at the nest by a potential predator. In *cyaneus* the call consists of eight "keks" per second at a frequency between 2 and 5 kHz (Cramp and Simmons 1980), while *hudsonius* emits six "keks" per second at a frequency between 0 and 6 kHz (MacWhirter and Bildstein 1996).

Habitat

hudsonius

As its old common name "Marsh Hawk" suggests, *hudsonius* prefers marshes, fresh and brackish wetlands, and damp meadows with undisturbed vegetation during the breeding season, especially in northeast and Midwest regions of North America. Upland prairies, dry grasslands, agricultural areas, and riparian woodlands up to little more than 2400 m above sea level are also used, but mainly in western North America, while dense forest habitats are avoided. The winter range can be more variable, with birds tending to frequent most open habitats, especially in lowlands (Apfelbaum and Seelbach 1983; MacWhirter and Bildstein 1996).

cyaneus

In Britain and Ireland, *cyaneus* breeds almost exclusively on moorland and in young coniferous forests. In Holland, sand dunes are occupied as breeding habitat, whereas in Scandinavia, they nest both on high conifer plateaus and around lowland sedge-fringed lakes. In winter, as in most *Circus* species, *cyaneus* may be found frequenting lowland plains and marshes (Simmons et al. 1987; Simmons 1988; Etheridge and Summers 2006).

Distribution, dispersal and migration

The *hudsonius* population breeds south of a line that runs roughly from northern Alaska, down along the southern shore of the Hudson Bay and into southern Quebec and the Maritime Provinces, and north of a line running from central California, through northern Texas, up to the Great Lakes and then south-east to New Jersey. It winters throughout much of the lower 48 United States, south through Central America, various Caribbean islands and into South America, but usually no further south than Colombia and Venezuela.

The *cyaneus* population breeds across Eurasia, south in Europe to Portugal and north to Finland, then east across Asia to the Kamchatka Peninsula in the north and south to eastern China. In Asia, some individuals winter as far south as Iran and northwest Pakistan, across to Indo-China and possibly the northern Philippines (Ferguson-Lees and Christie 2001). In autumn, there is a usually a short southerly migration, starting in late September. Females, and maybe non-breeding birds, that are dispersing earlier from the breeding territory, mainly undertake these short migrations. These birds generally appear in Ireland and northwest Europe (Belgium, France, etc.) by late October, while males do not arrive until mid-November. The northern most regions of the breeding range are almost completely deserted by males, which often complete a south-westerly migration in severe weather, while females can endure complete snow cover due to their ability to capture larger prey items.

Breeding behaviour

Mate choice

Both males and females engage in a display flight during the breeding period, termed "sky-dancing", the frequency of which is correlated with food availability (Simmons 1991). It has been noted that in Orkney, Scotland, female *cyaneus* displayed up to five times as often as males, but in New Brunswick (Canada) *hudsonius* males displayed 12 times as frequently as females (Simmons et al. 1987). Although the female:male sex ratio in Orkney (3:1) was more greatly skewed than the sex ratio of the population in New Brunswick (3:2), it is unknown if this imbalance accounts for the gender and frequency differences in display behavior or if it is reflection of some divergence in mating behavior since the two forms became fundamentally allopatric (Simmons et al. 1987).

Nest site

cyaneus There is a preference in *cyaneus* to place the nest in heather that is taller than the surrounding vegetation and a further preference for vegetation that is 40–50 cm high. In a study of 52 Scottish *cyaneus* nests, it was found that *cyaneus* showed a clear preference for nesting in heather (*Calluna vulgaris*) (Redpath et al. 1998). The average length of heather used to nest in was not that of the average length of heather. Nearly 50 % of birds nested in heather between 40 and 50 cm high, with 40 % of birds evenly occupying heather 30–40 and 50–60 cm high. It was also found in a separate study that out of 922 nests, 76 % were located in heather moorland. Other nest sites included upland grassland and open canopy and closed canopy woodlands (Redpath et al. 1998).

hudsonius *hudsonius* occupies a wide range of habitats. In a survey of 428 nests, 17 % were located in wet sedge meadows, 18 % were in freshwater reed marshes, 26 % were located in dry grasslands and 8 % were in agricultural fields (Apfelbaum and Seelbach 1983). The nest is usually constructed in standing water, on floating platforms that are raised above the water level, or in tall vegetation (e.g. reeds, cattails, etc.).

Male desertion

Male harriers generally supply food to the female who feeds the young herself, although when the young are old enough to feed themselves, males may provision them at the nest directly. After this initial period, by the time the chicks are about three weeks old, male investment in feeding the young often declines. In some instances food provisioning was found to cease, with the male deserting the nest completely (Simmons 2000). In one study of *hudsonius*, females at 7 out of 11 nests that were deserted by the male managed to fledge their young alone, while the nestlings in the other 4 nests died of starvation. Contrastingly, *cyaneus* females can rarely raise young without help from their mate (Simmons et al. 1987).

Conclusions

Circus cyaneus and *C. hudsonius* fulfil all the criteria set out by the BOU for assigning species rank to allopatric taxa (Helbig et al. 2002). They are fully diagnosable in several characters (i.e. 13, 4 and 3 characters for male, female and juvenile respectively) and have different DNA sequences. Should the two taxa ever become sympatric and create contact zones in the future, morphology, vocalization, breeding biology, and habitat should clearly act as prezygotic barriers.

Our finding of a genetic distance between the forms *hudsonius* and *cyaneus* of up to 2 % falls well within the range of other well-established species and taking into account other morphological and ecological differences found between the two forms, we suggest that *cyaneus* and *hudsonius* represent distinct evolutionary lineages and should be treated as separate species. We recommend the scientific name for the (currently nominate) Eurasian species remain as *Circus cyaneus* retaining the common name of Hen Harrier, and that the American form is given the scientific name of *Circus hudsonius* with the common name of Northern Harrier.

Additional files

Additional file 1: S1. Sample information for Cytochrome *b* sequencing. Taxonomic designations, museum collections, voucher numbers, collection site information and NCBI accession numbers for the frozen tissue samples used in this study (UW = University of Washington, Burke Museum, LSU = Louisiana State University Museum of Natural Science, MSWB = University of New Mexico, Museum of Southwestern Biology, SMNH = Swedish Museum of Natural History, ZMUC = University of Copenhagen Zoological Museum). NA* refers to samples that did not amplify under PCR and could not be used for further analysis.

Additional file 2: S4. DNA extraction and sequencing protocol. Detailed information providing DNA extraction protocols, primer usage, PCR amplification and purification, and DNA sequencing protocol for Cytochrome *b* sequencing.

Additional file 3: S5. Pairwise genetic distance matrix for Cytochrome *b*.

Additional file 4: S7. Pairwise genetic distance matrix for Cytochrome *c* oxidase subunit 1 (COI).

Additional file 5: S6. Pairwise genetic distance matrix for NADH dehydrogenase (ND1).

Additional file 6: S2. *Circus cyaneus* Museum measurements. Individual measurements for each of the 43 *Circus cyaneus* museum specimens of the forms *cyaneus* and *hudsonius*. *hudsonius* collection locations are given as standard US state and Canadian province abbreviations.

Additional file 7: S3. Measurements taken from literature. Measurements broken down by form and sex for eight characters found in the literature for either hudsonius or cyaneus. References for each measurement can be found in the main text.

Authors' contributions

GJE and JAM carried out Cytochrome *b* sequencing. GJE carried out literature review, museum visits and measurements, and phylogenetic and statistical analysis. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The dataset supporting the conclusions of this article are available in the GenBank repository, http://www.ncbi.nlm.nih.gov/nucleotide/. The accession numbers for each sequence can be found in Additional file 1: S1.

Consent for publication

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References

AOU (American Ornithologists' Union). Check-list of North American birds, 6th ed. Washington DC: American Ornithologists' Union; 1983.

Apfelbaum SI, Seelbach P. Nest tree, habitat selection and productivity of seven North American raptor species based on the Cornell University nest record card program. Raptor Res. 1983;17:97–113.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu Rev Ecol Syst. 1987;18:489–522.
Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Rapp BA, Wheeler DL. GenBank. Nucleic Acids Res. 2002;28:15–8.
Boehning-Gaese K, Gonzalez-Guzman LI, Brown JH. Constraints on dispersal and the evolution of the avifauna of the Northern Hemisphere. Evol Ecol. 1998;12:767–83.
BOU (The British Ornithologists' Union). A list of British birds. 2nd edn. London: BOU; 1915.
Chesser RT, Banks RC, Burns KJ, Cicero C, Dunn JL, Kratter AW, Lovette IJ, Navarro-Sigüenza AG, Rasmussen PC, Remsen J Jr. Fifty-sixth supplement to the American Ornithologists' Union: check-list of North American birds. Auk. 2015;132:748–64.
Chesser RT, Burns KJ, Cicero C, Dunn JL, Kratter AW, Lovette IJ, Rasmussen PC, Remsen JV, Rising JD, Stotz DF, Winker K. Fifty-seventh supplement to the American Ornithologists' Union: check-list of North American birds. Auk. 2016;133:544–60.
Cramp S, Simmons KEL. Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic. Vol II. Oxford: Oxford University Press; 1980.
Edwards G. The Ring-tailed Hawk. Natural History of Birds. Hudson Bay; 1750. p. 107.
Edwards G. The Blue Hawk. Glean Nat Hist. 1758; 1:33.
Edwards G. The Marsh Hawk. Glean Nat Hist. 1760; 2:291.
Etheridge B, Summers WS. Movements of British Hen Harriers *Circus cyaneus* outside the breeding season. Ring Migr. 2006;23:6–14.
Ferguson-Lees J, Christie D. Raptors of the world: a field guide. London: Christopher Helm; 2001.
Forsman D. The raptors of Europe and the Middle East: a handbook of field identification. London: T&AD Poyser; 1999.
Gill F, Donsker D. IOC world bird list (v 5.2); 2015. http://www.worldbirdnames.org. doi: 10.14344/IOC.ML.5.2.
Hasegawa M, Kishino H, Yano T. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol. 1985;22:160–74.
Helbig AJ, Knox AG, Parkin DT, Sangster G, Collinson M. Guidelines for assigning species rank. Ibis. 2002;144:518–25.
Helbig AJ, Seibold I, Kocum A, Liebers D, Irwin J, Bergmanis U, Meyburg BU, Scheller W, Stubbe M, Bensch S. Genetic differentiation and hybridization between greater and lesser spotted eagles (Accipitriformes: *Aquila clanga*, *A. pomarina*). J Ornithol. 2005; 146:226–234.
Johnsgard PA. Hawks, eagles and falcons of North America. Washington, DC: Smithsonian Institution Press; 1990.
Kruckenhauser L, Haring E, Pinsker W, Riesing MJ, Winkler H, Wink M, Gamauf A. Genetic vs. morphological differentiation of Old World buzzards (genus *Buteo*, Accipitridae). Zool Scripta. 2004;33:197–211.
Linné. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio duodecima, Reformata. 1766; p. 1–532.
MacWhirter RB, Bildstein KL. Northern Harrier (*Circus cyaneus*). In: Poole A, Gill F (eds) The birds of North America (No. 210); 1996.
Martin JP. From the Rarities Committee's files—'Northern Harrier' on Scilly: new to Britain. Brit Birds. 2008;101:394–407.
Mullarney K, Forsman D. Identification of Northern Harriers and vagrants in Ireland, Norfolk and Durham. Birding World. 2011;23:509–23.
Nabholz B, Lanfear R, Fuchs J. Body mass-corrected molecular rate for bird mitochondrial DNA. Mol Ecol. 2016;25(18):4438–49.
Oatley G, Simmons RE, Fuchs J. A molecular phylogeny of the harriers (*Circus*, Accipitridae) indicate the role of long distance dispersal and migration in diversification. Mol Phylogenet Evol. 2015;85:150–60.
Palmer RA. Handbook of North American birds. New Haven: Yale University Press; 1988.
Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. GenStat for Windows (11th edition). Hemel Hempstead: VSN International; 2008.
Pereira SL, Baker AJ. A mitogenomic timescale for birds detects variable phylogenetic rates of molecular evolution and refutes the standard molecular clock. Mol Biol Evol. 2006;23:1731–40.
Peters JL. Check-list of birds of the world. Cambridge, MA: Harvard University Press; 1931.
Rasmussen P, Anderton J (2005) Birds of South Asia: the Ripley Guide. 2 vols. Barcelona: Lynx Edicions and Smithsonian Institution; 2005.
Redpath R, Madders M, Donnelly E, Anderson B, Thirgood S, Martin A, McLeod D. Nest site selection by Hen Harriers in Scotland. Bird Study. 1998;45:51–61.
Sangster G, Collinson JM, Crochet P-A, Kirwan GM, Knox AG, Parkin DT, Votier SC. Taxonomic recommendations for Western Palearctic birds: 11th Report. Ibis. 2016;158:206–12.
Scharf WC, Hamerstrom F. A morphological comparison of two harrier populations. Raptor Res. 1975;9:27–32.
Simmons KEL. Food and the deceptive acquisition of mates by polygynous male harriers. Behav Ecol Sociobiol. 1988;23:83–92.
Simmons KEL. Comparisons and functions of sky-dancing displays of *Circus* harriers: untangling the Marsh Harrier complex. Ostrich. 1991;62:45–51.
Simmons RE. Harriers of the world. New York: Oxford University Press; 2000.
Simmons KEL, Barnard B, Smith PC. Reproductive behaviour of *Circus cyaneus* in North America and Europe: a comparison. Ornis Scand. 1987;18:33–41.
Swainson W, Richardson J. Fauna Boreali-Americana (Zoology of the northern parts of British America): the birds. London: John Murray; 1831.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–9.
Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinform. Chapter 2: Unit 2-3; 2002.
Thorpe JP. Juvenile Hen Harriers showing 'Marsh Hawk' characters. Brit Birds. 1988;81:377–82.
Watson D. The hen harrier. Berkhamsted: T & AD Poyser; 1977.
Weir JT, Schluter D. Calibrating the avian molecular clock. Mol Ecol. 2008;17:2321–8.
Wheeler BK. Raptors of eastern North America: the wheeler guides. Princeton: Princeton University Press; 2003.
Wheeler BK, Clarke WS. A photographic guide to North American raptors. London: Academic Press Ltd.; 1995.
Wink M. Phylogeny of old and new world vultures (Aves: Accipitridae and Cathartidae) inferred from nucleotide sequences of the mitochondrial cytochrome b gene. Zeitschrift Fur Naturforschung C: J Biosci. 1995;50:868–82.
Wink M, Sauer-Gürth H. Advances in the molecular systematics of African raptors. In: Chancellor RD, Meyburg B-U, editors. Raptors at risk. Berlin: WWGBP/Hancock House; 2000.
Wink M, Seibold I. Molecular phylogeny of Mediterranean raptors (Families Accipitridae and Falconidae). Biol Conserv Mediter Raptors Monogr. 1996;4:335–44.
Wink M, Heidrich P, Fentzloff C. A mtDNA phylogeny of sea eagles (genus *Haliaeetus*) based on nucleotide sequences of the cytochrome 6-gene. Biochem Syst Ecol. 1996;24:783–91.
Wink M, Seibold I, Lotfikhah F, Bednarek W. Molecular systematics of holarctic raptors (Order Falconiformes). In: Chancellor RD, Meyburg B-U, Ferrero JJ (eds) Holarctic birds of prey. Merida & Berlin: Adenex & WWGBP; 1998:29–48.
Witherby HF. A practical handbook of British birds. London: Witherby; 1920.