



The taxonomic position and breeding range of Golden Nightjar *Caprimulgus eximius* (Aves: Caprimulgidae)

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3 **The taxonomic position and breeding range of Golden Nightjar *Caprimulgus eximius* (Aves:**
4 **Caprimulgidae)**

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7 Yvonne Lawrie, Robert Swann¹, Peter Stronach², Yoav Perlman³ and J. Martin Collinson*

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10
11 University of Aberdeen,
12
13 School of Medicine, Medical Sciences and Nutrition,
14
15 Institute of Medical Sciences,
16
17 Foresterhill,
18
19 Aberdeen AB25 2ZD, UK.

20
21
22 *Corresponding author: m.collinson@abdn.ac.uk

23
24
25
26 ¹14 St.Vincent Road, Tain, Ross-shire

robert.swann@homecall.co.uk

27 ²Clachan, Boat of Garten, Inverness-shire PH24 3BX

peterstronach@gmail.com

28 ³ Israel Ornithological Center, Society for the Protection of Nature in Israel, 2 Hanegev St., Tel Aviv
29 66186, Israel

yoav.perlman@gmail.com

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35 **Abstract**

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37 Golden Nightjar *Caprimulgus eximius* is an apparently sedentary sub-Saharan species with a
38 breeding range extending from Senegal and Mauritania to Sudan. Although genetic studies of
39 nightjars and related Caprimulgiformes have been published previously, none has included Golden
40 Nightjar. In this study, mitochondrial and nuclear DNA of a Golden Nightjar found dead in Western
41 Sahara in April 2016 was sequenced and compared to other species in the genus *Caprimulgus*. It was
42 concluded with strong support that Golden Nightjar is closely related to Egyptian Nightjar *C.*
43 *aegyptius*. It is hypothesised that Golden and Egyptian Nightjars may have arisen by splitting of a
44 single ancestral species into migratory and sedentary populations.
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53 **Keywords: Golden Nightjar; Egyptian Nightjar *Caprimulgus; eximius; aegyptius; systematics;***
54 **molecular phylogeny; molecular sexing; Western Sahara; Nubian Nightjar; *nubicus*.**

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3 The avian Order Caprimulgiformes comprises oilbirds (family Steatornithidae), owl-nightjars
4 (Aegothelidae), frogmouths (Podargidae), potoos (Podargidae) and, most important in terms of
5 species number, the Caprimulgidae, including nightjars and New World nighthawks (del Hoyo et al.,
6 1999). Caprimulgid nightjars and nighthawks inhabit temperate and tropical zones worldwide. They
7 share substantially nocturnal and crepuscular aerial feeding strategies with cryptic plumage that acts
8 as camouflage at daytime roost sites. Such highly adaptive and constrained morphologies and
9 plumage patterns have the potential to frustrate systematic and phylogeographic studies.
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11 Phylogenetic studies of Caprimulgiformes based on DNA sequence data have been published and
12 compared to phylogenies based on morphological data (Barrowclough et al. 2006; Braun and
13 Huddleston 2009; Dumbacher et al. 2003; Larsen et al. 2007; Han et al. 2010; Mariaux and Braun
14 1996; Mayr 2010). They showed among other things that the genus *Caprimulgus* s.l. was polyphyletic
15 and that traditional classifications based on morphological criteria may not accurately reconstruct
16 patterns of evolution for these birds. Correct taxonomic arrangement of individual nightjar and
17 nighthawk species requires molecular data. Current taxonomy (Dickinson and Remsen 2014) reflects
18 the recent molecular data but many Caprimulgid species have not yet been sequenced.
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28 Omitted from previous studies was the Golden Nightjar *Caprimulgus eximius* Temminck
29 1826 of Sub-Saharan Africa. This attractive species has been poorly studied until recently (Jackson
30 2011), with few photographs and sound recordings (Cleere 1995; Jackson 2003). Because of its large
31 range, it is not considered to be threatened (Birdlife International, 2016). Its striking yellow-orange
32 plumage with black and white bars on most upperpart feathers is not approached by any other
33 nightjar species, though the general paleness and subtlety of plumage is reminiscent of other some
34 other desert and semi-desert species such as Egyptian Nightjar *C. aegyptius* and Sykes's Nightjar *C.*
35 *mahrattensis*. Vaurie (1960), in his description of Vaurie's Nightjar *C. centralasicus*, noted the
36 atypical speckled plumage and very long upper tail coverts of Golden Nightjar but placed it
37 tentatively between Egyptian Nightjar and a group consisting of Jungle Nightjar *C. indicus*, European
38 Nightjar *C. europaeus* and Red-necked Nightjar *C. ruficollis* (Vaurie 1960). The taxonomic affinities of
39 Golden Nightjar therefore remain uncertain. Two subspecies are commonly recognised: nominate *C.*
40 *e. eximius* of SW and Central Sudan, and *C. e. simplicior*, breeding from Central Chad to Senegal and
41 Mauritania (Dickinson and Remsen 2014), and likely also to breed in Western Sahara (described
42 below). The species appears to be sedentary, possibly with dispersive post-breeding movements (del
43 Hoyo et al. 1999).
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55 On 20 April 2016 a Golden Nightjar was found dead as roadkill by Jurrien van Deijk, Ruben
56 Vermeer, Daan Drukker and Jacob Lotz in the Oued Jenea in Oued Ad-Deheb province of Western
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3 Sahara at coordinates 22.678119 N, 14.492028 E (see <http://tinyurl.com/hlynyhr>). Images of the
4 specimen are presented in Figure 1. Plumage characteristics identify it as probably female (Fry et al.
5 1988; del Hoyo et al. 1999). The off-white tip of the outer tail feather was 19-20 mm which is normal
6 for females (15-25 mm) and is not shown in males (30-40 mm). Wing length of 178 mm and tail
7 length of 114 mm fell in the overlap zone for both sexes. The bird had a large brood patch,
8 suggesting active breeding. Up to 4 singing males had been found in this area during the month
9 (MahgrebOrnitho 2016), implying that Golden Nightjar breeds in this region.
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15 In order to determine the taxonomic affinity of Golden Nightjar, feather samples from the Oued
16 Jena specimen were used to isolate genomic DNA, using the DNA Micro Kit (QIAGEN, UK) according
17 to the manufacturer's instructions with addition of 0.01 M dithiothreitol to the digestion mix and
18 elution in 80 µl of QIAGEN buffer AE. PCR fragments encompassing the entire coding region of the
19 genes encoding cytochrome b (*cytb*) and cytochrome c oxidase subunit 1 (COI) were amplified from
20 samples using primers L14993, H16065, BirdF1 and BirdR2, and conditions described in Helbig et al.
21 (1995) and Hebert et al. (2004). Each 50 µl PCR reaction contained 28.5 µl of ddH₂O, 5 µl of 10x
22 Optibuffer, 1 µl of 50 mM MgCl₂ solution, 3 µl of dNTPs (2 mM each), 5 µl of forward and reverse
23 primers (10 mM each), 2 units (0.5 µl) of BIO-X-ACT Short thermostable DNA Polymerase (Bioline,
24 UK) and 2 µl of template DNA. Fragment of the nuclear *v-myc* myelocytomatosis viral oncogene-like
25 protein (MYC) gene were amplified using the MYC-F-01/MYC-R-04 and MYC-F-02/MYC-R-06 primers
26 (Harshman et al. 2003) as above with 35 cycles at annealing temperature 55°C and 30 s extension.
27 PCR products were separated by electrophoresis on a 1.5% agarose gel. The DNA from each gel
28 fragment was then isolated using the QIAquick Gel Extraction Kit (Qiagen, UK) according to the
29 manufacturer's protocols. Gel-extracted PCR products were sequenced by the Source BioScience
30 (Cambridge) DNA sequencing service.
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42 1008 bp of *cytb*, 695 bp of COI, and 545 bp of MYC were successfully sequenced from the
43 Golden Nightjar. Open reading frames were consistent with these genes being functional, not
44 pseudogenes, with no indels compared to database sequences. Sequences were deposited in the
45 European Nucleotide Archive with Accession numbers: LT671509 (*cytb*), LT671510 (COI), LT671511
46 (MYC). BLAST searches performed at <http://blast.ncbi.nlm.nih.gov/Blast.cgi> confirmed that for all
47 three genes, the most similar deposited sequences were of Egyptian Nightjar, from which there was
48 3.78% uncorrected divergence at the *cytb* locus. The 545 bp of MYC was 100% identical to the MYC
49 sequence from Egyptian Nightjar (Accession number GU586565) and 2-4 bp different (>99%
50 similarity) to multiple other nightjar sequences.
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3 Molecular analysis of phylogeny was performed, comparing Golden Nightjar with
4 representatives of the major clades of nightjars described by Han et al. (2000). Deposited gene
5 sequences were downloaded from GenBank from all previously sequenced species (see Table 1).
6 Sequences were aligned and appropriate models of evolution were selected using MEGA 7.0.
7 Bayesian analysis was performed using the program BEASTv1.8.3 (Drummond *et al.*, 2012) available
8 from (<http://beast.bio.ed.ac.uk>). MCMC chains were run for 10,000,000 generations with trees
9 sampled every 1000 generations using the lognormal uncorrelated relaxed clock model. All
10 remaining parameters, such as the base frequencies, gamma shape parameter, and root height of
11 the tree, were default. The phylogenetic tree estimate was carried out in TreeAnnotator v1.8.3 using
12 the maximum tree clade credibility target tree and median node height. The resulting inferred
13 phylogeny tree is presented as Figure 2. It confirmed with extremely high statistical support that
14 Golden Nightjar falls within the 'Old World' (true) *Caprimulgus* clade (Han et al., 2000) and yields a
15 sister relationship between Egyptian and Golden Nightjars.
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24 Bayesian and Maximum Likelihood analysis based on 771 bp of cytb using the same
25 sequences but also including a 771 bp fragment obtained from a juvenile Nubian Nightjar *C. nubicus*
26 sampled by YP at Ne'ot Hakikar, Israel (30.930712 N 35.390312 E), on 21 June 2006 also resolved the
27 sister relationship of Egyptian and Golden Nightjars and placed the Nubian Nightjar in a poorly
28 resolved polytomy of the *C. eximius/aegyptius* clade, *C. affinis*, and the *C.*
29 *nigriscapularis/pectoralis/policephalus* clade (Supplementary Figure S1). Further work will be
30 needed to determine its taxonomic affinity of Nubian Nightjar, but it is clear that it is not a sister
31 species to Golden or Egyptian Nightjars.
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38 For the smaller number of species for which both sequences (cytb and COI) were available, a
39 comparable Bayesian tree was produced using a 1703 bp concatemer of cytb and COI. The tree is
40 presented as Supplementary Figure S2 and is congruent with Figure 2 in placing Egyptian and Golden
41 Nightjars together with very high statistical support.
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47 Genetic sexing of the Golden Nightjar was performed by PCR using primer sets P2/P8
48 (Griffiths et al., 1998) and 2550F/2718R (Fridolfsson and Ellegren, 1999). Both P2/P8 and
49 2550F/2718R PCRs span introns of the sex-linked CHD1 gene, normally producing bands of different
50 sizes from the Z and W chromosomes, such that males should yield one PCR band and females two.
51 The P2/P8 PCR on Golden Nightjar yielded a single band of approximately 360 bp and the
52 independent 2550F/2718R PCR yielded a single band of approximately 440 bp (Figure 3). At face
53 value these data would suggest the bird was male. However the P2/P8 PCR is noninformative for
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3 many species of non-passerines because the Z and W chromosome bands are often of too similar
4 size to resolve on agarose gels (Griffiths et al. 1998 and pers. obs.). Whereas the 2550F/2718R PCR
5 usually produces well separated bands from non-passerines of 650-750 bp from the Z chromosome
6 and 440-480 bp from the W chromosome, in many species female samples only yield the shorter W
7 chromosome product (Fridolfsson and Ellegren, 1999). In the case of the Golden Nightjar, the single
8 band at ~440 bp is more consistent with a W chromosome product, hence we conclude that the bird
9 is most probably genetically female, consistent with the plumage characteristics. However it remains
10 possible that the morphological and plumage criteria are unreliable for determining sex. It is usually
11 accepted that male nightjars do not develop a brood patch, but Camacho et al. (2014) found brood
12 patches in breeding Red-necked Nightjars of both sexes, and it remains to be seen whether this
13 might occur in other *Caprimulgus*. Biometric criteria for sexing Golden Nightjars used here were
14 based on only 5 male and 5 female individuals (Fry et al. 1988) and secondary sexual features such
15 as the size and number of the white patches in the rectrices and remiges are known to be extremely
16 variable and may be subject to age-related and geographic variation (Forero et al., 1995; Forero and
17 Tella 1997). In the case of Golden Nightjar, more samples of known sex are required to delimit the
18 sexual, geographic and age-related variability of this species and to correlate with the results of
19 molecular work.
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33 Sequence data are not yet available for a further 18 of the 36 species currently included in
34 *Caprimulgus* by Dickinson and Remsen (2013), eight of which have a substantially or completely
35 African distribution. The semi-desert and scrubland-breeding Nubian Nightjar of northeast Africa and
36 the Middle East shares generally pale plumage coloration and striking primary pattern of Golden
37 Nightjar but our preliminary data analysis shows they are not sister species. Of the unsampled
38 African species, (Freckled Nightjar *C. tristigma*, Slender-tailed Nightjar *C. clarus*, Prigogine's Nightjar
39 *C. prigoginei*, Star-spotted Nightjar *C. stellatus*, Swamp Nightjar *C. natalensis*, Red-necked Nightjar *C.*
40 *ruficollis*, Donaldson-Smith's Nightjar *C. donaldsoni* and the poorly known Nechisar Nightjar *C. solala*)
41 none is intuitively more likely to be closely related to Golden Nightjar. The Star-spotted Nightjar is a
42 semi-desert species with some plumage similarity to Golden Nightjar but is considered to possible
43 form a superspecies with Plain Nightjar *C. inornatus* (del Hoyo et al. 1999). Further taxon sampling
44 will be required to fully resolve sister relationships and patterns of evolution of species in this genus.
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55 The phylogenetic trees in this study are broadly congruent with those produced previously in
56 that all major strongly supported nodes in Han et al. (2000) are recapitulated, suggesting that the
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3 strongly supported node linking Egyptian and Golden Nightjars (Figure 2) is robust. This study also
4 provides further evidence of a breeding range extension of Golden Nightjar to include Western
5 Sahara. The main breeding range of Golden Nightjar, from Senegal to the Sudan, closely matches
6 the wintering range of Egyptian Nightjar (del Hoyo et al. 1999). If the two are indeed sister species
7 then it is possible to speculate that their ancestral single species inhabited the desert and semi-
8 desert zone of Africa and split into sedentary and migratory populations, that subsequently
9 speciated. The relatively plain primary pattern Egyptian Nightjar contrasts strongly with the striking
10 black and white patches of Golden Nightjar and may have evolved coincident with speciation,
11 forming a pre-mating interspecific signal that acted as an accelerator for reproductive isolation.
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24
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27 paper.
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Table 1

| Species | Cytb | COI |
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| <i>Caprimulgus eximius</i> | LT671509 | LT671510 |
| <i>Systellura longirostris</i> | JQ988761 | JN801536 |
| <i>Antrostomus vociferus</i> | HQ696494 | AY666512 |
| <i>Systellura longirostris</i> | JQ988765 | |
| <i>Caprimulgus climacurus</i> | GU586687 | |
| <i>Antrostomus saturatus</i> | GU586657 | |
| <i>Antrostomus salvini</i> | GU586656 | |
| <i>Antrostomus rufus</i> | GU586655 | JQ174302 |
| <i>Caprimulgus rufigena</i> | GU586654 | |
| <i>Caprimulgus poliocephalus</i> | GU586651 | |
| <i>Caprimulgus pectoralis</i> | GU586650 | |
| <i>Caprimulgus nigriscapularis</i> | GU586647 | |
| <i>Caprimulgus madagascariensis</i> | GU586646 | |
| <i>Caprimulgus macrurus</i> | GU586644 | |
| <i>Caprimulgus manillensis</i> | GU586642 | |
| <i>Caprimulgus indicus</i> | GU586640 | |
| <i>Caprimulgus fossii</i> | GU586639 | |
| <i>Caprimulgus europaeus</i> | GU586638 | GU571785 |
| <i>Caprimulgus batesi</i> | GU586636 | |
| <i>Caprimulgus affinis</i> | GU586632 | |
| <i>Caprimulgus aegyptius</i> | GU586631 | JF498758 |
| <i>Antrostomus vociferus</i> | GU586635 | |
| <i>Setopagis whitelyi</i> | GU586659 | JQ174303 |
| <i>Antrostomus ridgwayi</i> | GU586653 | |
| <i>Setopagis parvula</i> | GU586649 | JQ174299 |
| <i>Hydropsalis maculicaudus</i> | GU586645 | JQ174294 |
| <i>Gactornis enarratus</i> | GU586637 | |
| <i>Nyctidromus anthonyi</i> | GU586633 | |
| <i>Caprimulgus carolinensis</i> | FJ588442 | JQ174284 |
| <i>Nyctipolus nigrescens</i> | FJ588446 | JQ174298 |
| <i>Hydropsalis cayennensis</i> | FJ588444 | JQ174285 |
| <i>Nyctibius grandis</i> | GU586676 | JQ175587 |

Accession numbers of DNA sequences used in this study.

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3 **Figure Legends**
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6 **Figure 1. Female Golden Nightjar. A.** Female Golden Nightjar, Aousserd Road, Western Sahara, 19
7 April 2016. **B.** Dry scrub habitat where Golden Nightjars were observed. **C.** The female Golden
8 Nightjar *Caprimulgus eximius* sampled in this study, Oued Jenea, Oued Ad-Deheb province of
9 Western Sahara, 20 April 2016. **D.** Vascularised brood patch indicating local breeding.
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14 **Figure 2. Bayesian phylogeny of nightjars (beast analysis) based on *cytb* (1008 bp) to identify the**
15 **systematic position of Golden Nightjar.** The tree was rooted with Great Potoo *Nyctibius grandis* as
16 an outgroup. Support from posterior probabilities is indicated at nodes. See Table 1 for full scientific
17 names and accession numbers.
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22 **Figure 3. Genetic sexing of Golden Nightjar.** Lane 1: P2/P8 PCR. Lane 2: 2550F/2718R PCR. Lane 3:
23 100 bp ladder. Arrow: 500 bp.
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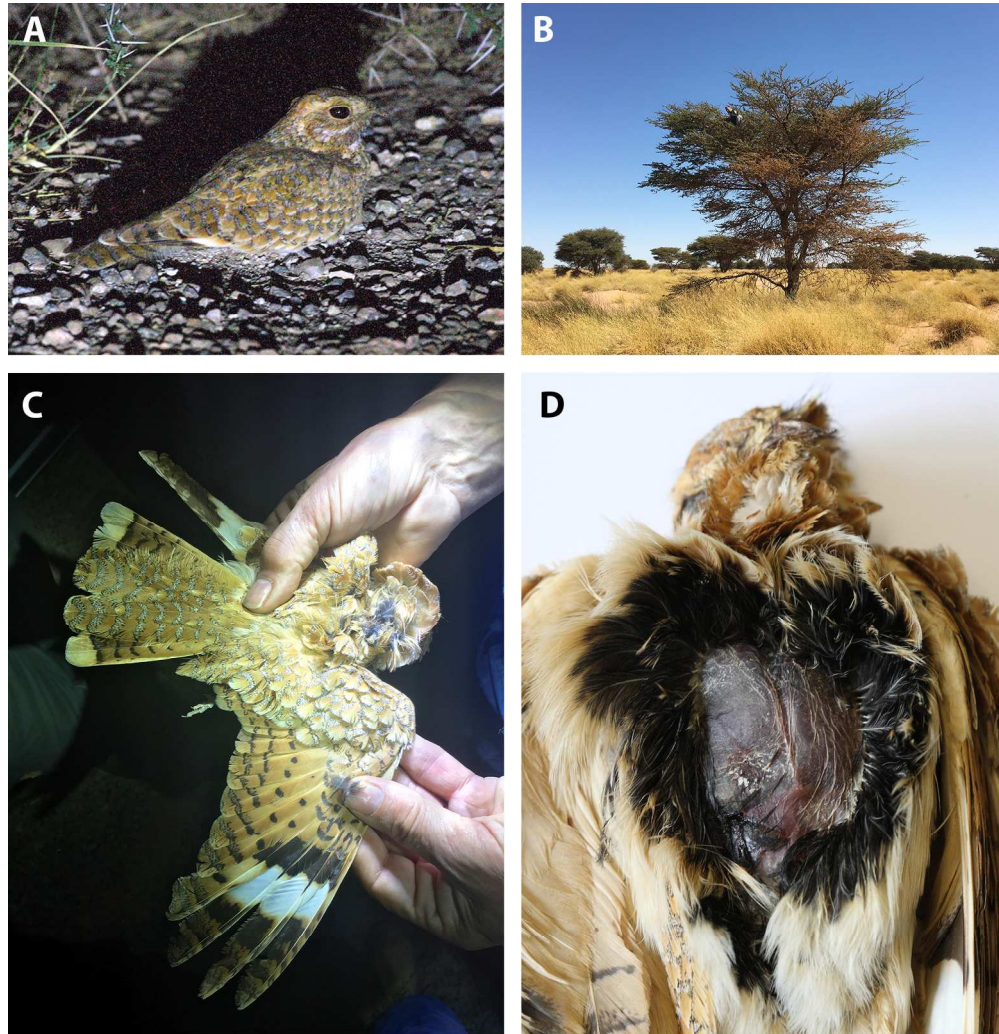


Figure 1. Female Golden Nightjar. A. Female Golden Nightjar, Aousserd Road, Western Sahara, 19 April 2016. B. Dry scrub habitat where Golden Nightjars were observed. C. The female Golden Nightjar *Caprimulgus eximius* sampled in this study, Oued Jenaa, Oued Ad-Deheb province of Western Sahara, 20 April 2016. D. Vascularised brood patch indicating local breeding.

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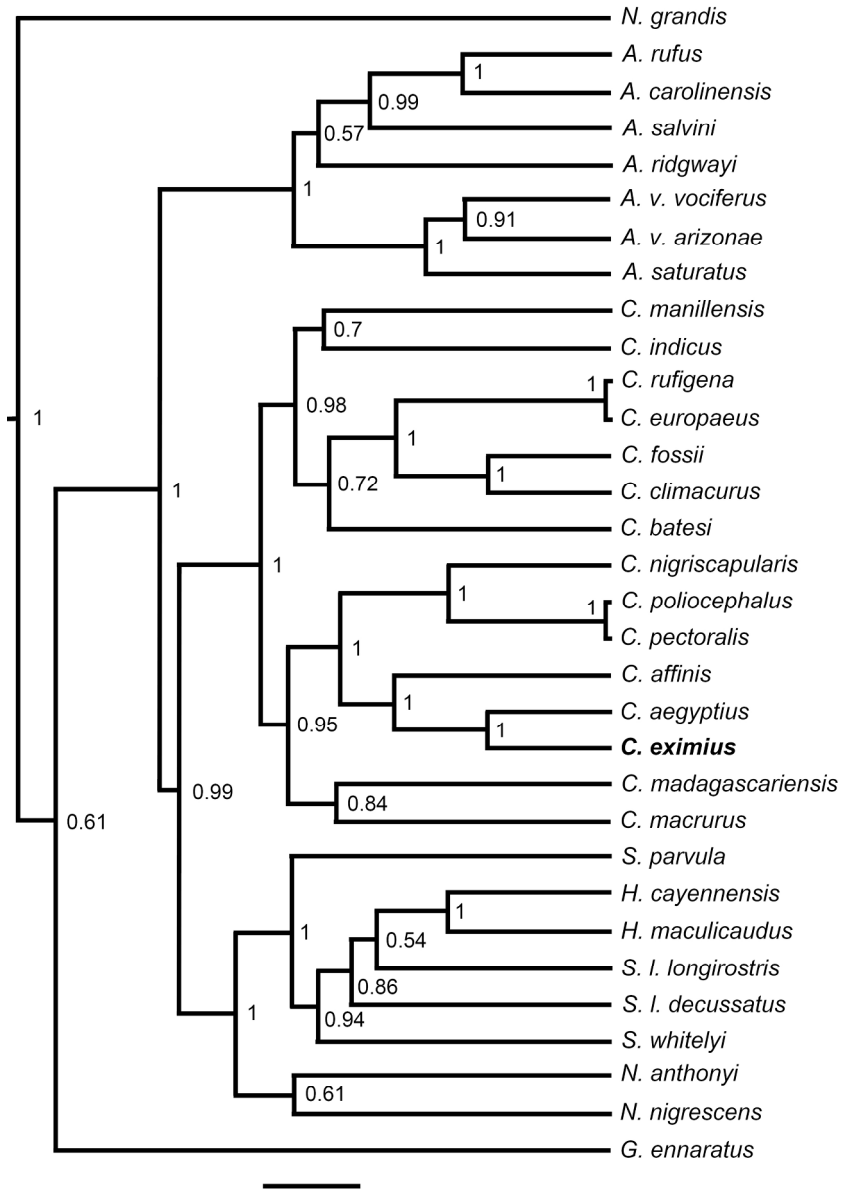


Figure 2. Bayesian phylogeny of nightjars (beast analysis) based on cytb (1008 bp) to identify the systematic position of Golden Nightjar. The tree was rooted with Great Potoo *Nyctibius grandis* as an outgroup. Support from posterior probabilities is indicated at nodes. See Table 1 for full scientific names and accession numbers.

200x281mm (300 x 300 DPI)

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2550F
P2/P8 2718R

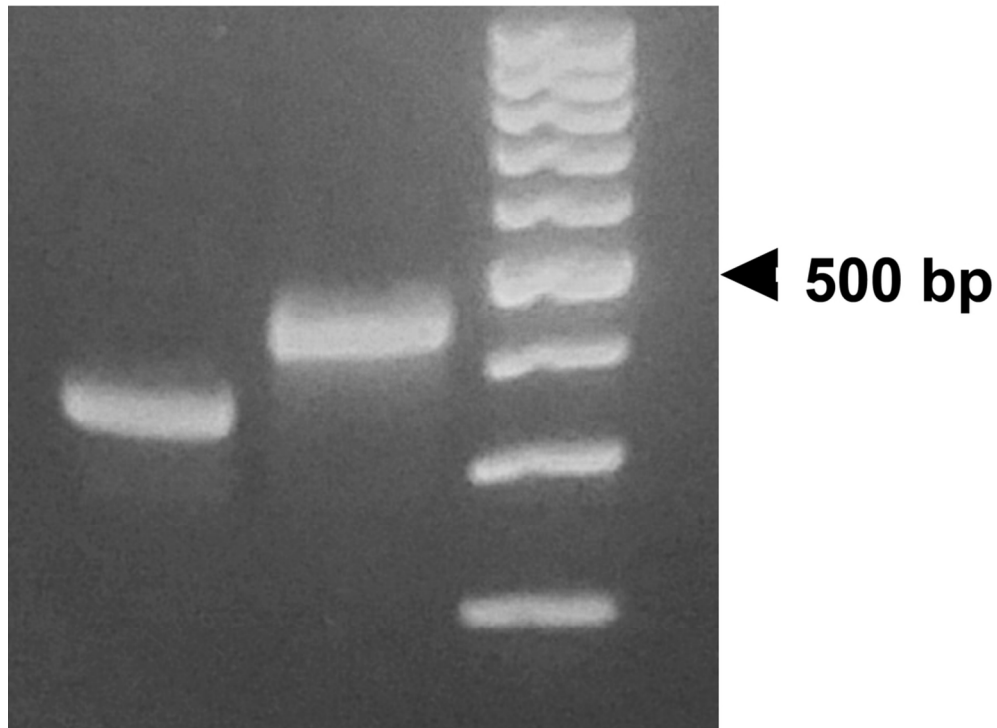


Figure 3. Genetic sexing of Golden Nightjar. Lane 1: P2/P8 PCR. Lane 2: 2550F/2718R PCR. Lane 3: 100 bp ladder. Arrow: 500 bp.

98x91mm (300 x 300 DPI)

1 **The taxonomic position and breeding range of Golden Nightjar *Caprimulgus eximius* (Aves:**
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3 **Caprimulgidae)**

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5 Yvonne Lawrie, Robert Swann¹, Peter Stronach², Yoav Perlman³ and J. Martin Collinson*

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7
8
9 University of Aberdeen,
10 School of Medicine, Medical Sciences and Nutrition,
11 Institute of Medical Sciences,
12 Foresterhill,
13 Aberdeen AB25 2ZD, UK.
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20 *Corresponding author: m.collinson@abdn.ac.uk
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28 **SUPPLEMENTARY MATERIAL**
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Figure S2

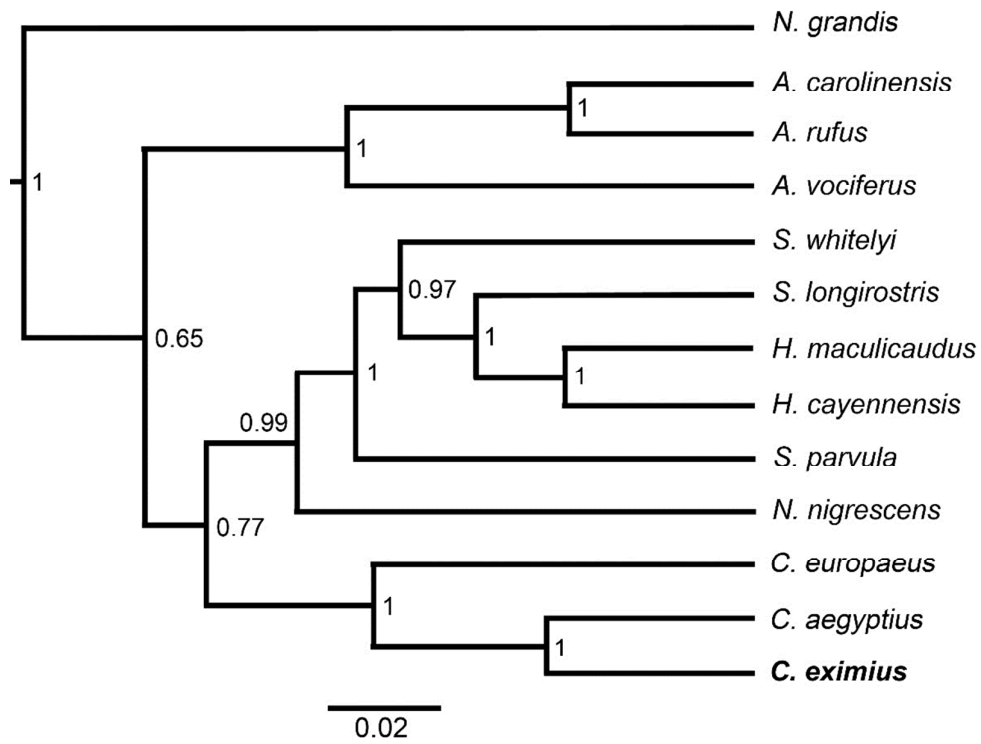


Figure Legend

Bayesian inference tree based on 1703 bp concatenation of cytb and COI for all relevant species for which both sequences were available. See main text for methodology.

Nubian Nightjar *Caprimulgus nubicus*

Partial cytb sequence will be completed and uploaded to GenBank as part of a future study of the systematics of this species.

TCTAGATCTGCCTAGCAACACAAATCCTAACCGGACTACTACTAGCTACACATTACACCGCAGACACAACCTG
GCCTTCTCATCTGTTGCCACACTTGCCGAAACGTACAATACGGCTGACTAATCCGTAATCTGCACGCAAATGG
AGCCTCACTCTTCTTCATTTGCAATTAACCTCCACATTGGACGAGGCCTATACTACGGATCCTACCTCTACAAAGA
AACCTGAAACACAGGAGTAATCCTCTTACTCACCTTAATAGCAACAGCCTTCGTAGGCTACGTCCTACCATGAG
GACAAATATCATTCTGAGGGGCTACAGTCATACCAACCTATTCTCAGCTATCCCATATATTGGCCAAACCTT
GTAGAATGAGCATGAGGTGGATTTTCCGTAGACAACCCACACTAACCCGATTTTTTGCCTACACTTCCTCCT
TCCCTTTATAATTGCCGGCTCACCTAATCCACCTAACATTCTCCATGAATCTGGCTCAAACAACCCCTCGG
AATTGTATCAAACCTGCGACAAAATTCCATTCCACCCCTATTTTTCCCTAAAAGACATCCTAGGCTTCGCACTAAT
ACTCACCCATTAATAACACTCGCCATATTCGCCCAAACCTGCTAGGGGACCCAGAAAACCTTACCCAGCAA
ATCCCCTAGTCACACCCCAATATCAAACCCGAGTGATACTTCTATTTGCATACGCCATCTTACGCTCAATCC
CGAACAAACTAGGAGGTGTCCTAGCCC