

# The rediscovery of *Strix butleri* (Hume, 1878) in Oman and Iran, with molecular resolution of the identity of *Strix omanensis* Robb, van den Berg and Constantine, 2013

Magnus S Robb<sup>1</sup>, George Sangster<sup>2,3\*</sup>, Mansour Aliabadian<sup>4,5</sup>, Arnoud B van den Berg<sup>6</sup>, Mark Constantine<sup>7</sup>, Martin Irestedt<sup>2</sup>, Ali Khani<sup>8</sup>, Seyed Babak Musavi<sup>9</sup>, João M G Nunes<sup>10</sup>, Maia Sarrouf Willson<sup>11</sup> and Alyn J Walsh<sup>12</sup>

<sup>1</sup>The Sound Approach, Rua Dr Pedro Almeida Lima 6, 2710-122 Sintra, Portugal.

<sup>2</sup>Department of Bioinformatics and Genetics, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden.

<sup>3</sup>Department of Zoology, Stockholm University, SE-10691 Stockholm, Sweden.

<sup>4</sup>Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Azadi Square, 9177948974, Mashhad, Iran.

<sup>5</sup>Research Department of Zoological Innovations, Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.

<sup>6</sup>The Sound Approach, Duinlustparkweg 98, 2082 EG Santpoort-Zuid, Netherlands.

<sup>7</sup>The Sound Approach, 12 Market Street, Poole, Dorset BH15 1NF, UK.

<sup>8</sup>Khorasan-e Razavi Provincial Office of the Department of the Environment, Mashhad, Iran.

<sup>9</sup>Postal code 7661675555, Khucheh Gochin, Golfam Street, Bolvar Shahid Bahonar, Bam, Iran.

<sup>10</sup>Parque Ecológico do Funchal, Estrada Regional 103, nr 259, Ribeira das Cales 9050, Monte I Funchal, Portugal.

<sup>11</sup>Environment Society of Oman, P.O. Box 3955, P.C. 112, Ruwi, Sultanate of Oman.

<sup>12</sup>Wildfowl Reserve, North Slobland, Wexford, Ireland.

\*Corresponding author: [g.sangster@planet.nl](mailto:g.sangster@planet.nl)

1 **ABSTRACT**

2

3 **Background:** Most species of owls (Strigidae) represent cryptic species and their taxonomic  
4 study is in flux. In recent years, two new species of owls of the genus *Strix* have been  
5 described from the Arabian peninsula by different research teams. It has been suggested that  
6 one of these species, *S. omanensis*, is not a valid species but taxonomic comparisons have  
7 been hampered by the lack of specimens of *S. omanensis*, and the poor state of the holotype  
8 of *S. butleri*.

9 **Methods:** Here we use new DNA sequence data to clarify the taxonomy and nomenclature  
10 of the *S. butleri* complex. We also report the capture of a single *S. butleri* in Mashhad, Iran.

11 **Results:** A cytochrome b sequence of *S. omanensis* was found to be identical to that of the  
12 holotype of *S. butleri*, indicating that the name *S. omanensis* is best regarded as a junior  
13 synonym of *S. butleri*. The identity of the *S. butleri* captured in Mashhad, Iran, was  
14 confirmed using DNA sequence data. This represents a major (1,400 km) range extension of  
15 this species.

16 **Conclusions:** The population discovered in Oman in 2013 and originally named '*S.*  
17 *omanensis*' actually represents the rediscovery of *S. butleri*, which was known from a single  
18 specimen and had not been recorded since 1878. The range of *S. butleri* extends into  
19 northeast Iran. Our study augments the body of evidence for the recognition of *S. butleri* and  
20 *S. hadorami* as separate species and highlights the importance of using multiple evidence to  
21 study cryptic owl species.

22

23 **Keywords:** molecular identification, nomenclature, phylogenetics, Strigidae, *Strix*,  
24 taxonomy

25 **INTRODUCTION**

26

27 Accurate taxonomic designations are important for most, if not all branches in biology. Even  
28 in birds, modern scientific studies continue to generate hypotheses of new species, often  
29 based on new data and multiple lines of evidence (Sangster 2009, Sangster & Luksenburg  
30 2015). Until the 1960s, studies of the taxonomic status of bird species relied almost  
31 exclusively on comparisons of morphological characters. By the 1960s, technological  
32 advances made it possible to obtain sound recordings in the field for taxonomic study  
33 (Lanyon 1960) and produce audiospectograms (sonagrams) which allowed objective  
34 comparison and measurement of acoustic characters. These techniques were first applied to  
35 the vocalizations of owls by van der Weyden (1973a, 1973b, 1974, 1975) and Marshall  
36 (1978). Subsequent studies of vocalizations have resulted in the discovery of many additional  
37 species of owls, a process which continues until the present (e.g. Sangster et al. 2013).

38 *Strix butleri* was described by Hume (1878) on the basis of a single specimen which  
39 was believed to have come from “Omara, on the Mekran Coast” (=Ormara), in what is now  
40 southern Pakistan (Fig. 1). Subsequently, small numbers of specimens from Egypt, Israel,  
41 Jordan, and Saudi Arabia have been assigned to this species (Goodman & Sabry 1984). In  
42 addition, the species is known from Sudan, Yemen and Oman (Mikkola 2012, BirdLife  
43 International & NatureServe 2014). However, there have been no subsequent specimens or  
44 sight records from north of the Persian Gulf, leading some to suggest that the type of *S.*  
45 *butleri* may have originated from the Arabian peninsula and been brought to Ormara over sea  
46 from Arabia (Roselaar & Aliabadian 2009, Kirwan et al. 2015).

47 In March 2013, Magnus Robb heard vocalisations of an unknown *Strix* owl in the Al  
48 Hajar range in northern Oman. In the course of four trips, sound recordings and photographs  
49 were obtained demonstrating that the population discovered in Oman represented a different  
50 species from ‘Hume’s Owl *S. butleri*’ as it was then understood (Robb et al. 2013). Robb et

51 al. (2013) documented the existence of two species in the Arabian peninsula, based on  
52 multiple differences in song, calls, and plumage, and described the Omani population as a  
53 new species, *Strix omanensis*. When examining the holotype of *S. butleri* in the Natural  
54 History Museum, Tring (BMNH 1886.2.1.994), they did not detect any major differences  
55 from the two other specimens of '*S. butleri*' in that collection. Nevertheless, they considered  
56 the possibility that the type of *S. butleri* may be same species as *S. omanensis*, and noted that  
57 "The eastern location [of the type specimen of *S. butleri*] raises the question whether it in fact  
58 could have concerned an Omani Owl [*S. omanensis*]. If it did, the scientific name now used  
59 for Hume's would become the scientific name of Omani while another scientific name would  
60 have to be chosen for Hume's" (Robb et al. 2013).

61 Kirwan et al. (2015) re-examined the type specimen of *S. butleri* and found that it  
62 differed from other specimens attributed to that species in multiple plumage and  
63 morphometric characters, indicating that these specimens belong to different species. This  
64 was corroborated by analysis of DNA sequences of 218 bp of the mitochondrial cytochrome  
65 b gene which showed a sequence divergence of about 10% between the holotype of *S. butleri*  
66 and other specimens of '*S. butleri*'. They described a new species, *S. hadorami*, to which  
67 they assigned all known specimens of '*S. butleri*' except the type of the latter. They did not  
68 examine DNA from the Omani population described as '*S. omanensis*'. However, they noted  
69 that the holotype *S. butleri* showed most of proposed diagnostic character states of *S.*  
70 *omanensis*. Kirwan et al. (2015) suspected that *S. omanensis*' may represent the same species  
71 as *S. butleri* and that the holotype of the latter may have originated from Oman.

72 Critical analysis of type specimens is crucial for the correct application of taxonomic  
73 names. Comparisons of the type of *S. butleri* with *S. omanensis* are hampered by the  
74 "miserable" state of the former (Meinertzhagen 1930) and the lack of a specimen of the  
75 latter. In such cases, comparison of DNA sequences may help to ascertain the taxonomic  
76 identity and validity of disputed species-level taxa.

77           In this study, we use DNA sequences of '*S. omanensis*' to clarify the taxonomic  
78 identity of *S. omanensis* and the nomenclature of the *S. butleri* complex. In addition, we use  
79 DNA identification techniques to assess the identity of a captured bird (tentatively identified  
80 as *S. butleri/S. omanensis*) in Mashhad, Iran, which represents the first record of the species  
81 north of the Persian Gulf since 1878.

82

83

## 84 **METHODS**

85

### 86 **Field work: Oman**

87 On 2 March 2015, Alyn Walsh and Magnus Robb caught an Omani Owl at the type locality,  
88 Al Jabal Al Akhdar, Al Hajar mountains, Al Batinah, Oman, using a 20 x 4 m mist net. In  
89 order to attract an owl to the net, they used playback of several CD tracks from Robb & The  
90 Sound Approach (2015) and a decoy owl, painted by Killian Mullarney to look like an  
91 Omani and 'perched' on a prominent acacia halfway along the net. After catching the owl,  
92 they took measurements, feathers, blood samples, photographs and a sound recording. The  
93 same measurements were the same as described in Kirwan et al. (2015), taken in the same  
94 way. For molecular analysis, they took three feathers from the breast, four tiny ones from the  
95 bend of the wing, and two blood samples. In addition they took photographs of the owl in the  
96 hand and after release, when it was perched on a thick branch.

97           The owl was identified as *S. omanensis* (sensu Robb et al. 2013) by the presence of  
98 several acoustic and morphological character states which were previously identified as  
99 diagnostic for this species (Robb et al. 2013). (i) Shortly before capture, the bird gave  
100 diagnostic four-note compound hooting, with the last two notes given in quick succession. In  
101 the hand, it showed (ii) orange-yellow eyes, (iii) bicoloured facial disc with dark grey-brown  
102 above and beside the eye and pale grey from just above the eye downwards, (iii) very dark,

103 greyish brown upperparts, (iv) ginger-buff to white underparts with long streaks (longitudinal  
104 black lines) but only weak transverse bars, and (v) a broad dark trailing edge to the  
105 underwing.

106

### 107 **Field work: Iran**

108 In the early morning of 23 January 2015, Ali Khani received news of an owl that had become  
109 entangled on the balcony of a house during the night. When he and Babak Musavi went to  
110 investigate, they concluded that since it had many feathers of Laughing Dove *Streptopelia*  
111 *senegalensis* around its legs and a blood-covered bill, it may have got in difficulties while  
112 hunting. The house was situated in a cultivated area near Vakilabad garden, just west of  
113 Mashhad, the second largest city of Iran. South and west of this garden there are barren,  
114 rocky slopes possibly offering suitable habitat for Omani Owls. These form part of the  
115 northern slopes of the Binalud range, which reaches its highest point (3211 m) at Mount  
116 Binalud, some 55 km to the west. Mashhad is c 80 km from the border with Turkmenistan,  
117 and over 1300 km from Ormara in Pakistan. They caught the owl, which appeared to be alert  
118 and healthy, and collected four feathers for molecular analysis. On releasing it, they took a  
119 series of photographs perched and in flight. Having had very little time to prepare for the  
120 encounter, they did not attempt to take blood samples or measurements.

121

### 122 **Laboratory procedures and phylogenetic analysis**

123 A blood sample and two feathers from Oman and a single feather from Iran were used for  
124 molecular identification. Genomic DNA was extracted using the Qiagen DNeasy Tissue Kit  
125 (Qiagen, Valencia, CA) following the protocol of the manufacturer. The lysis procedure was  
126 prolonged to 18 hours, and 20 µl of 1 M dithiothreitol (DTT) solution was added during to  
127 the initial lysis step.

128           The mitochondrial cytochrome b (cyt b) was amplified because this is the only  
129 marker for which sequences of the holotypes of *S. butleri* (BMNH 1886.2.1.994) and *S.*  
130 *hadorami* (BMNH 1965.M.5235) are available (Kirwan et al. 2015). Amplification was  
131 performed in two overlapping fragments. Primer sequences were newly designed, and are as  
132 follows: CytbStrixF1 (5'-GAATCTGCCTAATAGCCCAAATC-3'), CytbStrixR2 (5'-  
133 AAGCCACCTCAGGCTCATTCTAC-3'), CytbStrixR3 (5'-  
134 GGAGAGTGGGCGAAAGGTTATT-3'). The primer combination F1/R2 amplifies 345 bp  
135 and F1/R3 amplifies 806 bp. Both fragments fully cover the sequences of the holotypes of *S.*  
136 *butleri* and *S. hadorami*.

137           PCR products were cycle-sequenced in both directions using the Big Dye Terminator  
138 v1.1. Sequences were read on an ABI 3100 capillary sequencer (Applied Biosystems, Foster  
139 City, CA). Sequence fragments were aligned and visually edited using Lasergene Editseq  
140 (DNA Star, Madison, WI). Both sequences are deposited at GenBank (accession numbers  
141 KT428757–KT428758). DNA sequences of six other species of *Strix* were obtained from  
142 GenBank. *Tyto alba* was used as an outgroup. Genbank accession numbers and references to  
143 the original sources are given in Table 1.

144           Phylogenetic relationships were estimated with maximum likelihood (ML) analysis  
145 using MEGA5 (Tamura *et al.* 2011). Clade support for the ML analysis was assessed by  
146 1000 bootstrap replicates. The best-fit model was estimated with MEGA5 using the Akaike  
147 Information Criterion. The selected model was HKY + G. To further evaluate statistical  
148 support for the topology, we ran a Bayesian analysis using MrBAYES version 3.2.2  
149 (Ronquist et al. 2012). Default priors in MrBAYES were used. We ran four Metropolis-  
150 coupled MCMC chains for 1 million generations and sampled the topology every 100  
151 generations. Convergence between the two MrBayes runs was assessed by comparing the  
152 posterior probability estimates for both analyses using the program AWTY (Nylander *et al.*  
153 2008). The first 25% of the generations were discarded ('burn-in') and the posterior

154 probability was estimated for the remaining sampled generations. Uncorrected p pairwise  
155 sequence divergences were calculated in MEGA5 with complete deletion of nucleotide  
156 positions with missing data.

157 Nuclear copies of mitochondrial sequences (numts) may represent a problem in  
158 mtDNA studies (e.g. Den Tex *et al.* 2010). We used several lines of evidence to assess the  
159 authenticity of our sequences. First, electropherograms were inspected for double signal (two  
160 clear peaks at one or more nucleotides), which indicates a mixture of mitochondrial and  
161 nuclear sequences (Den Tex *et al.* 2010). Second, we checked the translated consensus  
162 sequence for the presence of frameshift mutations or stop codons, which are strong  
163 indications that a sequence does not represent that of a protein-coding gene. Finally, we  
164 checked whether nucleotide substitutions were primarily found at the third codon, which is  
165 expected when a sequence is of a protein-coding gene. In old numts, the distribution of  
166 substitutions is expected to be equal across all three codon positions (Zink & Barrowclough  
167 2008).

168

169

## 170 **RESULTS**

171

### 172 **Morphology: Oman (Fig. 2a and Fig. 2b)**

173 Morphometric data of the captured bird are given in Table 2.

174 *Structure.* Medium-sized owl with rounded head lacking ear-tufts, a well defined  
175 facial disc and typically large eyes. Tarsi long. Tail short. Wing-tips level with, or projecting  
176 marginally beyond end of tail, depending on posture.

177 *Head.* Facial disc pale grey, gradually becoming darker grey-brown above eye. Upper  
178 half of disc narrowly bordered dark brown; lower half with creamy or light buff 'ruff', finely  
179 stippled with dark spots. Prominent dark median crown-stripe beginning just above eye level,

180 widening slightly toward top of head and contrasting with two narrow clusters of whitish-  
181 tipped feathers either side, running from forehead onto crown. Pale grey forward-pointing  
182 facial feathering just above eye and bristly ‘moustache’ hardly contrasting with lower half of  
183 facial disc. Crown densely mottled dark on a lighter ground, sides of head with more ginger  
184 ground colour, gradually shading to off white toward lower nape. All feathers of sides and  
185 back of head pale-based and dark-tipped resulting in irregular pattern of light spots and dark  
186 blotches or bars following the contours of feather tracts. Largest whitish spots concentrated  
187 in nuchal band at back of head. Chin whitish, throat light buff, finely stippled dark.

188         *Upperparts.* Mantle, scapulars, back, rump and uppertail-coverts dark grey-brown  
189 with diffuse buff and whitish spots of varying size and intensity.

190         *Underparts.* Breast washed light ginger-buff, strongest (verging on rust-coloured) at  
191 sides, with loose arrangement of narrow dark shaft-streaks and few faint transverse bars.  
192 Belly and flank whitish with longer thin shaft-streaks and sparsely distributed, faintly marked  
193 buff-brown bars. Abdomen, undertail-coverts and thigh off-white, unmarked.

194         *Upperwing.* Primaries barred dark brown and greyish-buff, five light bars (including  
195 tip) interspaced with four broader dark bars. Secondaries similar but fewer bars (three light,  
196 three dark) and pattern with slightly less contrast than on primaries, especially toward base.  
197 Tertiaries brown, innermost with three narrow but distinct buff bars on the inner web, the  
198 middle and subterminal bars continuing onto the outer web. Alula dark grey-brown, longest  
199 feather apparently fresher and with three buff notches on outer web, shorter feathers plain.  
200 Greater and median secondary coverts brown with large whitish subterminal spot on outer  
201 webs of outermost feathers, smaller and less distinct pale markings on coverts closer to body.  
202 Lesser and marginal coverts more uniform dark brown. Greater primary coverts almost  
203 uniform dark brown with very subdued barred pattern.

204         *Underwing.* Outermost primary plain brown-grey with faint longitudinal streak on  
205 middle of inner web, rest of primaries boldly barred brown and white/buff-grey, contrast

206 between light and dark bars more pronounced at base where, toward inner primaries, white  
207 bars broadened and proximal dark bar much reduced in strength. Secondaries similar to inner  
208 primaries, extensively white at base merging imperceptibly with clean white greater coverts.  
209 Greater primary coverts white with bold dark tips to outer six feathers forming a prominent  
210 dark carpal-crescent. Remaining underwing coverts greyish with fine dark shaft-streaks,  
211 marginal coverts (leading edge of wing) white.

212 *Tail.* Upperside boldly barred dark brown and greyish-buff, three broad dark bars,  
213 and three or four narrow light bars, including tip. Light bars on central pair of rectrices  
214 reduced, especially on inner webs, so these feathers darker and less strongly patterned than  
215 the rest. Underside similarly marked to uppertail but pattern even bolder due to light bars  
216 being almost whitish. Three dark bars and up to three light bars visible beyond undertail  
217 coverts, width of light and dark bars more equal than on upperside.

218 *Bare parts.* Pupils black, iris orange-yellow with black surround; eyelid dark greyish.  
219 Bill pale green-grey. Tibia, tarsus and toes feathered whitish, soles light yellowish-buff,  
220 claws light horn-grey.

221

## 222 **Morphology: Iran (Fig. 2c and Fig. 2d)**

223 *Structure.* Medium-sized owl with rounded head lacking ear-tufts, a well defined facial disc  
224 and typically large eyes. Tarsi long. Tail short. Wing-tips level with, or projecting marginally  
225 beyond end of tail, depending on posture. Possibly not as long-legged as Omani individual;  
226 this may simply be due to the bird having been photographed in a more relaxed stance, with  
227 body plumage fluffed out concealing the true length of the tarsus.

228 *Plumage, general.* Overall impression is of bird that is lighter in colour, especially on  
229 the upperparts and folded upperwing, than individual from Oman. However, since all  
230 existing photos of ‘*omanensis*’ have been taken either at night, using flash, or of birds sitting

231 within roost-holes by day, comparisons with photos of Iranian owl (in low evening light,  
232 without the use of flash) need to be made with caution.

233 *Head.* Very similar to captured Omani individual. Buff colour on sides of head  
234 bordering upper part of facial disc a little paler and more washed-out but this is of doubtful  
235 significance. Facial disc grey, gradually becoming darker grey-brown above eye. Upper half  
236 of disc narrowly bordered dark brown; lower half with creamy or light buff 'ruff', finely  
237 stippled with dark spots. Prominent dark median crown-stripe beginning just above eye level,  
238 widening slightly toward top of head and contrasting with two narrow clusters of whitish-  
239 tipped feathers either side, running from forehead onto crown. Pale grey forward-pointing  
240 facial feathering just above eye and bristly 'moustache' hardly contrasting with lower half of  
241 facial disc. Crown densely mottled dark on a lighter ground, sides of head with paler buff  
242 ground colour, gradually shading to off white toward lower nape. Chin whitish, throat light  
243 buff, finely stippled dark.

244 *Upperparts.* Mantle, back, rump and upper-tail-coverts not visible in photographs;  
245 scapulars with buff and whitish spots but apparently lighter grey-brown ground colour than  
246 in captured '*omanensis*'. Note, however, that in one photo (Fig. 2d) where bird not  
247 illuminated by sun, brown of the upperparts and head appears considerably darker in tone.

248 *Underparts.* Breast washed light apricot-buff, strongest at sides and extending further  
249 down towards legs than in captured '*omanensis*', with loose arrangement of narrow dark  
250 shaft-streaks and few faint transverse bars. Belly, flank and undertail coverts whitish with  
251 longer thin shaft-streaks and sparsely distributed, faintly marked buff-brown bars. Abdomen  
252 and thigh off-white, unmarked.

253 *Upperwing.* Mostly based on photos of folded wing, though unsharp flight photo also  
254 informative. Remiges barred dark brown and pale buff, with pale buff tip. Tertials not clearly  
255 visible in photos. Alula dark grey-brown, all feathers notched with buff on outer web.  
256 Greater and median secondary coverts fairly pale brown with large whitish subterminal spot

257 on outer webs of outermost feathers, smaller and less distinct pale markings on coverts closer  
258 to body. Lesser and marginal coverts more uniform brown. Primary coverts distinctly barred,  
259 much more so than in captured '*omanensis*'.

260 *Underwing*. Not visible in photos.

261 *Tail*. Only partly visible in sharp photos, though upperside visible in unsharp flight  
262 photos. Upperside boldly barred dark brown and pale buff, three broad dark bars, and four  
263 narrow light bars, including tip. Underside similarly marked to uppertail but width of light  
264 and dark bars more equal. Three dark bars and up to three light bars visible beyond undertail  
265 coverts.

266 *Bare parts*. Pupils black, iris orange-yellow with black surround; eyelid dark greyish.  
267 Bill pale green-grey. Tibia, tarsus and toes feathered whitish, soles light yellowish-buff,  
268 claws apparently a bit blacker than in captured '*omanensis*', but probably due at least in part  
269 to different light conditions.

270

## 271 **Molecular identification**

272 We obtained 790 base pairs (bp) of cytochrome b of *S. omanensis* and 767 bp from the owl  
273 caught at Mashhad, Iran. We found no evidence of numts. Electropherograms showed no  
274 double signal; the alignment showed no stop codons, insertions or deletions; and most  
275 (65/78, 83%) nucleotide substitutions relative to the longest *S. hadorami* sequence available  
276 on GenBank (EU348994) were found in the third codon and resulted in only three amino acid  
277 substitutions.

278 The sequence of *S. omanensis* was identical to the short (218 bp) sequence available  
279 from the holotype of *S. butleri* (Genbank acc. no. KM459027). The sequences of *S.*  
280 *omanensis* and the Iranian owl were almost identical, differing in only two nucleotides  
281 (0.26%), both at third positions. Across 790 shared bp, the sequence of *S. omanensis* differed

282 from that of *S. hadorami* (EU348994) by 78 substitutions, corresponding to an uncorrected  
283 sequence divergence of 9.9%.

284 Phylogenies based on ML and BI produced identical phylogenies in which both *S.*  
285 *omanensis* and the owl caught at Mashhad, Iran clustered with the holotype of *S. butleri* (Fig.  
286 3). This was strongly supported in both ML (98%) and Bayesian analyses (1.0 PP). In these  
287 analyses, *S. hadorami* and *S. butleri* formed reciprocally monophyletic groups. Relationships  
288 with *S. woodfordii* were unresolved, most likely due to the small number of nucleotide sites  
289 analysed.

290

291

## 292 **DISCUSSION**

293

### 294 **Taxonomy and nomenclature**

295 Mitochondrial DNA (mtDNA) has long been a popular marker in taxonomic and molecular  
296 identification ('barcoding') studies of birds. This is due to its presence in high concentrations  
297 in tissue material, its smaller effective population size which results in faster fixation rates  
298 compared to nuclear DNA and, as a consequence, its ability to distinguish a large proportion  
299 of species (Zink & Barrowclough 2008, Ward 2009). Our study found that the cytochrome b  
300 sequence of a member of the population described as *S. omanensis* (Robb et al. 2013) and  
301 sampled at its type locality is identical to that of the holotype of *S. butleri*. This is a strong  
302 indication that *S. omanensis* and *S. butleri* belong to the same evolutionary lineage. However,  
303 there are some examples of valid species of birds that cannot be reliably distinguished using  
304 mtDNA markers. In most of these there is strong evidence from other data that these  
305 represent species (e.g. Crochet et al. 2002, Joseph et al. 2006, Irwin et al. 2009, Joseph et al.  
306 2009, Campagna et al. 2010, Päckert et al. 2012). Thus, a lack of fixed mtDNA differences  
307 cannot by itself be considered falsification of the existence of species taxa (de Queiroz 2007).

308 Despite this caveat, we believe that current evidence does not justify maintaining *S.*  
309 *omanensis* as a separate species because there is no positive evidence that it represents a  
310 separate lineage from *S. butleri*. Therefore, the name *Strix omanensis* Robb, van den Berg  
311 and Constantine, 2013 is best treated as a junior synonym of *Asio butleri* Hume, 1878 (now  
312 *Strix butleri*).

313 By providing evidence that the population in Oman previously known as ‘*S.*  
314 *omanensis*’ is *S. butleri*, our study augments the body of evidence supporting the treatment of  
315 *S. butleri* and *S. hadorami* as separate species. Whereas the evidence available to Kirwan et  
316 al. (2015) was limited to a specimen of *S. butleri* and two lines of evidence (DNA and  
317 morphology) differentiating it from *S. hadorami*, the hypothesis that these are species is now  
318 also supported by bioacoustic evidence, plumage data from photographs of multiple  
319 individuals of *S. butleri*, and DNA sequences of three individuals.

320 Demographic and genetic exchange between Omani and Iranian populations of *S.*  
321 *butleri* is probably limited by the Gulf of Oman and the Strait of Hormuz. Future studies  
322 should focus on making objective comparisons of the plumage and vocalizations of Omani  
323 and Iranian populations of *S. butleri*. This is not currently possible due to the absence of  
324 specimens from both countries, and of recordings from Iran, where there have been no  
325 further observations. More detailed molecular comparisons are warranted to investigate  
326 possible population structure and genetic diversity within *S. butleri*, which could inform both  
327 taxonomic and conservation genetic studies.

328 To avoid confusion, we propose to exclude ‘Hume’s Owl’ (and ‘Hume’s Tawny Owl’)  
329 as the English name for either species because this is an ambiguous name. Until the end of  
330 2014, it was used universally for what is now *S. hadorami*. At the same time it has historical  
331 links to *S. butleri*, the species actually described by Hume. Retaining it for either species may  
332 result in misunderstanding. Kirwan et al. (2015) proposed the name ‘Desert Tawny Owl’ for  
333 *S. hadorami*, but this may be shortened to ‘Desert Owl’ to avoid the implication of a close

334 relationship with Tawny Owl *S. aluco* or having to add a modifier such as ‘Forest’ to the  
335 latter name. We recommend the name ‘Omani Owl’ for *S. butleri* sensu stricto, because the  
336 only known population of this species is in Oman, with only single individuals ever having  
337 been located outside Oman.

338

### 339 **Rediscovery and distribution of *S. butleri***

340 Our study documents the extension of the range of *S. butleri* by 1,300 km to the Mashhad  
341 region in northeastern Iran, and its presence in the Al Hajar range of northern Oman (Fig. 1).  
342 Its range in Arabia may extend west to Wadi Wurayah National Park in the United Arab  
343 Emirates where it was identified in March 2015 by vocalizations (Jacky Judas pers comm)  
344 although further substantiation is desirable. Clearly, *S. butleri* is a highly elusive species  
345 which is difficult to study in the field. Further field work in Oman, the United Arab Emirates,  
346 Iran and Pakistan, perhaps aided by the use of song playback, is necessary to elucidate the  
347 range of *S. butleri*.

348

349

### 350 **ACKNOWLEDGEMENTS**

351

352 This study was financed and supported by The Sound Approach. It forms part of a broader  
353 Omani Owl conservation project conducted as a collaboration between The Sound Approach,  
354 BirdLife International and the Environment Society of Oman. We would like to thank the  
355 Omani Ministry of Social Development for approving this collaboration, and the Ministry of  
356 Environment and Climatic Affairs for granting us permission for fieldwork and to take  
357 genetic samples from a wild Omani Owl (permit nr 5/2015). The Office for Conservation of  
358 the Environment also advised us during the fieldwork phase of the project. We would like to  
359 thank Parque Ecológico do Funchal for making it possible for João Nunes to join us, and

360 David Tierney for his forbearance with Alyn Walsh's long absences. Andrew Spalton  
361 provided welcome advice and good company in the field. In the days immediately following  
362 the discovery in Iran, Richard Porter arranged for The Sound Approach and the Iranian team  
363 to work together, for which we are extremely grateful. We thank Niloofar Alaye for her  
364 assistance with the molecular work. Killian Mullarney gave valuable feedback on the  
365 manuscript.

366

367

## 368 **REFERENCES**

369

370 BirdLife International, NatureServe (2014) Bird species distribution maps of the world.

371 BirdLife International, Cambridge, UK and NatureServe, Arlington, USA.

372 Braun MJ, Huddleston CJ (2009) A molecular phylogenetic survey of caprimulgiform  
373 nightbirds illustrates the utility of non-coding sequences. *Mol Phylogen Evol* 53:948–  
374 960.

375 Campagna L, Lijtmaer DA, Kerr KCR, Barreira AS, Hebert PDN, Loughheed SC, Tubaro PL  
376 (2010) DNA barcodes provide new evidence of a recent radiation in the genus  
377 *Sporophila* (Aves: Passeriformes). *Mol Ecol Res* 10:449–458.

378 Crochet P-A, Lebreton J-D, Bonhomme F (2002) Systematics of large white-headed gulls:  
379 Patterns of mitochondrial DNA variation in western European taxa. *Auk* 119:603–620.

380 Den Tex R, Maldonado JE, Thorington R, Leonard JA (2010) Nuclear copies of  
381 mitochondrial genes: another problem for ancient DNA. *Genetica* 138:979–984.

382 Desmond MJ, Parsons TJ, Powers PO, Savidge JA (2001) An initial examination of  
383 mitochondrial DNA structure in burrowing owl populations. *J Raptor Res* 35:274–281.

- 384 Fjeldså J, Mayr G, Jønsson K, Irestedt M (2013) On the true identity of Bluntschli's Vanga  
385 *Hypositta perdita* Peters, 1996, a presumed extinct species of Vangidae. Bull Br  
386 Ornithol Club 133:72–75.
- 387 Goodman SM, Sabry H (1984) A specimen record of Hume's Tawny Owl *Strix butleri* from  
388 Egypt. Bull Br Ornithol Club 104:79–84.
- 389 Hausknecht R, Jacobs S, Müller J, Zink R, Frey H, Solheim R, Vrezec A, Kristin A, Mihok J,  
390 Kergalve I, Saurola P, Kuehn R (2014) Phylogeographic analysis and genetic cluster  
391 recognition for the conservation of Ural Owls (*Strix uralensis*) in Europe. J Ornithol  
392 155:121–134.
- 393 Hume AO (1878) *Asio butleri*, sp. nov.? Stray Feathers 7:316–318.
- 394 Irwin DE, Rubtsov AS, Panov EN (2009) Mitochondrial introgression and replacement  
395 between yellowhammers (*Emberiza citrinella*) and pine buntings (*Emberiza*  
396 *leucocephalos*) (Aves: Passeriformes). Biol J Linn Soc 98:422–438.
- 397 Joseph L, Adcock GJ, Linde C, Omland KE, Heinsohn R, Chesser RT, Roshier D (2009) A  
398 tangled tale of two teal: population history of the grey *Anas gracilis* and chestnut teal  
399 *A. castanea* of Australia. J Avian Biol 40:430–439.
- 400 Joseph L, Wilke T, Ten Have J, Chesser RT (2006) Implications of mitochondrial DNA  
401 polyphyly in two ecologically undifferentiated but morphologically distinct migratory  
402 birds, the masked and white-browed woodswallows *Artamus* spp. of inland Australia. J  
403 Avian Biol 37:625–636.
- 404 Kirwan GM, Schweizer M, Copete JL (2015) Multiple lines of evidence confirm that Hume's  
405 Owl *Strix butleri* (A. O. Hume, 1878) is two species, with description of an unnamed  
406 species (Aves: Non-Passeriformes: Strigidae). Zootaxa 3904:28–50.
- 407 Lanyon WE (1960) The Middle American populations of the Crested Flycatcher *Myiarchus*  
408 *tyrannulus*. Condor 62:341–350.

- 409 Lim GS, Balke M, Meier R (2012) Determining species boundaries in a world full of rarity:  
410 singletons, species delimitation methods. *Syst Biol* 61:165–169.
- 411 Marshall JT (1978) Systematics of smaller Asian night birds based on voice. (LP and  
412 booklet.) *Ornithol Monogr* 25:1–58.
- 413 Meinertzhagen R (1930) *Nicoll's Birds of Egypt. Vol. 1.* Hugh Rees, London
- 414 Mikkola H (2012) *Owls of the World: A Photographic Guide.* Christopher Helm, London,
- 415 Nguembock B, Fjeldså J, Couloux A, Pasquet E (2008) Phylogeny of *Laniarius*: Molecular  
416 data reveal *L. liberatus* synonymous with *L. erlangeri* and “plumage coloration” as  
417 unreliable morphological characters for defining species and species groups. *Mol*  
418 *Phylogen Evol* 48:396–407.
- 419 Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there  
420 yet?): a system for graphical exploration of MCMC convergence in Bayesian  
421 phylogenetics. *Bioinformatics* 2008:581–583.
- 422 Omote K, Nishida C, Dick MH, Masuda R (2013) Limited phylogenetic distribution of a  
423 long tandem-repeat cluster in the mitochondrial control region in *Bubo* (Aves,  
424 Strigidae) and cluster variation in Blakiston's fish owl (*Bubo blakistoni*). *Mol Phylogen*  
425 *Evol* 66:889–897.
- 426 Päckert M, Martens J, Wink M, Feigl A, Tietze DT (2012) Molecular phylogeny of Old  
427 World swifts (Aves: Apodiformes, Apodidae, *Apus* and *Tachymarptis*) based on  
428 mitochondrial and nuclear markers. *Mol Phylogen Evol* 63:606–616.
- 429 Robb MS, van den Berg AB, Constantine M (2013) A new species of *Strix* owl from Oman.  
430 *Dutch Birding* 35:275–310.
- 431 Robb M, *The Sound Approach* (2015). Undiscovered owls. The Sound Approach, Poole.
- 432 Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L,  
433 Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic  
434 inference and model choice across a large model space. *Syst Biol* 61:539–542.

- 435 Roselaar CS, Aliabadian M (2009) Review of rare birds in Iran, 1860s–1960s. *Podoces* 4:1–  
436 27.
- 437 Sangster G (2009) Increasing numbers of bird species result from taxonomic progress, not  
438 taxonomic inflation. *Proc R Soc B* 276:3185–3191.
- 439 Sangster G, Luksenburg JA (2015) Declining rates of species described per taxonomist:  
440 slowdown of progress or a side-effect of improved quality in taxonomy? *Syst Biol*  
441 64:144–151.
- 442 Sangster G., King BF, Trainor CR, Verbelen P (2013) A new species of scops owl (*Otus*)  
443 from Lombok, Indonesia. *PLoS ONE* 8(2):e53712. doi:10.1371/journal.pone.0053712
- 444 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular  
445 evolutionary genetics analysis using maximum likelihood, evolutionary distance, and  
446 maximum parsimony methods. *Mol Biol Evol* 28:2731–2739.
- 447 van der Weyden WJ (1973a) Vocal affinities of the African and European Scops Owls *Otus*  
448 *scops* (Strigidae). *Bull l'Inst Fr Afr Noire* 35 sér A, No. 3:716–722.
- 449 van der Weyden WJ (1973b) Geographical variation in the territorial song of the White-faced  
450 Scops Owl *Otus leucotis*. *Ibis* 115:129–131.
- 451 van der Weyden WJ (1974) Vocal affinities of the Puerto Rican and Vermiculated Screech  
452 Owls (*Otus nudipes* and *Otus guatemalae*). *Ibis* 116:369–372.
- 453 van der Weyden WJ (1975) Scops and screech owls: vocal evidence for a basic subdivision in  
454 the genus *Otus* (Strigidae). *Ardea* 63:65–77.
- 455 Van Eijk P (2013) Presumed second locality for Omani Owl. *Dutch Birding* 35:387–388.
- 456 Ward RD (2009) DNA barcode divergence among species and genera of birds and fishes.  
457 *Mol Ecol Res* 9:1077–1085.
- 458 Wink M, Heidrich P (1999) Molecular evolution and systematics of the owls (Strigiformes).  
459 In: König C, Weick F, Becking J-H, *Owls: a guide to the owls of the world*. Pica Press,  
460 Robertsbridge, pp 39–57.

- 461 Wink M, El-Sayed A-A, Sauer-Gürth H, Gonzalez J (2009) Molecular phylogeny of owls  
462 (Strigiformes) inferred from DNA sequences of the mitochondrial cytochrome b and  
463 the nuclear RAG-1 gene. *Ardea* 97:581–591.
- 464 Zink RM, Barrowclough GF (2008) Mitochondrial DNA under siege in avian  
465 phylogeography. *Mol Ecol* 17:2107–2121.

466 **Table 1.** Genbank accession numbers of samples used in molecular analyses.

467

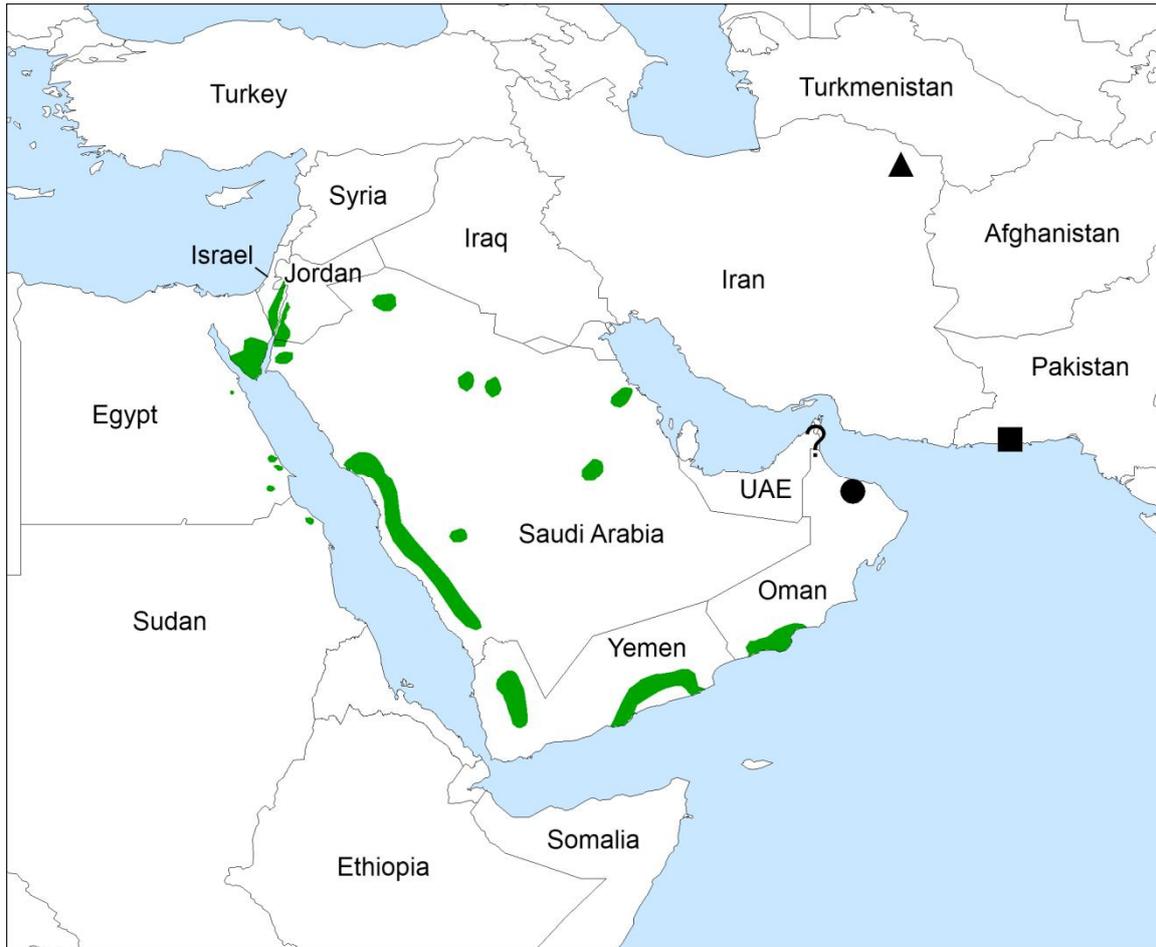
| <b>Taxon</b>                         | <b>GenBank accession number</b> | <b>Source</b>             |
|--------------------------------------|---------------------------------|---------------------------|
| <i>Strix omanensis</i> (Oman)        | KT428757                        | This study                |
| <i>Strix butleri</i> (Iran)          | KT428758                        | This study                |
| <i>Strix butleri</i> (holotype)      | KM459027                        | Kirwan et al. (2015)      |
| <i>Strix hadorami</i>                | AJ003912                        | Wink & Heidrich (1999)    |
| <i>Strix hadorami</i>                | AJ003913                        | Wink & Heidrich (1999)    |
| <i>Strix hadorami</i>                | EU348994                        | Wink et al. (2009)        |
| <i>Strix hadorami</i> (holotype)     | KM459028                        | Kirwan et al. (2015)      |
| <i>Strix woodfordii nigricantior</i> | EU348995                        | Wink et al. (2009)        |
| <i>Strix woodfordii</i>              | AJ004065                        | Wink & Heidrich (1999)    |
| <i>Strix woodfordii</i>              | AJ004066                        | Wink & Heidrich (1999)    |
| <i>Strix woodfordii woodfordii</i>   | AJ004064                        | Wink & Heidrich (1999)    |
| <i>Strix uralensis</i>               | JX092123                        | Hausknecht et al. (2014)  |
| <i>Strix uralensis</i>               | AB741546                        | Omote et al. (2013)       |
| <i>Strix aluco</i>                   | AJ004045                        | Wink & Heidrich (1999)    |
| <i>Strix aluco</i>                   | AJ004057                        | Wink & Heidrich (1999)    |
| <i>Strix nebulosa</i>                | AJ004058                        | Wink & Heidrich (1999)    |
| <i>Strix nebulosa</i>                | AJ004059                        | Wink & Heidrich (1999)    |
| <i>Strix rufipes</i>                 | AJ004060                        | Wink & Heidrich (1999)    |
| <i>Strix rufipes</i>                 | AJ004061                        | Wink & Heidrich (1999)    |
| <i>Strix varia</i>                   | AF448260                        | Desmond et al. (2001)     |
| <i>Tyto alba</i>                     | FJ588458                        | Braun & Huddleston (2009) |

468

469 **Table 2.** Morphometric data obtained from an individual of '*S. omanensis*' (= *S. butleri*)  
470 caught in the Al Hajar range, northern Oman on 2 March 2015.  
471

| <b>Variable</b>                         | <b>State</b>             |
|---|--------------------------|
| Tarsus                                  | 67.4mm                   |
| Wing                                    | 255 mm                   |
| Tail                                    | 142 mm                   |
| Tail graduation                         | 15 mm                    |
| Bill (upper mandible from skull to tip) | 31.85 mm                 |
| Bill (skull to nostrils)                | 17.7 mm                  |
| Bill (skull to centre of curve)         | 24 mm                    |
| Bill depth at end of feathering         | 14.0 mm                  |
| Bill depth from top of cere             | 16.0 mm                  |
| Weight                                  | 220 g                    |
| Moult                                   | p1 + p2 old on left wing |
| Primary 1 to wingtip                    | 56 mm                    |
| P2 to wingtip                           | 13 mm                    |
| P3 to wingtip                           | 0 mm                     |
| P4 to wingtip                           | 0 mm                     |
| P5 to wingtip                           | 8 mm                     |
| P6 to wingtip                           | 33 mm                    |
| P7 to wingtip                           | 50 mm                    |
| P8 to wingtip                           | 60 mm                    |
| P9 to wingtip                           | 71 mm                    |
| P10 to wingtip                          | 80 mm                    |
| Secondary 1 – wingtip                   | 93 mm                    |
| P1 falls                                | between 7 + 8            |
| P2 falls                                | between 5 + 6            |

472



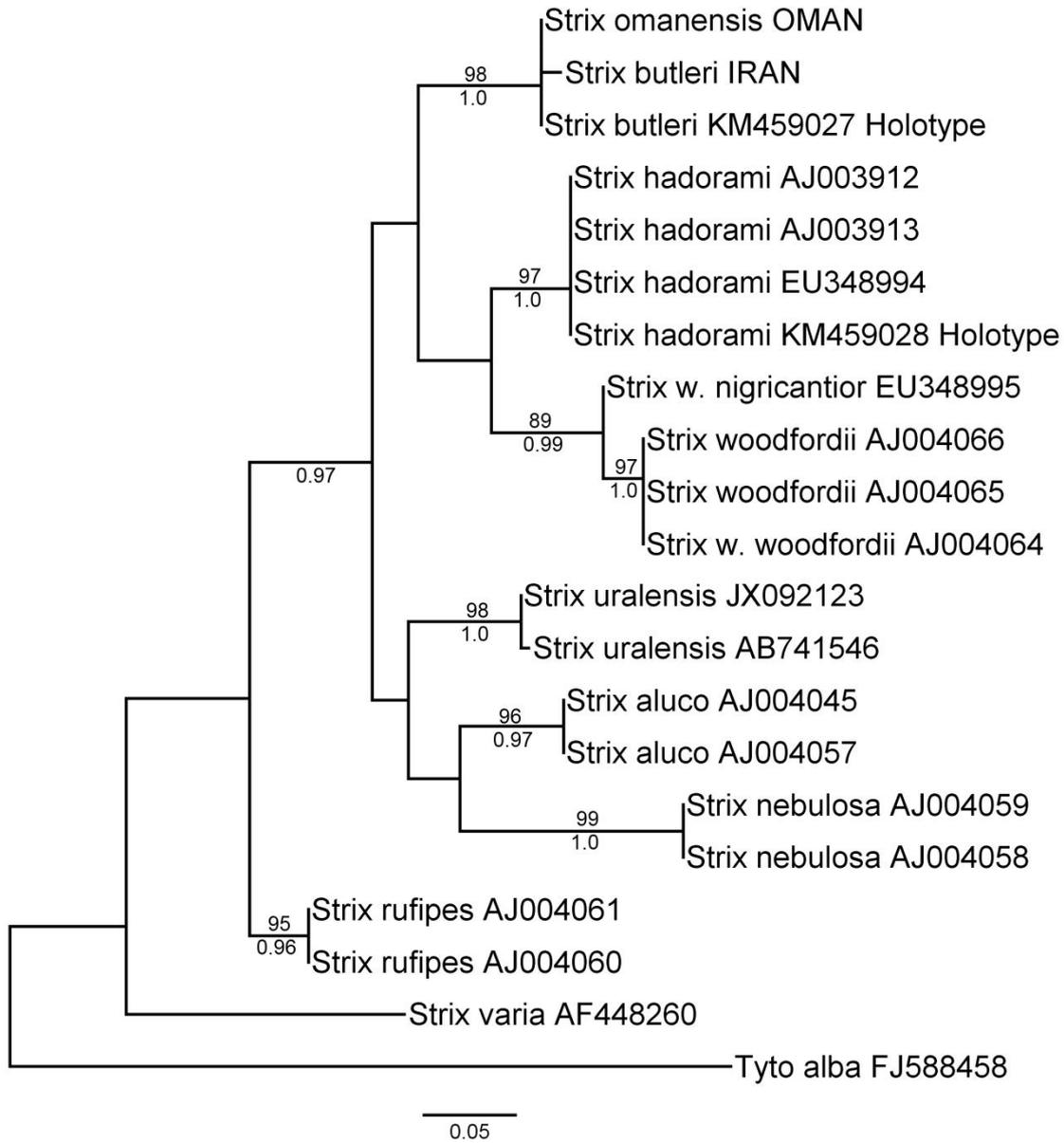
473  
474  
475  
476  
477  
478  
479

Fig. 1. Map showing the known distribution of *Strix hadorami* (green) and *S. butleri* (black). Symbols indicate the type localities of '*S. omanensis*' (circle) and *S. butleri* (square), and the new record in NE Iran (triangle). The question mark denotes a hearing record of *S. butleri* in Wadi Wurayah National Park, United Arab Emirates, which requires substantiation.



480  
481  
482  
483  
484  
485

Fig. 2. Photographs of (a, b) *Strix butleri* captured at the type locality of '*Strix omanensis*', Al Hajar range, Oman, 2 March 2015 (Magnus S. Robb & Alyn J. Walsh) and (c, d) *Strix butleri* after release, Mashhad, Iran, 23 January 2015 (Seyed Babak Musavi).



486  
487  
488  
489  
490  
491  
492  
493

Fig. 3. Maximum likelihood phylogeny of *Strix* owls based on 218 bp of cytochrome b, showing the position of *Strix omanensis* Robb, van den Berg & Constantine, 2013 sampled at its type locality and the owl sampled in Mashhad, Iran in January 2015. Maximum Likelihood bootstrap support values (>80%) and Bayesian Posterior Probabilities (>0.95) are given above and below branches, respectively.