

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 Robb¹, George Sangster^{2,3*}, Mansour Aliabadian^{4,5}, Arnoud B van den Berg⁶, Mark Constantine⁷, Martin Irestedt², Ali Khani⁸, Seyed Babak Musavi⁹, João M G Nunes¹⁰, Maia Sarrouf Willson¹¹ and Alyn J Walsh¹²

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

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

³Department of Zoology, Stockholm University, SE-10691 Stockholm, Sweden.

⁴Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Azadi Square, 9177948974, Mashhad, Iran.

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

⁶The Sound Approach, Duinlustparkweg 98, 2082 EG Santpoort-Zuid, Netherlands.

⁷The Sound Approach, 12 Market Street, Poole, Dorset BH15 1NF, UK.

⁸Khorasan-e Razavi Provincial Office of the Department of the Environment, Mashhad, Iran.

⁹Postal code 7661675555, Khucheh Gochin, Golfam Street, Bolvar Shahid Bahonar, Bam, Iran.

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

¹¹Environment Society of Oman, P.O. Box 3955, P.C. 112, Ruwi, Sultanate of Oman.

¹²Wildfowl Reserve, North Slobland, Wexford, Ireland.

*Corresponding author: 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.

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

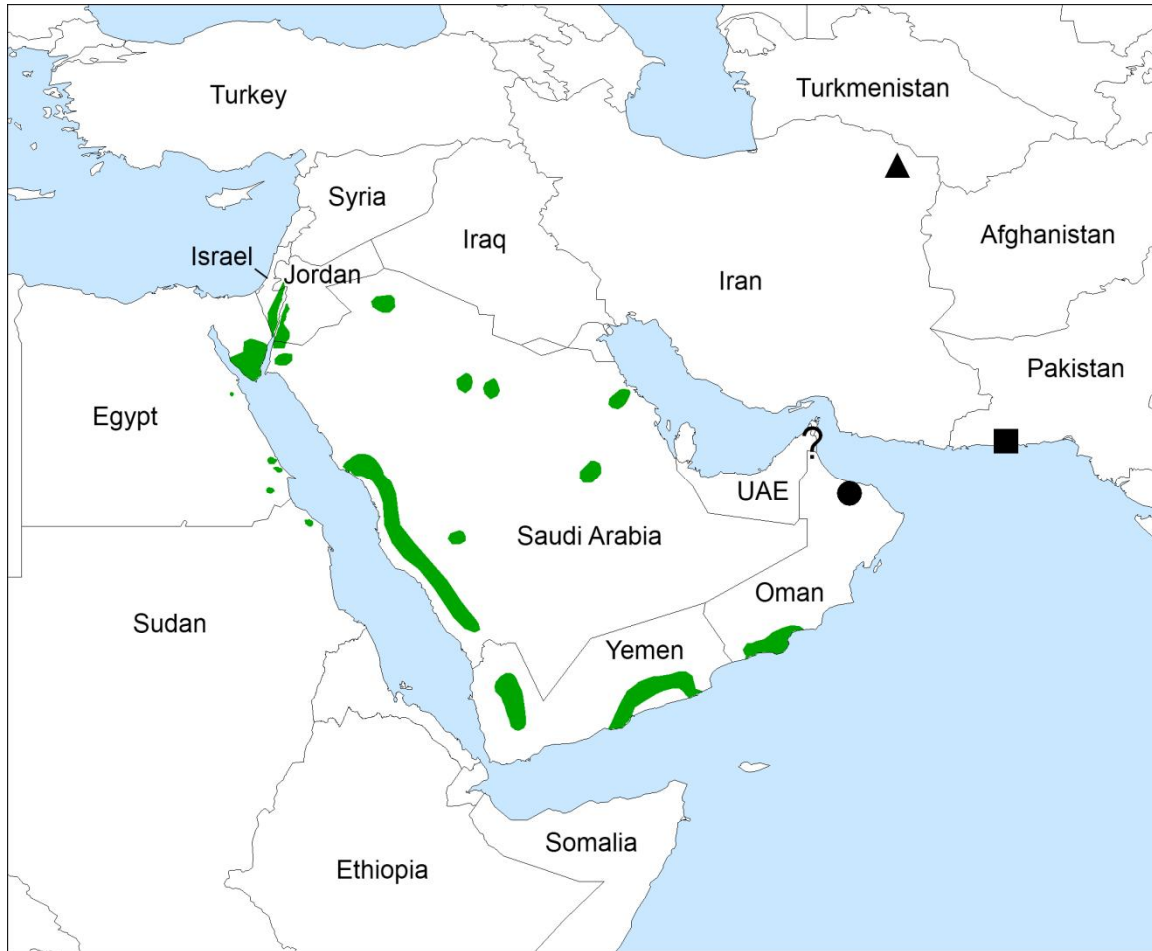
| Taxon | GenBank accession number | Source |
|--------------------------------------|---------------------------------|---------------------------|
| <i>Strix omanensis</i> (Oman) | KT428757 | This study |
| <i>Strix butleri</i> (Iran) | KT428758 | This study |
| <i>Strix butleri</i> (holotype) | KM459027 | 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

| Variable | State |
|---|--------------------------|
| 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.