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2 **Genetic diversity and divergence in the endangered Cape Verde warbler *Acrocephalus brevipennis***

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## 14 **Abstract**

15 Genetic factors play an important role in the long-term persistence of populations and species, and  
16 conservation strategies should take such factors into account. We use neutral molecular markers to  
17 assess diversity and divergence between the three remaining island populations of a little-studied  
18 endemic passerine, the Cape Verde warbler, *Acrocephalus brevipennis*. Variation at both  
19 microsatellite loci and the cytochrome b gene reveal low diversity within the species overall, but  
20 considerable divergence among the populations on Santiago, Fogo and São Nicolau islands. The  
21 genetic markers show a gradient of genetic diversity with population size, with the smallest  
22 population of S. Nicolau being the least diverse, and the largest, Santiago, the most diverse. The  
23 more geographically isolated population on S. Nicolau is also more divergent from Santiago and Fogo  
24 than these two are from each other. The Cape Verde warbler diverged from its sister species, the  
25 greater swamp warbler *Acrocephalus rufescens*, within the last million years, and the three  
26 populations became isolated from one another 165,000–199,000 years ago. There is also evidence of  
27 population bottlenecks, especially in the smallest and most isolated population of S. Nicolau. This  
28 population seems to have decreased during the last century, with potential for further decreases  
29 and even extinction. As the three populations are genetically distinct, with no evidence of gene flow  
30 between them, we argue they should be treated as separate management units for the successful  
31 conservation of this species.

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34 Keywords: microsatellites, cytochrome b, island passerine, endemic species, management units,  
35 Macaronesia

36

## 37 Introduction

38 Over the last few centuries many once common species have become threatened or extinct  
39 throughout the world, mainly due to anthropogenic impacts ([Baillie et al. 2004](#); [Butchart et al. 2010](#)).  
40 Many of these declines and extinctions have occurred on islands ([Steadman 1995](#); [Brooks et al.](#)  
41 [2002](#); [Sax & Gaines 2008](#)). Island species tend to be more vulnerable to extinction threats than  
42 mainland species for many reasons ([Frankham 1998](#); [Sax & Gaines 2008](#)), including smaller  
43 population and range sizes, increased vulnerability to stochastic events, evolutionary/prey naivety,  
44 lower levels of genetic diversity and decreased gene flow ([Frankham 1998](#); [Frankham & Ralls 1998](#);  
45 [Griffin et al. 2000](#); [Sax & Gaines 2008](#); [Frankham et al. 2009](#); [Sih et al. 2010](#)). Islands and  
46 archipelagos are often the focus of conservation actions not only because they contain threatened  
47 species but also because they can be biodiversity hotspots due to their high levels of endemism  
48 ([Myers et al. 2000](#); [Emerson 2002](#); [Kier et al. 2009](#)). Genetic factors can contribute significantly to  
49 extinction risk in the wild ([Frankham & Ralls 1998](#); [Saccheri et al. 1998](#); [Spielman et al. 2004](#);  
50 [Frankham 2005](#)). Higher levels of genetic variation within a population provide more potential to  
51 adapt, for example to environmental changes and novel predators and parasites, whereas small,  
52 genetically depauperate populations are disadvantaged in this regard, and can also suffer from  
53 inbreeding depression ([Crnokrak & Roff 1999](#); [Hedrick & Kalinowski 2000](#); [Brook et al. 2002](#)). Such  
54 factors can drive small populations into an extinction vortex ([Gilpin & Soulé 1986](#)). Consequently,  
55 where possible, management plans for threatened species should take genetic factors into account  
56 ([Brook et al. 2002](#); [Frankham 2005](#)).

57 Genetic data can be used to resolve taxonomic uncertainties, define management units and  
58 assess the extinction risk of a species, being therefore important in informing conservation and  
59 helping mitigate extinction ([Frankham et al. 2009](#)). This is especially important when populations  
60 have become isolated from one another, or fragmented ([Moritz 1994b](#); [Fouquet et al. 2010](#); [Garcia-](#)  
61 [del-Rey et al. 2013](#)). In an attempt to develop a framework for prioritizing conservation efforts, the

62 terms 'evolutionarily significant units' (ESUs) and conservation 'management units' (MUs), mostly  
63 defined by genetic criteria, were coined ([Ryder 1986](#); [Moritz 1994a](#)). Although there are many  
64 different definitions (see [Fraser & Bernatchez 2001](#); [Funk et al. 2012 for reviews](#)), in a genetic  
65 framework ESUs should be reciprocally monophyletic for mitochondrial DNA and significantly  
66 divergent at nuclear loci ([Moritz 1994b](#); [Avice 2004](#)), whereas the concept of MUs is applied to  
67 populations which are significantly divergent at nuclear or mitochondrial loci ([Ryder 1986](#); [Moritz](#)  
68 [1994a](#)). In other terms, ESUs identify historically isolated populations, and MUs can be defined as  
69 populations which show incomplete phylogenetic divergence but currently lack gene flow ([Moritz](#)  
70 [1994b](#)). Although such definitions are not necessarily equivalent to subspecies ([Moritz 1994b](#)), they  
71 provide a first quantitative basis for defining conservation priorities below the species level ([Funk et](#)  
72 [al. 2012](#)).

73 Different types of genetic markers can have different mutation and recombination rates or  
74 transmission pathways, thus they can reveal different aspects of a species' evolutionary history  
75 ([Avice 2004](#)). Microsatellites have fast mutation rates ([Selkoe & Toonen 2006](#)), and can be used to  
76 infer current population structure, demography and gene flow (e.g., [Illera et al. 2007](#); [Dool et al.](#)  
77 [2013](#); [Ferchaud et al. 2015](#); [Xenikoudakis et al. 2015](#)). Furthermore, because of their high levels of  
78 polymorphism they are also useful for assessing demographic bottlenecks ([Sunnucks 2000](#); [Garza &](#)  
79 [Williamson 2001](#); [Williamson-Natesan 2005](#)). In contrast, mitochondrial DNA has slower mutation  
80 rates, and is commonly used to infer population history dating to, and before, the Last Glacial  
81 Maximum ([Avice et al. 1987](#)) circa 23 Kya ago ([Clark et al. 2009](#)). Combining data from microsatellites  
82 and mitochondrial DNA can provide a clearer picture of diversity within, and divergence among,  
83 populations, and can help reconstruct their relative demographic and phylogenetic histories  
84 ([Reynolds et al. 2015](#)). Additionally, patterns of variation at neutral markers can provide information  
85 about colonisation, gene flow, phylogeny and historical relations between populations (e.g., [Hille et](#)  
86 [al. 2003](#); [Dool et al. 2013](#); [Dutta et al. 2013](#); [Ferchaud et al. 2015](#); [Padilla et al. 2015](#)). Finally, genetic

87 markers can be used to assess whether a lack of diversity may be contributing to current declines in  
88 the population ([Allendorf & Luikart 2007](#); [Frankham et al. 2009](#)) and to determine if augmentation  
89 ([Weeks et al. 2011](#)) may be required to alleviate current problems, or ensure maximal adaptive  
90 potential is retained in the species for the future.

91 The Cape Verde warbler *Acrocephalus brevipennis* is an endangered passerine endemic to  
92 Cape Verde, a volcanic archipelago ca. 500 km off West Africa, between 14–18°N and 22–26°W in  
93 the Atlantic Ocean (Fig 1). It is most closely related to the greater swamp warbler *Acrocephalus*  
94 *rufescens* of sub-Saharan Africa ([Leisler et al. 1997](#); [Fregin et al. 2009](#)). The Cape Verde warbler was  
95 discovered about 150 years ago on the island of São Nicolau ([Keulemans 1866](#)), and in the late 19th  
96 century it was known to exist at least on Santiago, S. Nicolau and Brava ([Hazevoet 1993](#)). During the  
97 20th century the population decreased, possibly due to habitat loss caused by droughts ([Hazevoet](#)  
98 [1995](#)) and by 1995 the bird was thought to be confined to Santiago ([Hazevoet 1995](#); [BirdLife](#)  
99 [International 2016](#)). However, in 1998 the bird was rediscovered on S. Nicolau ([Hazevoet et al.](#)  
100 [1999](#)), where the most recent published estimates suggested just 8–10 breeding pairs remain  
101 ([Hazevoet et al. 1999](#); [Donald et al. 2004](#)). In 2004, another population, estimated at ca. 500  
102 breeding pairs, was found on Fogo ([Hering & Hering 2005](#); [Hering & Fuchs 2009](#)). Fogo and Santiago,  
103 in the southern Sotavento group of islands, are separated by ca. 60 km, whereas S. Nicolau, in the  
104 northwestern Barlavento group, is separated from the former two islands by more than 160 km (Fig  
105 1). Such oceanic barriers can prevent gene flow in *Acrocephalus* warblers ([Komdeur et al. 2004](#);  
106 [Cibois et al. 2008](#)), especially between the Sotavento and Barlavento groups ([Hazevoet 1995](#); [Hille et](#)  
107 [al. 2003](#)), which could have important implications for this species' conservation. Currently, the Cape  
108 Verde warbler's global population is estimated at a maximum of 2,200–3,000 individuals, and is  
109 thought to be decreasing ([BirdLife International 2016](#)), but lack of census information means that  
110 such estimates may be somewhat unreliable ([BirdLife International 2016](#)). The species has legal  
111 protection status in Cape Verde but to date no countrywide conservation strategy has been

112 developed. An understanding of the genetic variation within, and divergence between, the three  
113 warbler populations is clearly important to the success of any future conservation programme  
114 ([Frankham et al. 2009](#); [McCartney-Melstad & Shaffer 2015](#)).

115 Here we use neutral molecular markers (mtDNA and microsatellites) to study the three  
116 known remaining populations of the Cape Verde warbler. Our aims were to clarify the degree of  
117 genetic divergence between the bird and its sister species, the greater swamp warbler, to assess  
118 genetic relationships between Cape Verde warbler populations, and to measure genetic diversity  
119 within each population so that this knowledge can be used to inform future conservation actions.

120

## 121 **Materials and methods**

### 122 **Sample collection and DNA extraction**

123 Samples were collected from November 2013 to January 2014, and September to November 2014,  
124 the main breeding season ([Hazevoet 1995](#)). We surveyed for warblers across all potential suitable  
125 habitat types and altitudes (the maximum altitude where a bird was sampled was 1,384 m above sea  
126 level) on each of the three islands to ensure that coverage was as comprehensive as possible (Fig 1,  
127 Table S1). We also conducted extensive transect and point counts in January 2014 on Brava (2  
128 people x 4 days), where the bird was present until 1969 ([Hazevoet 1995](#)), and in September 2014 on  
129 Santo Antão (2 people x 7 days), where the vegetated valleys were thought to provide suitable  
130 habitat ([Hazevoet 1995](#)). When detected, birds were attracted with conspecific song playback and  
131 caught with mist nets. Each caught bird was fitted with a unique combination of a numbered Cape  
132 Verde metal ring, and three UV-resistant plastic colour rings. We determined if the birds were adults  
133 or juveniles based on previously described criteria for this and other warbler species ([Komdeur 1991](#);  
134 [Cramp & Perrins 1992](#); [Baker 1997](#)), and recorded each sampling location with a Garmin eTrex® H  
135 GPS. A small ca. 40 µl blood sample was collected from each bird by brachial venipuncture and

136 stored at room temperature in 800 µl absolute ethanol. We aimed to sample a minimum of 25–30  
137 unrelated individuals per island, as this is sufficient for accurate estimation of genetic parameters in  
138 microsatellite analyses ([Hale et al. 2012](#)). In addition, we used seven other Cape Verde warbler  
139 samples from S. Jorge dos Órgãos (central Santiago), seven greater swamp warbler samples (one  
140 from Senegal and six from Nigeria) and three Seychelles warbler *Acrocephalus sechellensis* samples  
141 (details on additional samples, sampling sites, dates, and bird age determination in Supplementary  
142 Material and Table S1). DNA was extracted using a salt extraction protocol following [Richardson et](#)  
143 [al. \(2001\)](#). Individuals were molecularly sexed following [Griffiths et al. \(1998\)](#).

144

## 145 **Molecular markers**

### 146 *Cytochrome b*

147 We amplified and sequenced a 1,150 bp fragment of mtDNA comprising the last 13 nucleotides of  
148 the ND5 gene, 1,128 nucleotides of the cytochrome b, and 9 nucleotides in between the two genes  
149 (hereafter “cytochrome b”, for simplicity). To avoid amplifying nuclear copies of the mitochondrial  
150 DNA ([numts](#); [Sorenson & Quinn 1998](#)), we re-designed the primers ND5 and mt-F from [Helbig et al.](#)  
151 [\(2005\)](#). We used BLAST ([Altschul et al. 1997](#)) to map the primer sequence against the mitochondrial  
152 genome of the Eurasian reed warbler *Acrocephalus scirpaceus*, ([accession number AM889139](#), [Singh](#)  
153 [et al. 2008](#)) in GenBank ([Benson et al. 2013](#)) and re-designed them to match that genome. We  
154 checked the redesigned primer pair (ND5-Acro 5'-GGCCTAATCAAAGCCTAC-3' and mt-F-Acro 5'-  
155 GGCTTACAAGACCAATGTTT-3') for compatible annealing temperatures, hairpins, complementarity,  
156 primer-dimer formation and % GC content using Primer3 ([Koressaar & Remm 2007](#); [Untergasser et](#)  
157 [al. 2012](#)). Polymerase chain reactions (PCRs) were performed in 15 µl total volume, consisting of 7.5  
158 µl TopTaq Master Mix (Qiagen, West Sussex, UK), 0.6 µl (10 µM) each primer, 4.8 µl ddH<sub>2</sub>O and 1.5  
159 µl DNA. Thermal cycle conditions were 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 55°C

160 for 45 s and 72°C for 1 min, with a final extension step at 72°C for 10 min and a cooling step at 20°C  
161 for 5 min. PCR products were quantified in a 1.5% agarose gel and inspected for signs of non-specific  
162 annealing, i.e. ghost bands ([Sorenson & Quinn 1998](#); [Bensasson et al. 2001](#)). Successfully amplified  
163 products were cleaned with 5 µl of Exo and FastAP (Life Technologies, UK) to 10 µl of PCR product,  
164 and sequenced in both directions (Eurofins, Germany). PCRs were repeated twice to confirm unique  
165 haplotypes and to resolve any ambiguous sites.

166

### 167 *Microsatellites*

168 Individuals were genotyped at 17 polymorphic microsatellite loci, using multiplex combinations  
169 adapted from Spurgin et al. ([2014; Table S2](#)), using primers designed for the Seychelles warbler  
170 ([Richardson et al. 2000](#)). Each PCR included 1 µl Qiagen PCR multiplex master mix, 1 µl primer mix  
171 and 1 µl pre-dried DNA. The PCR cycling conditions were 15 min at 95°C, followed by 30 cycles of 30  
172 s at 94°C, 1 min 30 s at 55 or 56°C and 1 min at 72°C, followed by a final extension step of 30 min at  
173 60°C. PCR products were separated on an ABI 3730 DNA analyser (Applied Biosystems, UK), and  
174 allele sizes were assigned using GeneMapper 4.0 software (Applied Biosystems, UK). Genotypes  
175 were checked visually. PCRs were repeated twice to confirm unique alleles. In cases where two or  
176 more amplifications resulted in conflicting genotypes for the same individual we considered the  
177 individual as heterozygous. We calculated microsatellite error rate per locus according to Pompanon  
178 et al. ([2005](#)). One of the markers, Ase6, could not be reliably scored due to stutter, so it was  
179 excluded from further analyses.

180

### 181 **Data analyses**

#### 182 *Cytochrome b*

183 Unless stated otherwise, statistical analyses were performed in R v. 2.14.1 ([R Core Team 2016](#)).  
184 Sequences were visually inspected in FinchTV v. 1.4.0 (Geospiza Inc., Seattle, WA, USA) and

~



185 manually aligned in BioEdit v. 7.0.9.0 ([Hall 1999](#)), against partial CDS sequences of a Cape Verde and  
186 a greater swamp warbler (from Senegal) previously published ([Fregin et al. 2009](#)) – accession  
187 numbers FJ883026 and FJ883037, respectively. The sequences were translated in MEGA v. 6  
188 ([Tamura et al. 2013](#)), and checked for stop codons. We calculated the number of haplotypes and  
189 segregating sites, haplotype and nucleotide diversity, Tajima's D ([Tajima 1989](#)) and Fu's FS ([Fu 1997](#))  
190 in DnaSP v. 5 ([Librado & Rozas 2009](#)), and pairwise  $F_{ST}$  values ([Wright 1951](#); [Weir & Cockerham 1984](#))  
191 in Arlequin v. 3.5 ([Excoffier & Lischer 2010](#)). Significance of  $F_{ST}$  values was tested with 10,000  
192 permutations. We calculated mean genetic distances within and among the three populations of  
193 Cape Verde warbler and the greater swamp warbler in DnaSP, using the pairwise distance model. A  
194 phylogenetic tree was constructed using the maximum likelihood method implemented in MEGA v.  
195 6, based on the Tamura-Nei model of evolution ([Tamura & Nei 1993](#)) as selected by the Bayesian  
196 Information Criterion in the same software. We used 1,000 bootstrap replicates as a test of  
197 phylogeny, and a discrete Gamma distribution with four categories to model evolutionary rate  
198 differences among sites. However, haplotype networks are usually more appropriate to visualise  
199 relationships between haplotypes in intraspecific datasets than phylogenetic trees ([Bandelt et al.](#)  
200 [1999](#); [Posada & Crandall 2001](#)). Therefore, a median joining haplotype network was also built in  
201 Network v. 4.6.1.3 ([Bandelt et al. 1999](#)).

202

### 203 *Time to most recent common ancestor*

204 Time to most recent common ancestor (tMRCA) of the Cape Verde and greater swamp warblers was  
205 estimated using a Bayesian phylogenetic approach in BEAST v.2.3.0 ([Drummond et al. 2012](#);  
206 [Bouckaert et al. 2014](#)). We estimated the best-fit model of nucleotide substitution in jModelTest v.  
207 2.1.7 ([Guindon & Gascuel 2003](#); [Darriba et al. 2012](#)), which tests for the best model of evolution using  
208 the Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC) and decision theory (DT).  
209 AIC selected GTR+I whereas BIC and DT selected HKY+G. We therefore used the HKY model of

210 substitution ([Hasegawa et al. 1985](#)) with a gamma site model comprising four rate categories ([Yang](#)  
211 [1994](#)) and an estimated gamma shape parameter. Effective sample sizes (ESS) for all model  
212 parameters exceeded the recommended minimum 200 when using the HKY model, so this model  
213 was considered appropriate. The dataset was composed of unique haplotypes of Cape Verde and  
214 greater swamp warblers, using the Seychelles warbler as an outgroup. Codons were partitioned into  
215 3 (1 + 2 + 3, frame 2) to allow each nucleotide position within the codon to be modelled separately.  
216 Empirical substitution model state frequencies were used along with an estimated HKY kappa  
217 parameter. We used a substitution rate of 0.0105 per site per million years, which corresponds to a  
218 2.1% divergence rate, generally applicable to passerine cytochrome b sequences ([Weir & Schluter](#)  
219 [2008](#)). Molecular clock rate variation is highly unlikely in our dataset, which comprises a single gene  
220 across closely related species with resulting low sequence information content. We therefore  
221 employed a strict molecular clock. As neither fossil nor relevant biogeographical calibration points  
222 were available for this clade of passerines, we utilised published molecular phylogenies to provide  
223 loose bounds for the priors. We specified a uniform distribution between 1 and 12 Mya for the  
224 tMRCA of the Cape Verde, greater swamp and Seychelles warblers group, as 12 Mya is the estimated  
225 divergence time of the small and large groups of *Acrocephalus* warblers; Cape Verde, greater swamp  
226 and Seychelles warblers are members of the latter group ([Price 2008](#); [Leisler & Schulze-Hagen 2011](#)).  
227 We used a diffuse uniform prior bound between the present and 1 Mya to estimate tMRCA of Cape  
228 Verde and greater swamp warblers and the same for the tMRCA of the three Cape Verde warbler  
229 populations. This is because, even though a divergence time has never been proposed, previous  
230 phylogenetic analyses suggests that these two sister species are so similar, when compared to the  
231 dated splits in the *Acrocephalus* phylogeny, that they are not likely to have diverged more than 1  
232 Mya ([Leisler et al. 1997](#); [Fregin et al. 2009](#); [Leisler & Schulze-Hagen 2011](#)). Finally, the birth rate and  
233 clock rate priors were both assigned diffuse gamma distributions ( $\alpha = 0.001$ ,  $\beta = 1000$ ) ([Drummond &](#)  
234 [Bouckaert 2014](#)). The Markov chain Monte Carlo (MCMC) simulations were run with a standard 10%

235 burn-in. We did four runs, with a total of 100 million iterations, to check mixing and convergence,  
236 which was assessed with ESS. Runs were combined using LogCombiner ([Rambaut & Drummond](#)  
237 [2015](#)) and the results were visualised using Tracer v.1.6 ([Rambaut et al. 2015](#)).

238

### 239 *Microsatellite analysis*

240 For each microsatellite locus and population we tested for deviations from Hardy–Weinberg  
241 equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP v. 4.0.10 ([Raymond & Rousset](#)  
242 [1995](#)). We applied a Bonferroni sequential correction to control for type I errors ([Rice 1989](#)). Null  
243 allele estimates were calculated in MICROCHECKER ([van Oosterhout et al. 2004](#)). Allelic richness and  
244 number of private alleles in each population were calculated after controlling for differences in  
245 sample size, using a rarefaction approach implemented in HP-RARE ([Kalinowski 2005](#)). Inbreeding  
246 coefficients ( $F_{IS}$ ) were calculated with GENEPOP. We tested for population structure by calculating  
247 global and pairwise  $F_{ST}$  values across the three Cape Verde warbler populations. Global and pairwise  
248  $F_{ST}$  estimates, observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity were calculated using Arlequin v. 3.5  
249 ([Excoffier & Lischer 2010](#)).

250 To determine the most likely number of genetic clusters (K), a Bayesian algorithm was  
251 implemented in STRUCTURE v. 2.3 ([Pritchard et al. 2000](#)). The goal was to detect overall population  
252 structure in the Cape Verde warbler, so we included information on population of origin in the  
253 analysis. We used a no-admixture ancestry model and independent allele frequencies. However, as a  
254 no-prior model may be better for revealing subtle similarities, or possible gene flow between  
255 populations ([Pritchard et al. 2000](#); [Falush et al. 2007](#); [Hubisz et al. 2009](#)), we also ran a model using  
256 admixture and correlated allele frequencies, disregarding prior information on sampling location. For  
257 both models, we undertook four runs of 500,000 MCMC repetitions with a burn-in of 20,000 at each  
258 clustering level for  $K = 1–6$ . To assess structure within the Santiago population, we ran both analyses  
259 using only the Santiago birds. The number of clusters best fitting the data was determined using

260 both log probabilities and the ad hoc  $\Delta K$  test ([Evanno et al. 2005](#)), implemented in STRUCTURE  
261 HARVESTER ([Earl & vonHoldt 2012](#)). Graphical results were visualised using DISTRUCT version 1.1  
262 ([Rosenberg 2004](#)).

263

#### 264 *Bottleneck tests*

265 We used three methods to assess evidence of population bottlenecks: the heterozygosity excess test  
266 ([Cornuet & Luikart 1996](#)) implemented in BOTTLENECK v. 1.2.02 ([Piry et al. 1999](#)); the mode shift  
267 test ([Luikart et al. 1998](#)), implemented in the same software; and the M-ratio test ([Garza &](#)  
268 [Williamson 2001](#)), as well as a modified version of this last test which accounts for monomorphic  
269 alleles ([Excoffier et al. 2005](#)), in Arlequin. Heterozygosity and mode-shift tests use excess  
270 heterozygosity and allele frequency data to detect recent bottlenecks, i.e. bottlenecks that have  
271 occurred within the past dozen generations ([Luikart et al. 1998](#)). M-ratio tests ([Garza & Williamson](#)  
272 [2001](#); [Excoffier et al. 2005](#)) are based on the reasoning that a bottlenecked population will lose some  
273 rare alleles, and this loss will not depend on allele size; therefore a bottlenecked population will  
274 show gaps in the allele size distribution. M-ratio tests can detect bottleneck signatures over longer  
275 periods than heterozygosity or shift mode tests, as M-ratios are likely to change more slowly than  
276 heterozygosity after a bottleneck ([Garza & Williamson 2001](#); [Williamson-Natesan 2005](#); [Peery et al.](#)  
277 [2012](#)).

278 The distances between these islands are unequal (see Fig 1), and dispersal patterns can be  
279 differently influenced by the direction of the prevailing winds ([Hazevoet 1995](#); [Correia 1996](#); [Hille et](#)  
280 [al. 2003](#)). The islands have different geography, age, and habitat types available for the birds  
281 ([Hazevoet 1995](#)) and there are only three populations. It is therefore not possible to ascertain the  
282 effects of isolation by distance ([Wright 1943](#)), resistance (a metric which combines Euclidean  
283 distances and spatial heterogeneity) ([McRae & Nürnberger 2006](#)) or environment factors ([Wang &](#)  
284 [Bradburd 2014](#)) on the genetic structure of the Cape Verde warbler using these data.

**286 Results***287 Cytochrome b*

288 We observed 16 cytochrome b haplotypes in the Cape Verde warbler, five haplotypes in the greater  
289 swamp warbler and one haplotype in the Seychelles warbler (Table 1; Fig 2 and 3). The Cape Verde  
290 warbler populations do not share haplotypes except for the most common one, shared between  
291 Santiago and Fogo (Fig 2 and 3). The sequences have been deposited in the National Center for  
292 Biotechnology Information (NCBI) GenBank database (accession nos. KX518324–KX518347). Where  
293 the same haplotype was found in two different populations, we submitted two identical sequences.  
294 Basic diversity indexes are given in Table 1. Pairwise genetic distances between haplotype sequences  
295 within and among Cape Verde warbler populations were very low in all cases, ranging between 0.1%  
296 and 0.5% (Table 2). The Santiago population (the largest) showed the highest diversity within the  
297 Cape Verde warbler, in terms of number of haplotypes, number of segregating sites, haplotype and  
298 nucleotide diversity (Table 1, Fig 4). Nevertheless, haplotype and nucleotide diversity in the greater  
299 swamp warbler were much higher, despite the limited number of samples screened (Table 1). The  
300 population of S. Nicolau exhibits the lowest levels of diversity, while Fogo is intermediate (Table 1,  
301 Fig 4). Diversity levels for the Cape Verde warbler population overall are similar to those for the  
302 Santiago population (Table 1). The neutrality tests (Tajima's D and Fu's FS), which reflect population  
303 expansion or contraction in the absence of selective forces, were not significant but showed  
304 tendencies for population expansion on Santiago, and contraction on Fogo and S. Nicolau (Table 1).

305 The maximum likelihood phylogenetic tree and the haplotype network show that the greater  
306 swamp warbler haplotypes cluster in one distinct group (Fig 2 and 3). Among the Cape Verde  
307 warbler haplotypes, some nodes have low bootstrap support values because of the low information  
308 content of the sequences, as seen in their very low genetic distances (Table 2). Importantly, the S.

309 Nicolau haplotypes seem to have derived from the most common haplotype (labelled CVW\_ST\_FG in  
310 Fig 2), which is found on Santiago and Fogo but not on S. Nicolau (Fig 2 and 3). Apart from this most  
311 common haplotype, there are no shared haplotypes between the three populations.

312

### 313 *Time to the most recent common ancestor*

314 The tMRCA estimate was sampled from an approximately log-normal distribution; hence geometric  
315 means are provided ([Morrison 2008](#)). The tMRCA for the Cape Verde and greater swamp warbler  
316 was estimated at about 292 Kya, with upper confidence limits well within the last million years  
317 (Table 3). The tMRCA of S. Nicolau and the two Sotavento populations was estimated at 199 Kya,  
318 and the tMRCA for Santiago and Fogo at 165 Kya, both with upper 95% confidence limits within the  
319 last 650 thousand years (Table 3). The lower 95% confidence limits for the tMRCA for all Cape Verde  
320 warbler populations, and for the Sotavento populations, was over 30 Kya ago, i.e., prior to the Last  
321 Glacial Maximum ([Clark et al. 2009](#)).

322

### 323 *Microsatellites*

324 Genetic diversity measurements are given in Table 4. The estimated error rate per locus was 0.25%.  
325 Evidence for null alleles was found in microsatellite loci Ase11 and Ase22 with null allele frequencies  
326 of 0.309 and 0.164, respectively, using the Oosterhout algorithm ([van Oosterhout et al. 2004](#)), so  
327 these were removed from subsequent analyses. Ase13 and Ase48 were in strong linkage  
328 disequilibrium ( $P < 0.001$ ) across all populations. There was no difference between results of  
329 summary statistics using either only Ase13 or only Ase48 (Mantel test on  $F_{ST}$  matrices,  $P = 0.172$ ;  
330 Wilcoxon tests on  $H_e$  and  $H_o$ , all  $P > 0.742$ ), so Ase48 was removed from further analyses. After  
331 removing these markers, we found no deviations from HWE or significant LD between loci.  
332 Heterozygosity, allelic richness and number of private alleles show a clear gradient, with highest

333 values in Santiago, intermediate in Fogo and lowest in S. Nicolau, concurring with the mtDNA results  
334 (Table 4 and Fig 4). The inbreeding coefficient ( $F_{is}$ ) is very close to random expectation for all the  
335 populations of the Cape Verde warbler, i.e. between -0.0030 and 0.0047 (Table 4).

336 Global  $F_{ST}$  was 0.33 for cytochrome b and 0.16 for microsatellites (both  $P < 0.001$ ), showing  
337 pronounced overall differentiation among the populations. Pairwise  $F_{ST}$  values are shown in Table 5.  
338 Differentiation between the Santiago and Fogo populations was moderate to high, and high  
339 between those populations and that of S. Nicolau. The pattern of differentiation identified among  
340 populations was the same for mitochondrial and microsatellite markers.

341 The Evanno method supports two main genetic clusters ( $k = 2$ ; highest  $\Delta K = 342.51$ , Fig S1  
342 and S2), one including the S. Nicolau population and one with the Sotavento populations (Santiago  
343 and Fogo, Fig S2). However, when  $k = 3$  the plot shows clear separation between the three island  
344 populations (Fig 5). This indicates that even though the main differences are between S. Nicolau and  
345 the Sotavento populations, there are also marked differences between Santiago and Fogo. For the  
346 analyses using only the Santiago population, the best  $k = 1$  (results not shown), showing no evidence  
347 of structure within the Santiago population.

348

#### 349 *Bottleneck tests*

350 All tests show a clear signal of a past population bottleneck in the S. Nicolau population (Table 6).  
351 The M-ratio tests and modified M-ratio tests range between 0.15 and 0.30 (Table 6), well below the  
352 critical value of 0.68 ([Garza & Williamson 2001](#)), suggesting bottlenecks have occurred on all islands.  
353 However the shift mode and heterozygosity tests (using the strict stepwise mutation model) did not  
354 detect bottlenecks for the Santiago and Fogo populations (Table 6).

355

## 356 **Discussion**

357 Overall we found low genetic diversity within, but considerable divergence among, the three  
358 remaining populations of Cape Verde warbler. There is a gradient of genetic diversity decreasing  
359 from Santiago through Fogo to S. Nicolau (Fig 4). The gradient of genetic diversity from the larger to  
360 the smaller island is consistent with expectations derived from population and island size theories  
361 ([MacArthur & Wilson 1967](#); [Frankham 1996](#); [Frankham et al. 2009](#)). Santiago possibly holds the  
362 largest population, estimated at ca. 500 pairs in the 1980s, when the warbler was thought to be  
363 absent from the north part of the island ([Hazevoet 1995](#)). As birds can now be found all across this  
364 island, if the density and territory size are relatively constant, the actual population size is likely to be  
365 considerably higher (pers. obs.). However, to our knowledge there is no information on density or  
366 territory sizes for this island, and no censuses that could provide a reliable basis for an accurate  
367 estimate have been carried out on Santiago; thus estimates must be taken with extreme caution  
368 ([BirdLife International 2016](#)). Clearly, it would be advisable to undertake thorough population  
369 censuses on Santiago to better inform conservation. The population on Fogo is medium-sized, with  
370 ca. 500 pairs ([Hering & Fuchs 2009](#)) and birds limited to the well-vegetated north-eastern part of the  
371 island. On S. Nicolau there is a very small population (8-10 pairs; [Hazevoet et al. 1999](#); [Donald et al.](#)  
372 [2004, 12-13 pairs; this study](#)) limited to the reed patches and mango tree valleys within a small (< 20  
373 km<sup>2</sup>) central part of the island (pers. obs.).

374 Genetic divergence is greatest between S. Nicolau and the two Sotavento populations of  
375 Santiago and Fogo (Table 5). Our results are similar to those of previous studies addressing genetic  
376 divergence in other Cape Verdean taxa including geckos ([Arnold et al. 2008](#); [Vasconcelos et al. 2012](#)),  
377 skinks ([Brehm et al. 2001](#); [Brown et al. 2001](#)) and kestrels ([Hille et al. 2003](#)). There is usually a clear  
378 genetic differentiation between the populations on the Barlavento and Sotavento island groups  
379 (e.g., [Brown et al. 2001](#); [Hille et al. 2003](#)), except where the focal species occupy micro-habitat  
380 niches and/or have very limited dispersal capacities. In such cases, they can be genetically divergent



381 even among closely located islands ([Brehm et al. 2001](#); [Arnold et al. 2008](#); [Vasconcelos et al. 2012](#));  
382 nevertheless the differentiation between Barlavento and Sotavento island groups is always present.

383         The warbler population on Santiago is widespread, inhabits a diverse range of habitats, from  
384 sugarcane plantations to mountain forests, and is genetically the most diverse of the three (Tables 1  
385 and 5, Fig 3 and 4), but we did not find any population substructure. Considering that, based on area  
386 of occurrence of the warbler alone, it is potentially the largest population, and the fact that it is the  
387 most genetically diverse, we would argue that the Santiago population is currently of least  
388 conservation concern of the three populations. Although the Fogo population was only discovered in  
389 2004 ([Hering & Hering 2005](#)), our results indicate that the island was colonised well before the Last  
390 Glacial Maximum (> 30 Kya) and has been isolated and diverging ever since. This population seems  
391 to be of medium conservation concern. Taken together, our results indicate that the split between  
392 the Santiago and Fogo populations was more recent than between those two and S. Nicolau. Current  
393 demographic trends in any of the three populations are unknown.

394         When the Cape Verde warbler was discovered on S. Nicolau in the 1860s, it was said to be  
395 “numerous” ([Keulemans 1866](#)). However, by the 1920s it was considered extinct on the island, and  
396 was only rediscovered in the 1990s ([Hazevoet 1995](#); [Hazevoet et al. 1999](#)). One specimen, collected  
397 in the 1970s and housed in the Zoological Centre in Lisbon, is testimony that a remnant population  
398 persisted between the 1920s and the 1990s ([Hazevoet et al. 1999](#)). Our results support the  
399 occurrence of a severe bottleneck in this population, in agreement with the anecdotal evidence  
400 ([Hazevoet et al. 1999](#); [Donald et al. 2004](#)). This population has lower diversity, different  
401 microsatellite allele frequencies and private mitochondrial alleles when compared to Santiago and  
402 Fogo. Phylogenetically the S. Nicolau birds group with the other Cape Verde warbler populations  
403 rather than with the greater swamp warbler (Fig 2). Additionally, the tMRCA of S. Nicolau and the  
404 two Sotavento populations is 199 kya. Consequently, the current S. Nicolau population cannot have  
405 been derived from birds that arrived from Santiago or Fogo in the late 20<sup>th</sup> century. It is also highly

406 unlikely that *S. Nicolau* was recolonised by greater swamp warblers dispersing directly from Africa  
407 after the original population was extinct. There is a strong indication that a bottleneck has occurred  
408 in this population, and based on our own sampling effort we estimate a maximum of 20–25 breeding  
409 pairs currently on the island. *S. Nicolau* is known to have suffered from severe droughts during the  
410 last century ([Sena-Martins & Moreno 1986](#); [Hazevoet 1995](#); [Correia 1996](#)) and this and other factors,  
411 such as predation and/or pathogens, may have reduced the warbler population. However, we  
412 cannot accurately estimate by how much it has decreased given that there is no reported estimate  
413 of its original size, and assessing possible habitat loss is beyond the scope of this paper. Thus, any  
414 links between bottlenecks and constraints on population size are only speculative at this point.  
415 Clearly, the warbler population on *S. Nicolau* should be considered to be of high conservation  
416 concern.

417         The estimated tMRCA between the three Cape Verde warbler populations (165–199 Kya) is  
418 not that much more recent than the tMRCA between the Cape Verde and greater swamp warblers  
419 (292 Kya; Table 3), suggesting that there was little gene flow between the Cape Verde warbler  
420 populations after the colonisation of the islands. However, divergence dates have an associated  
421 error (95% HPD; Table 3) and the use of different markers, substitution rates, calibration methods or  
422 molecular clocks can lead to variation in estimated tMRCA and divergence times (e.g. [Ho 2007](#);  
423 [Yoshikawa et al. 2008](#); [Cibois et al. 2011](#)). Consequently tMRCA must be treated with caution. For  
424 example, the molecular substitution rate we used was 2.1% sequence divergence per million years  
425 ([Weir & Schluter 2008](#)). However, there is evidence that no universal molecular clock rate exists for  
426 birds, and that substitution rates can be higher than 2.1% for recently diverged sister species or for  
427 intraspecific datasets ([García-Moreno 2004](#); [Lovette 2004](#); [Subramanian et al. 2009](#)). Nevertheless, it  
428 is clear that the Cape Verde warbler diverged from its sister species within the last million years  
429 (geometric mean estimate = 292 Kya), as previously suggested ([Hazevoet 1995](#); [Fregin et al. 2009](#)),  
430 and that the three Cape Verde warbler populations have been isolated from each other since before

431 the Last Glacial Maximum (> 30 Kya) and perhaps much longer (199 Kya). That Santiago and Fogo  
432 share the same most common cytochrome b haplotype (Fig 2 and 3), and have a tMRCA estimate of  
433 165 Kya suggest that the split between these two populations was more recent than the split  
434 between them and S. Nicolau (199 Kya), with which they share no cytochrome b haplotypes.

435 Our results indicate that the populations have been through bottlenecks of variable  
436 intensity, with particularly strong evidence for the S. Nicolau population (Table 6). The M-ratio,  
437 which tests for occurrence of gaps in allele size range, can still show a bottleneck signal long after  
438 the signal is no longer detectable with heterozygosity or shift mode tests ([Garza & Williamson 2001](#);  
439 [Williamson-Natesan 2005](#); [Peery et al. 2012](#)). It is tempting to infer that, even though all the warbler  
440 populations appear to have undergone bottlenecks, the one affecting S. Nicolau occurred more  
441 recently, or is still ongoing, while in Fogo and Santiago the populations have stopped decreasing, or  
442 are even increasing in size. Contrasting results from M-ratio and heterozygosity tests are commonly  
443 used to infer differences in the timing of bottlenecks ([Garza & Williamson 2001](#); [Williamson-Natesan](#)  
444 [2005](#); [Peery et al. 2012](#)), but these tests can be unequally affected if assumptions are violated ([Peery](#)  
445 [et al. 2012](#)), so results must be interpreted with caution. Therefore, even though our data show that  
446 the S. Nicolau population has been recently, or still is, bottlenecked, and that evidence for  
447 bottlenecks in the other two populations is not as strong, this does not exclude the possibility that  
448 the other populations are currently decreasing as well.

449 Despite extensive surveys, we did not find warblers on either Brava or Santo Antão,  
450 consistent with results from previous work (H. Dinis & E. Ramos, *pers. comm.*). This means that the  
451 Brava population, last reported in 1969 ([Hazevoet 1995](#)), is very likely to be extinct. A possible  
452 explanation is that, as the second smallest island in the archipelago, it would not have had sufficient  
453 suitable habitat to support a population through the drought periods that the archipelago suffered  
454 in the 1970s and 1980s ([Hazevoet 1995](#); [Correia 1996](#)). In the case of Santo Antão, a lack of historical

455 information means we are unable to make any inferences about the possible presence of a  
456 population there in the past.

457         The three populations of the Cape Verde warbler fall under the definition of MUs, but not  
458 under the definition of ESUs. There is no evidence of gene flow among them, they are genetically  
459 distinct, but there is sharing of one mitochondrial haplotype at least between Fogo and Santiago.  
460 The population of S. Nicolau is especially distinct, as it does not share mitochondrial haplotypes with  
461 either of the Sotavento populations, even though they are not reciprocally monophyletic. From a  
462 conservation genetics perspective, it may appear prudent to manage all three populations as  
463 separate units, such as suggested for similar situations in other taxa ([Fouquet et al. 2010](#); [Garcia-del-](#)  
464 [Rey et al. 2013](#); [Reynolds et al. 2015](#)). However, the decision to preserve each population's genetic  
465 uniqueness in the face of future risks of inbreeding depression or a limited adaptive potential is not  
466 straightforward ([Amos & Balmford 2001](#); [Miller et al. 2009](#); [Weeks et al. 2011](#); [Coleman et al. 2013](#);  
467 [Weeks et al. 2016](#)). Augmentation of the number of individuals on some islands, to help prevent  
468 inbreeding and maximise adaptive potential, may be beneficial to the populations' chances of  
469 survival ([Weeks et al. 2011](#); [Weeks et al. 2016](#)). However, such actions could potentially lead to  
470 outbreeding depression ([Edmands 2007](#)) and the relative potential costs and benefits must therefore  
471 be assessed carefully. In this work, we did not investigate potential direct threats to any of the three  
472 populations, such as pathogens, predation or habitat destruction; we discuss our findings solely from  
473 a conservation genetics perspective.

474

#### 475 **Conservation conclusions and recommendations**

476 Our genetic data suggest that each of the three remaining populations of the Cape Verde warbler  
477 should be treated as different MUs ([Ryder 1986](#); [Moritz 1994b](#)), with different conservation  
478 priorities (high on S. Nicolau, medium on Fogo, low on Santiago). Special attention should be given

479 to the more genetically divergent (and most genetically depauperate) population on S. Nicolau.  
480 Territory occupancy is currently being monitored at several locations on Santiago and Fogo (A.  
481 Rendall, J. Mascarenhas, A. Rodrigues, *pers. comm.*). Fewer conservation actions have been directed  
482 to S. Nicolau, where monitoring has been irregular over the last few years (L. Oliveira, *pers. comm.*).  
483 From previous surveys ([Hazevoet et al. 1999](#); [Donald et al. 2004](#)) and our own sampling effort, we  
484 estimate that S. Nicolau currently holds a maximum of 20–25 breeding pairs (minimum 12; *pers.*  
485 *obs.*) and therefore could be considered to be on the verge of extinction. At the moment, the  
486 relative roles of genetic versus ecological factors in constraining population sizes on any of the  
487 islands are not clear and further information on this topic is required to help inform conservation  
488 actions. It is important to undertake baseline studies on this species (e.g. population sizes estimates,  
489 breeding behaviour, productivity, diet, habitat and predation), preferably on all islands as ecological  
490 differences might exist between populations. Until such studies are undertaken, managers should  
491 incorporate in their plans actions that take this bird's habitat into account. For example, in the  
492 Natural Park of Serra Malagueta, not all the invasive shrubs were removed in a recent restoration  
493 action because some warblers were nesting there (J. Mascarenhas, *pers. comm.*). Direct  
494 conservation actions and the use of augmentation in the population of S. Nicolau should be  
495 considered to help conserve this population. We hope that this genetic analysis will be of use to  
496 practitioners in refining action plans to maximise the conservation outcome for this endemic  
497 species.

498

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565

## 566 **Author contributions**

567 DSR designed the study with input from IB and NJC. HRB, DSR, IB and NJC worked to raise the  
568 funding. HRB collected the samples, except where stated otherwise, did the molecular work and  
569 drafted the manuscript. HRB and DJW analysed the data with input from DSR. All authors had input  
570 into earlier drafts and agreed on the final manuscript.

571

## 572 **Data accession statement**

573 Haplotype sequences have been submitted to GenBank (accession numbers KX518324 -KX518347).

574

## 575 **Conflict of interest**

576 The authors declare that they have no conflict of interest.

577

**578 Compliance with ethical standards**

579 This work has been approved by the BIO Ethical Review Committee of the University of East Anglia.

580

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865 **Figure captions**

866 **Fig. 1** The Cape Verde archipelago with the locations where Cape Verde warblers were sampled in 2013 and 2014.

867 Inset shows the archipelago in relation to West Africa. For details on sampling sites and dates, see main text  
868 and supplementary material

869 **Fig. 2** Maximum likelihood phylogenetic analysis of the Cape Verde warbler (CVW, n = 145) and greater swamp  
870 warbler (GSW, n = 7) species, based on cytochrome b; haplotypes names are coded by island (ST = Santiago,  
871 FG = Fogo, SN = S. Nicolau); asterisks mark haplotypes only found on Fogo or S. Nicolau; the Seychelles  
872 warbler haplotype (SW, n = 3) is used as an outgroup

873 **Fig. 3** Median-joining haplotype network based on cytochrome b of the Cape Verde warbler (CVW, n = 145) and the  
874 greater swamp warbler (GSW, n = 7). Haplotypes found in the Cape Verde warbler populations are  
875 represented in dark grey for Santiago, light grey for Fogo and white for S. Nicolau; the central haplotype is  
876 the most common one (labelled CVW\_ST\_FG in Fig. 2); haplotypes found in the greater swamp warbler, in  
877 medium grey, cluster separately. The size of the circles is proportional to the number of individuals that  
878 share that haplotype. Black dots represent intermediate (unsampled) haplotypes, with 1 bp between  
879 consecutive haplotypes

880 **Fig. 4** Gradient of decreasing genetic diversity with estimated population size: rarefied allelic richness (full circles, n =  
881 131; error bars = SD) and number of haplotypes (empty circles, n = 145) for each of the Cape Verde warbler  
882 (CVW) populations, ordered by decreasing population size from Santiago to S. Nicolau

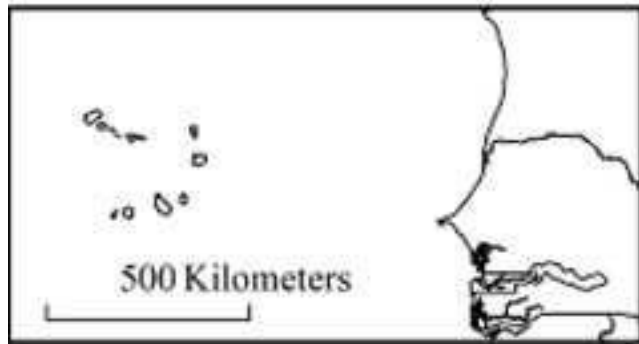
883 **Fig. 5** STRUCTURE plot showing k = 3 distinct genetic clusters in the Cape Verde warbler samples where Santiago, n =  
884 66; Fogo, n = 38; and S. Nicolau, n = 27. Each bar represents an individual's proportional probability of  
885 membership to each cluster, given in dark grey, light grey and white, respectively

886

Santo Antão



S. Nicolau



Santiago



Brava



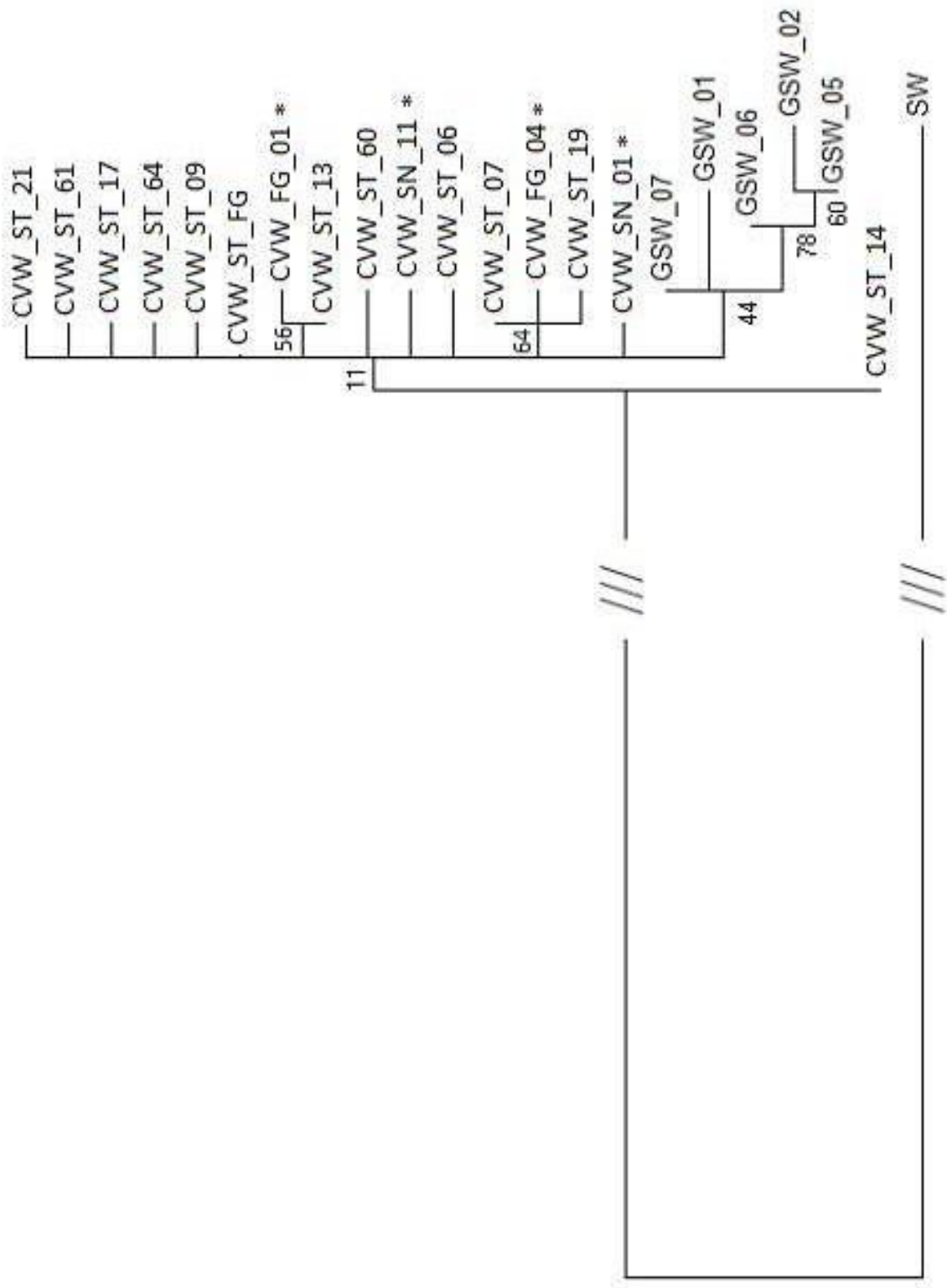
Fogo



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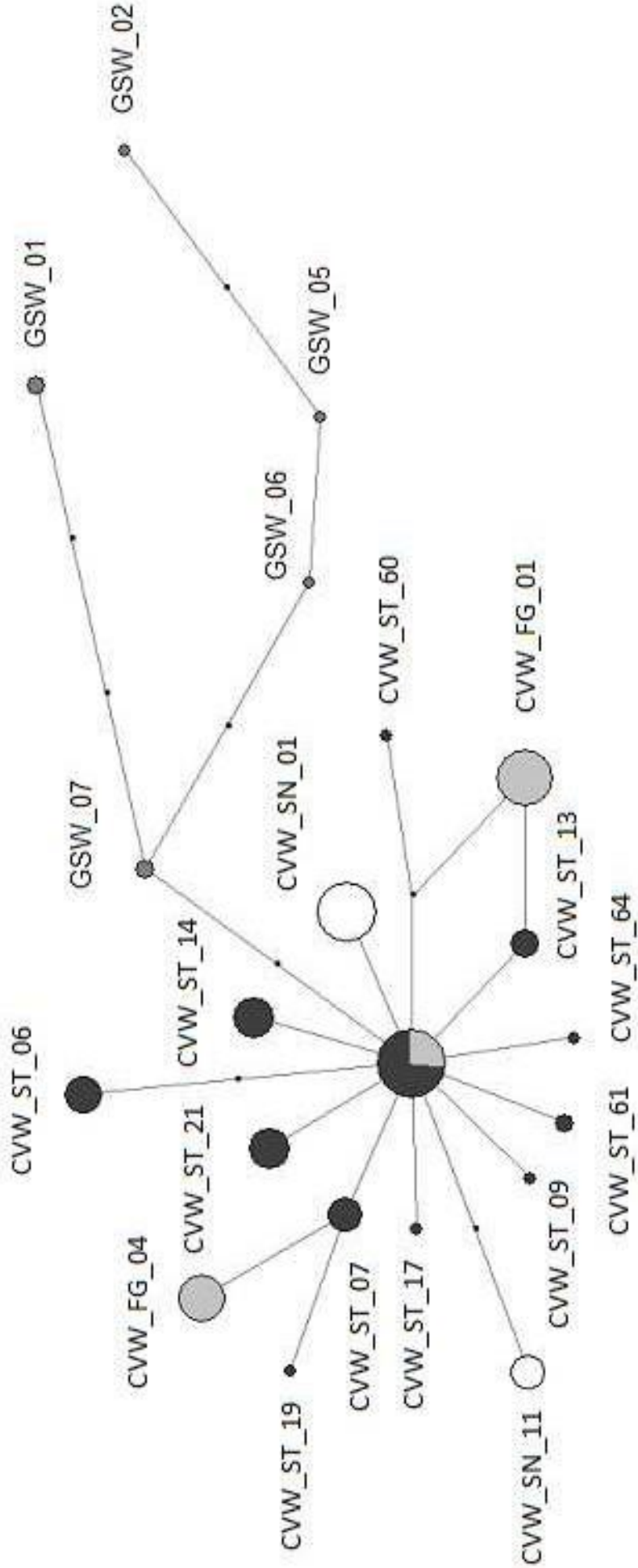
100 Kilometers



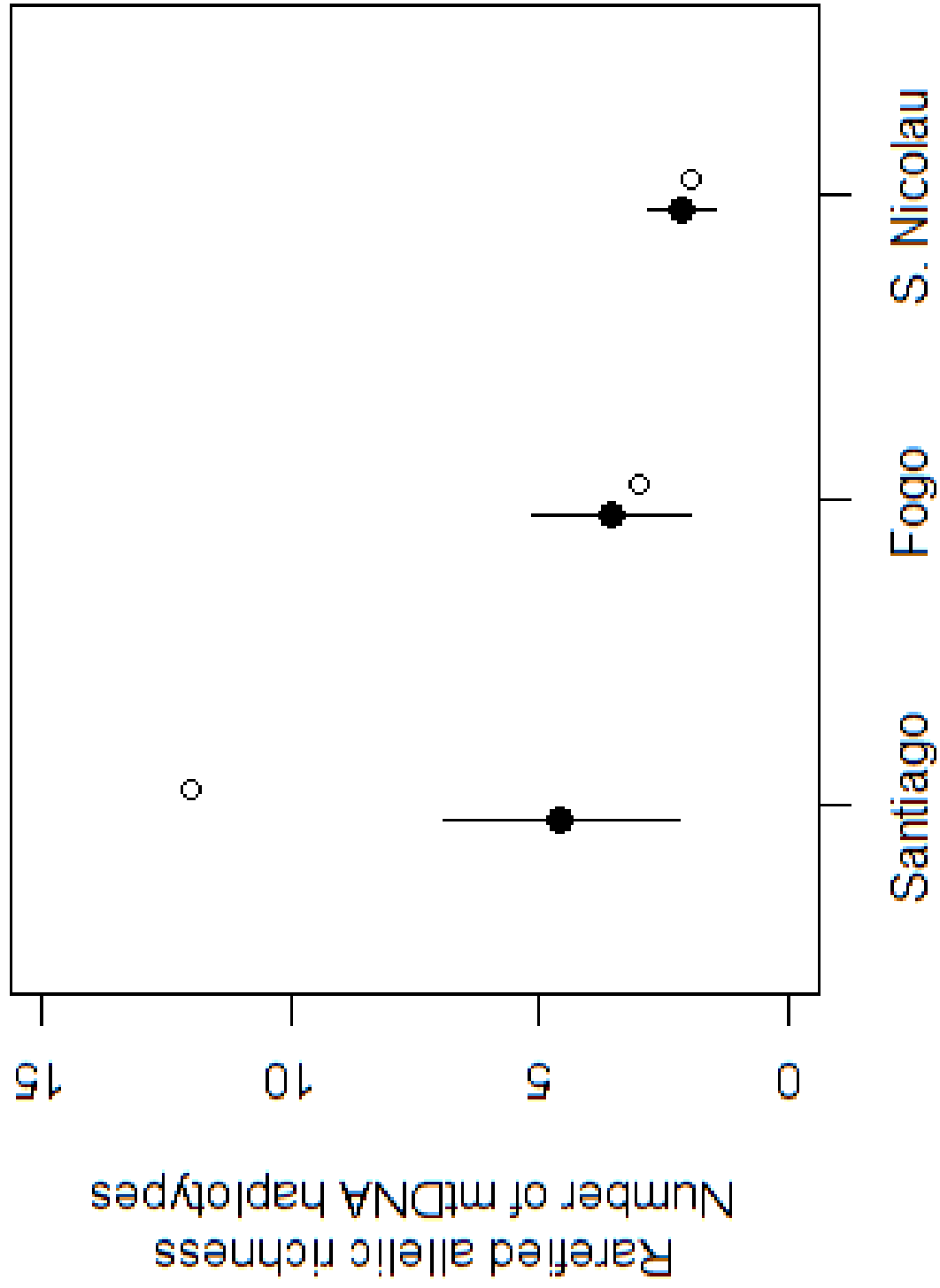


0.01

Greater swamp warbler



Cape Verde warbler





Santiago

Fogo

S. Nicolau



1 **Tables**

2 **Table 1** Genetic diversity and demography in the Cape Verde warbler (CVW) and greater swamp  
 3 warbler (GSW) based on cytochrome b; N = number of individuals sequenced; S =  
 4 segregating sites; h = number of haplotypes; Hd = haplotype diversity; Pi = nucleotide  
 5 diversity; for Tajima's D, all  $P > 0.05$  and for Fu's FS, all  $P > 0.02$  (non-significant)

Population	N	S	h	Hd ± SD	Pi ± SD	Tajima's D	Fu's FS
All CVW	145	17	16	0.887 ± 0.011	0.00182 ± 0.00009	-0.887	-4.335
Santiago	72	13	12	0.841 ± 0.024	0.00134 ± 0.00011	-1.222	-4.356
Fogo	43	4	3	0.642 ± 0.035	0.00172 ± 0.00008	2.601	4.450
S. Nicolau	30	3	2	0.370 ± 0.084	0.00097 ± 0.00022	1.097	3.704
GSW	7	8	5	0.905 ± 0.103	0.00315 ± 0.00063	0.569	-0.333

6  
 7 **Table 2** Estimates of evolutionary divergence among haplotype pairs (pairwise genetic  
 8 distances), between the Cape Verde warbler populations and greater swamp warbler  
 9 (GSW), based on cytochrome b sequences: within (diagonal in underlined italic) and  
 10 among population (below diagonal)

Population	Santiago	Fogo	S. Nicolau	GSW
Santiago	<u>0.001</u>			
Fogo	0.002	<u>0.002</u>		
S. Nicolau	0.002	0.002	<u>0.001</u>	
GSW	0.004	0.005	0.005	<u>0.003</u>

11  
 12  
 13 **Table 3** Estimates of the time to the most recent common ancestor (tMRCA; geometric mean  
 14 shown) for the Seychelles warbler (SW), Cape Verde warbler (CVW) and greater swamp  
 15 warbler (GSW), based on the cytochrome b; HPD = highest posterior density; Mya =  
 16 million years; Kya = thousand years

Split	tMRCA	95% HPD
Seychelles warbler / Cape Verde warbler and greater swamp warbler	3.23 Mya	1.00–9.63 Mya
Cape Verde warbler / greater swamp warbler	292 Kya	67–872 Kya
S. Nicolau / Santiago and Fogo	199 Kya	40–615 Kya
Santiago / Fogo	165 Kya	32–511 Kya

17

18 **Table 4** Genetic diversity for the Cape Verde warbler (CVW) populations, based on 13  
 19 microsatellite loci and using only unrelated birds (N); observed ( $H_o$ ) and expected ( $H_e$ )  
 20 heterozygosity, allelic richness, private alleles and inbreeding coefficient ( $F_{IS}$ )

Population	N	$H_o \pm SD$	$H_e \pm SD$	Allelic richness $\pm SD$	Private alleles $\pm SD$	$F_{IS}$
All CVW	131	0.483 $\pm$ 0.181	0.543 $\pm$ 0.207	5.536 $\pm$ 3327	na	0.1100
Santiago	66	0.567 $\pm$ 0.196	0.565 $\pm$ 0.195	4.598 $\pm$ 2.446	1.290 $\pm$ 1.271	-0.0030
Fogo	38	0.507 $\pm$ 0.241	0.509 $\pm$ 0.228	3.574 $\pm$ 1.651	0.362 $\pm$ 0.596	0.0047
S. Nicolau	27	0.355 $\pm$ 0.120	0.355 $\pm$ 0.126	2.151 $\pm$ 0.685	0.183 $\pm$ 0.376	0.0018

21

22 **Table 5** Pairwise  $F_{ST}$  values for the Cape Verde warbler populations of Santiago, Fogo and S.  
 23 Nicolau showing microsatellite pairwise  $F_{ST}$  above the solid line and Cytochrome b  $F_{ST}$  values  
 24 below; all values are significant at  $P < 0.001$

Population	Santiago	Fogo	S. Nicolau
Santiago		0.062	0.232
Fogo	0.256		0.275
S. Nicolau	0.349	0.450	

25

26 **Table 6** Wilcoxon test for heterozygosity excess  $P$ -values (IAM = infinite allele model; TPM = two  
 27 phase model with either 70% or 90% of stepwise mutations and SMM = stepwise

28 mutation model), mode shift test and Garza-Williamson's M and modified M ratio  
 29 indexes for bottleneck detection in the three Cape Verde warbler populations

Population	IAM	TPM		SMM	Shift mode test	M index	Modified M index
		(70%)	(90%)				
<b>Santiago</b>	0.0006	0.0210	0.0327	0.1465	no	0.307 ±0.117	0.289 ±0.120
<b>Fogo</b>	0.0002	0.0057	0.0061	0.0640	no	0.272 ±0.135	0.221 ±0.098
<b>S. Nicolau</b>	0.0002	0.0005	0.0005	0.0024	yes	0.201 ±0.106	0.151 ±0.101

30

31