Phylogenetic placement and life history trait imputation for Grenada Dove Leptotila wellsi

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Abstract

Phylogenetic analyses can be used to resolve taxonomic uncertainties and reconstruct a species’ evolutionary history. This can be combined with ecological data to predict missing life history traits which are important for creation of conservation management strategies. We investigated the evolutionary and life history of the ‘Critically Endangered’ Grenada Dove Leptotila wellsi by estimating its phylogenetic placement and using this new phylogeny to test the accuracy of phylogenetic comparative methods for estimating both documented and unknown life history traits. We extracted DNA from two Grenada Dove samples and obtained sequences from three mitochondrial markers: Cytochrome oxidase 1 (COI), NADH dehydrogenase 2 (ND2) and Cytochrome b (Cyt b); and one nuclear marker: β-Fibrinogen intron 7 (β-FIB). We present the first genetic data obtained for the Grenada Dove. Our data identify the Grey-Chested Dove Leptotila cassini as the species which shares both a most recent common ancestor, with an estimated divergence of approximately 2.53 million years ago, and the smallest genetic distance (P = 0.0303) with the Grenada Dove. Life history trait values for the Grenada Dove predicted from our analyses using phylogenetic imputation are: clutch size = 2 (± 0.09) eggs; clutches per year = 1.4 (± 0.81); incubation time = 14.2 (± 0.75) days; hatching weight = 3.8 g (± 1.05) and single imputation: fledging age (genus median) = 15.5 days, longevity (genus median) = 8.6 years. This study contributes novel information regarding evolutionary history and life history characteristics to inform long-term conservation actions for a ‘Critically Endangered’ species.

Introduction

Over 12% of extant bird species are threatened with extinction (Brooks et al. 2008), 58.8% of which are island species (Tershy et al. 2015). Natural extinction rates are higher in island populations than in mainland populations. Sensitivity to environmental change, predation by introduced predators, and small population sizes are all common characteristics among island avifaunas; therefore, island endemic species are at an increased risk of extinction (Frankham 1998). Taxonomic patterns in extinction risk suggest that families containing high numbers of endemic species are more susceptible to extinction (Lockwood et al. 2000). Resolution of unknown taxonomic and evolutionary relationships can therefore contribute to a greater understanding of extinction vulnerabilities among bird groups (Lockwood et al. 2000, Johnson et al. 2007).

Advances in systematic biology have allowed phylogenetic information to be combined with phenotypic data to further infer the evolutionary processes, based on the assumption that phenotypic traits within groups of species will be influenced by shared ancestry (Cressler et al. 2015, Bastide et al. 2018). Recently, comparative phylogenetic methods for missing data imputation have been employed to predict physiological adaptations, conservation status, and functional life history traits for data deficient species (Riek and Bruggeman 2013, Swenson 2014, González-del-Pliego et al. 2019, James et al. 2021). Predictive models are particularly useful for estimation of trait values when species are rare and elusive, locations are remote, and fiscal constraints limit field surveys (Wood et al. 2018, Horswill et al. 2021). Life history traits such as generation time, longevity, male and female maturity, clutch size, and incubation and fledging times can be used to evaluate the capability of a species to adapt to climate change and habitat fragmentation as well as being essential to estimate population trends (Mace et al. 2008, Pearson et al. 2014, Storchová and Hofák 2018, Cuervo and Møller 2019, Horswill et al. 2021).

Pigeons and doves (Columbiformes; Columbidae) are one of the oldest lineages of birds, inhabiting a wide range of habitats on six of the seven continents, despite which their evolutionary history is still poorly resolved (Pereira et al. 2007, Soares et al. 2016). Neotropical columbids may form the most ancient lineage (Johnson and Clayton 2000b, Shapiro et al.
but have been little studied, with fundamental taxonomy, conservation status, ecological and life-history data still largely unknown (Brooks et al. 2008, Devenish-Nelson et al. 2019). Therefore, gathering information about Neotropical species is essential and urgent for conservation of this diverse group of birds (Latta 2012).

The Neotropical columbid genus Leptotila comprises 11 morphologically similar species, of which one, the Grenada Dove L. wellsi, is ‘Critically Endangered’ (BirdLife International 2018). Endemic to Grenada in the eastern Caribbean Sea, the Grenada Dove is found in two seemingly isolated populations in the west and south-west of the island (Rusk et al. 2008, Rusk 2017). The most recent population assessment estimated that 160 ± 30 individuals remain of this species and showed a decreasing population trajectory (Rivera-Milán et al. 2015, BirdLife International 2018). Important details about the species’ biology, such as longevity and incubation period, are currently unreported. The Grenada Dove was once considered a subspecies of the Grey-fronted Dove L. rufaxilla until reclassified as a distinct species based on morphological and acoustic evidence (Blockstein and Hardy 1989). However, its genetic distinctiveness and relationships have never been investigated. Anceodatal evidence indicates that the Grenada Dove is closely related to the Caribbean Dove L. jamaicensis due to morphological similarities (Anthony Jeremiah, Head of Forest and National Parks Department, Government of Grenada, pers. comm.).

By investigating both the evolutionary and life history of the Grenada Dove, and by estimating its phylogenetic placement, we were able to predict currently unreported life history traits. We extracted DNA from Grenada Dove samples and obtained sequences from three mitochondrial DNA markers: Cytochrome oxidase I (COI), NADH dehydrogenase 2 (ND2) and Cytochrome b (Cyt b); and one nuclear marker: β-Fibrinogen intron 7 (β-FIB). We used these markers first to infer the evolutionary history of this species among other Neotropical columbid species. We then used phylogenetic comparative methods for missing data imputation, based on this evolutionary history, to predict unknown life history trait values to inform conservation management decisions.

**Methods**

**DNA Extraction and PCR**

Non-invasively collected eggshell and feather samples (n = 2) were collected in 2018 and 2017, respectively. Samples were transported by airmail to the UK in June 2018 where they were cleaned with 70% ethanol and stored at −20°C. DNA barcoding was used to confirm species identification for the samples using methods outlined in Patel et al. (2010). As this is the first time Grenada dove DNA has been obtained, there is no reference available for this species. However, as it is the only member of its genus resident on Grenada, where all of our samples were collected, we conducted a BLAST® search with the resulting data and found that sequences obtained for this study were consistent with previously obtained sequences for Leptotila. We used the optimised method for DNA extraction, using the QIAGEN DNeasy® Blood and Tissue kit and the primerless PCR protocol, outlined in Peters et al. (2019).

The substrate from the primerless PCR process was used to amplify the mitochondrial markers Cyt b (882 bp), COI (613 bp) and ND2 (949 bp) and the nuclear marker β-FIB (902 bp). Primer sequences and the corresponding annealing temperatures are given in Supporting Information 1. PCR cycling parameters for all except the COI region were: initial denaturation at 95°C for 5 min, 45 cycles of 95°C for 30 s, 50–60°C (primer specific, see Appendix S1 in the online supplementary material) for 30 s, 72°C for 60 s and a final extension at 72°C for 5 min; and for the COI region: 95°C for 5 min, 10 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, 35 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 60 s and a final extension at 72°C for 5 min. Where regions could not be amplified using a single primer set, additional primers were designed using Primer3 (Untergasser et al. 2012) allowing a small amplicon strategy to be used where regions were amplified in smaller overlapping sections (as per Peters et al. 2019).

Prior to sequencing, samples were purified as per the manufacturer’s protocol using the QIAquick PCR Purification kit (QIAGEN Inc., Crawley). Preparation and submission of samples were carried out according to the Eurofins Genomics guidelines for the MixS2Seq kit (Eurofins Genomics, Luxembourg) where sequencing was performed using Sanger sequencing methods. As the primerless PCR technique has the potential to increase the chance of sequencing error, amplification and sequencing were performed in triplicate in order to obtain consensus data for each region (Peters et al. 2019, Weber et al. 2000). Sequence reconstruction and alignments were conducted using Sequencer 5.4.6 (Gene Codes Corporation 2017).

**Phylogenetics**

Sequences from 24 Neotropical columbids with Common Crane Grus grus as an outgroup were obtained for all markers from Genbank (Benson et al. 2013; Table 1). Phylogenetic tree construction was performed using MEGAX 10.2.4 (Kumar et al. 2018). We used Maximum Likelihood to fit 24 different nucleotide substitution models and used Akaike information criterion (AIC) and Bayesian information criterion (BIC) to select the best models (Schwarz 1978, Akaike 1987, Nei and Kumar 2000).

The Hasegawa-Kishino-Yano (HKY) model using a discrete Gamma distribution (+G) was the best-supported model for the combined mitochondrial and nuclear DNA dataset of 3,346 bp and thus evolutionary analyses were performed using this model with 500 bootstrap replicates (Hasegawa et al. 1985). In order to check the consistency of this tree we also built the tree using the *BEAST approach implemented in BEAST v2.6.6 (Heled and Drummond 2010, Bouckaert et al. 2019) and the resulting species tree provided the same topology as the concatenated tree (for details see Appendix S2). As mtDNA tends to have a larger proportion of variable bases than nDNA (Allio et al. 2017), we also inferred the position of the Grenada Dove within the Leptotila genus based on the mtDNA and nDNA separately, to provide a comparison between the two marker types, using the following best-supported models as per AIC and BIC: General Time Reversible model (GTR), parameter set [1, 0.849], alleles 1, respectively, for a total of 1,307 nucleotides (Tavaré 1986), and Tamura 3-parameter model, for a total of 902 bp of nDNA (Nei and Kumar 2000). We also inferred the position of the Grenada Dove in relation to L. jamaicensis, using a reduced genetic dataset using the Hasegawa-Kishino-Yano model (+G, parameter set [1, 0.849], alleles 1, respectively, for a total of 2,720 bp of ND2, Cyt b and β-FIB.

Time-calibrated phylogeographic reconstructions were reconstructed to infer molecular-based estimates of divergence by applying the RelTime-ML method following the protocol outlined in Mello (2018). Fossil calibrations are widely used for molecular dating, but no fossil records are available for the taxa used in this study (Ksepka et al. 2015, Peters and McClennen 2015). We therefore used confidence intervals of minimum and maximum boundary estimations from the TimeTree database, which provides
divergence time estimates from published studies integrated with a geological timescale, to calibrate divergence time (Kumar et al. 2017, Mello 2018, Tamura et al. 2012, 2018).

**Life history trait imputation**

To predict life history traits for the Grenada Dove we used multivariate phylogenetic comparative methods for missing data imputation implemented in the RPhylopars package version 0.3.0 (Goolsby et al. 2017) in R version 3.6.2 (R Core Team 2020). This approach uses a multivariate procedure of phylogenetic covariance across species to predict differences in life history traits (Goolsby et al. 2017, James et al. 2020). We imputed phylogenetic covariance across 24 Neotropical columbid species. Twelve life history traits - clutch size, clutches per year, incubation time, hatching weight, fledging age, longevity, generation length, male and female maturity, no-sex body mass (unreported sex; Myhrvold et al. 2015), and male and female body mass - were obtained for each of these species, where available, from primary and grey literature (Appendix S3; Myhrvold et al. 2015, HBW Alive 2020).

Available life history trait values for the Grenada Dove were a clutch size of two eggs (Rusk, unpubl. data), an imputed generation length of 4.2 years calculated using the IUCN generation length calculator (BirdLife International 2018), a fledging age of 14 days from focal nest observations and an estimate of two or three nesting attempts per year (Rusk, unpubl. data). We imputed these traits to determine whether multivariate phylogenetic comparative methods generated similar values. We fitted evolutionary models to the dataset. We focused on the genus Columbina by predicting known values, which we removed individually from the dataset. We contained the most known data, which allowed us to sequentially remove a total of 20% of these known values for each trait with minimal decrease in the accuracy of predictions (Penone et al. 2014). We tested the accuracy of the RPhylopars method by predicting known values, which we removed individually from the dataset. We focused on the genus Columbina as this genus contained the most known data, which allowed us to sequentially remove a total of 20% of these known values for each trait with minimal decrease in the accuracy of predictions (Penone et al. 2014). We compared the predicted values from RPhylopars to the

| Species Complete mtDNA genome β-FIB COI ND2 Cyt b |
|-----------------------------------------------|---------------|---------------|---------------|---------------|
| Colimbina squammata N/A AF182651.1 3 EF373368.1 16 KJ645747.1 25 AF483347.1 21 |
| Columba livia KF936376.1 111 AY082415.1 17 N/A N/A N/A |
| Columba inca N/A AF182650.1 13 DQ433527.1 6 KJ645733.1 25 AF182683.1 1 |
| Columba minuta N/A AF182652.1 4 JQ174506.1 20 KJ645741.1 23 KJ639100.2 26 |
| Columba passerina N/A AF182653.1 13 JN801583.1 20 KJ645740.1 23 KF294046.1 19 |
| Columba picui N/A AF182654.1 13 FJ027422.1 1 KJ645737.1 12 KJ639094.2 26 |
| Columba talpacoti N/A KJ658681.1 25 FJ027429.1 1 KJ645744.1 25 KJ639101.2 25 |
| Grus grus FJ769849.1 12 DQ811960.1 2 N/A N/A N/A |
| Leptotila cassini N/A HQ993561.1 6 JQ175250.1 25 FJ175707.1 12 HQ993505.1 16 |
| Leptotila jamaicensis NA AF279706.1 1 NA HQ993543.1 14 AF279716.1 14 |
| Leptotila megakura N/A AF182664.1 13 FJ027741.1 1 HQ993545.1 14 AF483342.1 21 |
| Leptotila plumbeiceps NA AF279717.1 1 JQ175252.1 25 HQ993544.1 16 AF279707.1 21 |
| Leptotila rufaxilla N/A HQ993560.1 1 JQ175255.1 25 AF251546.1 14 HQ993504.1 16 |
| Leptotila verreauxi HM460214.1 15 HQ993559.1 1 N/A N/A N/A |
| Patagioenas fasciata KX902239.1 22 AF353465.1 1 N/A N/A N/A |
| Patagioenas leucocephala N/A AF182656.1 13 JQ175689.1 25 AY274070.1 12 AF182689.1 14 |
| Patagioenas plumbea N/A AF182658 1 JQ175696.1 25 AF251547 14 AF182691 4 |
| Patagioenas speciosa N/A AF279712 1 JQ175700.1 25 AF353442 1 AF279711 1 |
| Streptopelia decaocto NC_037513.1 18 AF353449.1 1 N/A N/A N/A |
| Streptopelia roseogrisea N/A AF353450 1 JN801382.1 24 AF353419 1 AF353399 5 |
| Zenaida asiatica N/A AF258324.1 13 DQ433527.1 1 AF251543.1 1 AF251533.1 1 |
| Zenaida auriculata HM460211.1 15 AF182667.1 13 N/A N/A N/A |
| Zenaida aurita N/A AF258323.1 1 JN639032.1 14 AF251542 1 AF182704.1 4 |
| Zenaida galapagoensis N/A AF258322.1 13 JQ420133.1 13 AF251539 1 AF251531 1 |
| Zenaida macroura NC_031863.1 22 AY082416.1 17 N/A N/A N/A |

Sources: 1(Blackburn et al. 2003); 2(Ericson et al. 2008); 3(Johnson & Clayton, 1999a); 4(Johnson & Clayton, 2000b); 5(Johnson & Weckstein, 2011); 6(Kerr et al. 2006); 7(Kerr et al. 2007); 8(Krajewski et al., 2010); 9(Krajewski et al., 2010); 10(Li et al., 2013); 11(Miller et al., 2006); 12(Monceau et al., 2012); 13(Monconse et al., 2012); 14(Pacheco et al., 2011); 15(Pereira et al., 2007); 16(Pychliko and More, 2003); 17(Saunders et al., 2014); 18(Schindel et al., 2011); 19(Shapiro et al., 2002); 20(Soares et al., 2008); 21(Sorenson et al., 2003); 22(Stoeckle et al., 2011); 23(Sweet and Johnson, 2015); 24(Tavares et al., 2011)
true value to assess the accuracy of predictions. We also calculated the median values, used in some life-history imputation without explicit phylogenetic information (e.g. Bird et al. 2020), for fledging age and longevity.

**Results**

**Phylogenetics**

There were no polymorphisms observed between the two Grenada Dove sequences. There were 27 nucleotide polymorphisms across 3,346 bp that were unique to the Grenada Dove and not present in any other *Leptotila* species used in this investigation, consisting of 21 transition mutations, two transversion mutations and one transition/transversion mutation (depending on the *Leptotila* species) in the mtDNA and three transitional mutations in the nDNA β-FIB gene. The phylogenetic tree constructed using concatenated sequence data - Cyt b (882 bp), COI (613 bp), ND2 (949 bp), and β-FIB (902 bp) - for Neotropical columbids indicates that the Grenada Dove falls within the monophyletic group formed by *Leptotila* species (Figure 1a). The Grenada Dove shared a most recent common ancestor with its sister group containing *L. cassinii* and *L. plumbeiceps* approximately 2.53 million years ago (mya) (Figure 2a). The smallest genetic distance (*P* = 0.0303) where 100 nucleotides were polymorphic (Table 2) was identified between the Grenada Dove and *L. cassinii*. The phylogenetic tree built with the reduced data and containing *L. jamaicensis* (Figure 3) still shows a sister relationship between the Grenada Dove and *L. cassinii* and *L. plumbeiceps*.

The mitochondrial phylogenetic trees shown in Figures 1b and 2b suggest that the Grenada Dove shared its most recent common ancestor with *L. cassinii* and *L. plumbeiceps* approximately 2.18 mya, which is consistent with the phylogenetic tree constructed with the concatenated data set in Figure 1a. The nuclear phylogenetic trees shown in Figures 1c and 2c suggest that the Grenada Dove shared its most recent common ancestor with *L. megalura* and *L. rufaxilla* approximately 2.75 mya, dissimilar to the most recent common ancestor shown by the phylogenetic trees constructed with the combined (Figure 1a and 2a) and mtDNA (Figure 1b and 2b) datasets. Analysis of the β-FIB gene reveals an indel (Appendix S4) in *L. cassinii* and *L. plumbeiceps* sequences which is not shared by the Grenada Dove or any of the other *Leptotila* species included in this study.

**Life history trait imputation**

Strong phylogenetic signals were revealed for clutch size, no-sex body mass, and male and female body mass, whereas weak phylogenetic signal was detected for clutches per year, incubation time, hatching weight, fledging age, longevity, and generation length (Appendix S5). Model validation (Figure 4) showed lower variation relative to each trait around seven of the twelve life history trait

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**Figure 1.** Phylogenetic analysis for 24 Neotropical columbid species and outgroup using the Maximum Likelihood method. Grenada Dove sequences indicated by the circular symbol; a. all mitochondrial and nuclear sequence data estimated using the Hasegawa-Kishino-Yano model (+G, parameter = 0.2281); b. mtDNA using General Time Reversible model ([+I], 38.01% sites); c. β-fibrinogen using the Tamura 3-parameter model. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site and nodes supported by bootstrap values of >80%.
predictions including clutch size, clutches per year, incubation
time, hatching weight, fledging age, longevity, and generation
length and were therefore considered accurate. However, the
remaining five life history trait predictions including male and
female maturity, no-sex body mass, and male and female body
mass showed a high level of variation from the true value and
therefore were discounted. Despite suggested trait accuracy from
the model validation for fledging age and longevity, we report large
standard error values in addition to weak phylogenetic signal, and
as such, genus medians were considered a more accurate represen-
tation for these traits. Known values for generation length for
Leptotila are themselves imputed using the IUCN generation length
calculator, and thus we discounted this trait from our results.

Overall, six life history trait values were predicted for the Grenada
Dove with confidence (Figure 5) using multivariate and single
imputation procedures: a. clutch size $= 2 (±0.09 \text{ SE})$ eggs,
b. clutches per year $= 1.4 (±0.81 \text{ S.E.})$, c. incubation time$=14.2
(±0.75 \text{ SE})$ days, d. hatching weight $= 3.8 \text{ g} (±1.05 \text{ SE})$ grams,
fledging age $= 15.5 \text{ days (genus median)}$ days, f. longevity $= 8.6 \text{ years (genus median)}$.

Discussion

This study reports the first genetic data obtained for the Grenada
Dove and supports a sister relationship with Grey-chested Dove
L. cassinii along with Grey-headed Dove L. plumbeiceps, whose
ranges span Central America into Colombia (BirdLife International
2021). We provide estimations for six previously unpublished life
history traits for the Grenada Dove some of which, such as clutch
size and clutches per year, have been found to be correlated with
extinction risk (Parlato et al. 2015).

Leptotila cassinii was the species with the smallest genetic dis-
tance from the Grenada Dove ($P = 0.0303$) with 100 bp nucleotide
polymorphisms based on concatenated sequence data and phylo-
genetic reconstruction. The most recent common ancestor was
shared approximately 2.53 mya between L. cassinii, L. plumbeiceps,
and the Grenada Dove. However, when the mtDNA and nDNA are
considered separately our results show disparity. The mtDNA
shows the same relationship displayed by the concatenated data
described above, but nDNA analysis revealed that the most recent
common ancestor was shared between L. rufaxilla, L. megalura,
and the Grenada Dove. The mito-nuclear incongruence may have
arisen for a variety of reasons; a larger amount of mtDNA sequence
data was used providing a stronger phylogenetic signal, the
differing characteristics between these markers such as differential lineage sorting and linkage disequilibrium, all of which can lead to ambiguous patterns of variation and evolutionary inferences (Hurst and Jiggins 2005, Rubinoff and Holland 2005). Overall, we identify *L. cassinii* and *L. plumbeiceps* as the species sharing the most recent common ancestor with the Grenada Dove, however, we recommend caution due to the disparity between the mitochondrial or nuclear phylogeny. Nevertheless, our result was unexpected as *L. cassinii* is predominantly a Central American species and has a larger geographical distance from Grenada than *L. rufaxilla*, of which the Grenada Dove was previously thought to be a subspecies. We reveal a genetic difference \( (P = 0.04) \) and 126 nucleotide polymorphisms between the Grenada Dove and *L. rufaxilla*. Our findings support the work of Blockstein and Hardy (1989) whose morphological analysis (showing differing morphological traits such as cinnamon underwing on primary feathers, a greater extent

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**Figure 3.** Phylogenetic analysis for 25 Neotropical columbid species and outgroup using the Maximum Likelihood method. Grenada Dove sequences indicated by the circular symbol and *Leptotila jamaicensis* are indicated by a square using the Hasegawa-Kishino-Yano model (\( +G \), parameter = 0.2496). Tree drawn to scale, with branch lengths measured in the number of substitutions per site and nodes supported by bootstrap values of >80%.

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of white abdomen and a lesser extent of white on the tail tips) and sonographic data (no response was made by the Grenada Dove to L. rufaxilla playback presentations) led to the reclassification of the Grenada Dove as a distinct taxon. Anecdotal evidence suggested the Grenada Dove is closely related to L. jamaicensis (Anthony Jeremiah, Head of Forest and National Parks Department, Government of Grenada, pers. comm.), however our results show that the Grenada Dove shares common ancestors with four other Leptotila more recently than its shared ancestor with L. jamaicensis and with a greater genetic distance and number of nucleotide polymorphisms in comparison. The evolutionary history of the Leptotila genus was inferred by Johnson and Weckstein (2011) using a molecular phylogeny to investigate the role of the Central American land-bridge in avian dispersal-driven diversification events. Their results show that this genus diverged into two clades at a time that coincides with the closure of the Isthmus of Panama forming the Central American land-bridge. Their study suggests that the northern clade dispersed southwards from North America across the Panama Isthmus (Johnson and Weckstein 2011). Our results indicate that the Grenada Dove is placed within the southern clade and not with the northern clade containing L. jamaicensis. We hypothesise that there was a shared evolutionary history with an upwards dispersal event from South America by the ancestor shared by L. rufaxilla, L. cassinii and the Grenada Dove with a range expansion to Central America and the southern islands of the Lesser Antilles. Dispersal over the barriers presented by the South American Andes and the Caribbean Sea implies reduced gene flow between the ancestral populations and eventual isolation. This is supported by the absence of the indel in the Grenada Dove nDNA, suggesting this mutation occurred after divergence from the shared common ancestor with L. cassinii and L. plumbeiceps. Our data support the hypothesis that allopatric speciation due to isolation of the Grenada Dove and exposure to differing selective pressures resulted in the evolution of Grenada Dove as a distinct species.

Our analyses were able to predict six life history values for the Grenada Dove. Values for clutch size, clutches per year and fledging age agreed with documented values from focal observations (Rusk, unpubl. data). With our model estimations of only two eggs per clutch and 1.4 clutches per year the Grenada Dove has a relatively slow breeding rate, which has been shown to be correlated with extinction risk (Robinson et al. 2010, Hutchings et al. 2013). Given that IUCN generated a 4.2 year generation length and with a low predicted longevity (genus median = 8.6 years), these values suggest this species would be slow to recover after a major population decline (Bird et al. 2020). As not all traits are phylogenetically conserved (Kamilar and Cooper 2013), we tested for phylogenetic signal and found that signal strength varied amongst traits. Clutch size and generation length both exhibited strong phylogenetic signals, as is expected owing to their correlation with body mass, which indicates phylogenetic conservatism of these life history traits (Kamilar and Cooper 2013, Calhoon et al. 2014). All other traits exhibited low phylogenetic signal strength, which is not uncommon in traits such as these that often evolve as a response to differing environmental conditions (Kamilar and Cooper 2013, Martin et al. 2018). Although our model validation has suggested that many of our predicted values are accurate, these results have to

Figure 4. Plot displaying the squared difference between the eb model predicted value and the true value divided by the trait median using the genus Columbina along with Normalized Root Mean Squared Error [NRMSE (Penone et al. 2014, James et al. 2021)]. Raw data are represented by the ° and the first, second and third quartile are represented by the box plot. Mean Squared Error per life history trait.
be used cautiously, and would benefit from validation from field data (Ando 2019, Bolton et al. 2016).

When comparing predicted values for life history traits for the Grenada Dove and *L. cassini* (the species we reveal shares the most recent common ancestor and the smallest genetic distance) and *L. rufaxilla* (the species it was once considered a subspecies of) we find that a clutch size of two and more than one clutch per year were shared between all three species. The Grenada Dove and *L. cassini* both having a hatching weight of over 3 g and an incubation time of around 14 days whereas *L. rufaxilla* has a slightly lower hatching weight of around 2 g and a slightly higher incubation time of 15 days. While there is no great difference in life history traits between studied members of the *Leptotila* genus, as expected, we find that the species with life history traits most similar to the Grenada Dove is *L. cassini* which is the species we also reveal has the smallest genetic distance.
The more favourable conservation status of the other *Leptotila* species examined in this study may be attributed to their much larger ranges and availability of suitable habitat (BirdLife International 2021). We reinforce the need for increasing suitable habitat available to the Grenada Dove to reduce extinction risk, both through habitat restoration and protection, as has been outlined in the recovery and action plan for this species, as well as identification of additional habitat for establishing new populations and enabling population increase (Rusk et al. 2008).

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**Supplementary Materials.** To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0959270922000065.

**References**

Allo, R., Donega, S., Galtier, N. and Naboutz, B. (2017) Large variation in the ratio of mitochondrial to nuclear mutation rates across animals: Implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. *Mol. Biol. Evol.* 34: 2762–2772.

Ando, H. (2019) Genetic and ecological conservation issues for oceanic island birds, revealed by a combination of the latest molecular techniques and conventional field work. *Ecol. Res.* 34: 255–264.

Bastide, P., Solís-Lemus, C., Kriebel, R., Sparks, K. W. and Ané, C. (2018) Phylogenetic comparative methods on phylogenetic networks with reticulation. *Syst. Biol.* 67: 800–820.

Benson, D.A., Cavaunagh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. and Sayers, E. W. (2013) GenBank. *Nucleic Acids. Res.* 41: 36–42.

Bird, J. P., Martin, R., Akçakaya, H. R., Gilroy, J., Burfield, I. J., Garnett, S., Symes, A., Taylor, J., Şekerciąoğlu, Ç. H. and Butchart, S. H. M. (2020) Generation lengths of the world’s birds and their implications for extinction risk. *Conserv. Biol.* 34: 1252–1261.

BirdLife International (2018) *Leptotila wellsi*. The IUCN Red List of Threatened Species. https://www.iucnredlist.org/species/22690874/131031811 (Accessed 19 February 2020).

BirdLife International (2021) Data Zone Species search http://datazone.birdlife.org/species/search (Accessed 6 September 2021).

Blockstein, D. E. and Hardy, J. W. (1989) The Grenada dove (*Leptotila wellsi*) is a distinct species. *Auk* 106: 334–340.

Bolton, N. M., Van Oosterhout, C., Collar, N. J. and Bell, D. J. (2016) Population constraints on the Grenada Dove *Leptotila wellsi*: preliminary findings and proposals from south-west Grenada. *Bird Conserv. internatn.* 26: 205–213.

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavrishuhkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F., Müller, N. F., Ogilvie, H. A., Du Plessis, L., Popinga, A., Rambaut, A., Sivertoni, I., Suchard, M. A., Wu, C. H., Xie, D., Zhang, C., Stadler, T. and Drummond A. J. (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 15: 1–28.

Brooks, T. M., Collar, N. J., Green, R. E., Marsden, S. J. and Pain, D. J. (2008) The science of bird conservation. *Bird Conserv. Internatn.* 18: 2–12.

Calhoon, E. A., Jimenez, A. G., Harper, J. M., Jurkowitz, M. S. and Williams, J. B. (2014) Linkages between mitochondrial lipids and life history in temperate and tropical bird. *Physiol. Biochem. Zoölog.* 87: 265–275.

Cressler, C., Butler, M. and King, A. (2015) Detecting adaptive evolution in phylogenetic comparative analysis using the Ornstein-Uhlenbeck model. *Syst. Biol.* 64: 953–968.

Cuervo, J. J. and Moller, A. P. (2019) Demographic, ecological, and life-history traits associated with bird population response to landscape fragmentation in Europe. *Landsc. Ecol.* 9: 1–13.

Devenish-Nelson, E. S., Weidemann, D., Townsend, J. and Nelson, H. P. (2019) Patterns in island endemic forest-dependent bird research: the Caribbean as a case-study. *Biodivers. Conserv.* 28: 1885–1904.

Dowsett, D. E. and Hardy, J. W. (1989) The Grenada dove (*Leptotila wellsi*). *Handbook of the birds of the world alive | HBW Alive* (2020).}

Hasegawa, M., Kishino, H. and Yano. T. (1985) Dating of the human-ape divergence by a molecular clock method. *J. Mol. Evol.* 22: 160–174.

Hewitt, G. D. D. and Jiggins, F. M. (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts. *Proc. R. Soc. B.* 272: 1525–1534.

Johnson, K. P. and Clayton, D. H. (2000a) A molecular phylogeny of the dove genus *Zenaida* mitochondrial and nuclear DNA sequences. *Condor* 102: 864–870.

Johnson, K. P. and Clayton, D. H. (2000b) Nuclear and mitochondrial genes contain similar phylogenetic signal for pigeons and doves (*Aves: Columbiformes*). *Mol. Phylogenet. Evol.* 14: 141–151.

Kerr, K. C. R., Lijima, D. A., Barreira, A. S., Hebert, P. D. N. and Tabaro, P. L. (2009) Probing evolutionary patterns in neotropical birds through DNA barcodes. *PloS ONE* 4: 4379.

Kerr, K. C. R., Stockdale, M. Y., Dove, C. J., Weigt, L. A., Francis, C. M. and Hebert, P. D. N. (2006) *Zenaida asiatica* voucher FMNH 430791 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial GenBank:
Tamura, K., Battistuzzi, F. U., Billing-Ross, P., Murillo, O., Filipski, A. and Kumar, S. (2012) Estimating divergence times in large molecular phylogenies. *PNAS* **109**: 19333–19338.

Tamura, K., Tao, Q. and Kumar, S. (2018) Theoretical foundation of the reltime method for estimating divergence times from variable evolutionary rates. *Mol. Biol. Evol.* **35**: 1770–1782.

Tavaré, S. (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. *Lect. Math. Life Sci.* **17**: 57–86.

Tavares, E. S., Gonçalves, P., Miyaki, C. Y. and Baker, A. J. (2011) DNA barcode detects high genetic structure within neotropical bird species. *PLoS ONE* **6**: 1–10.

Tershy, B. R., Shen, K. W., Newton, K. M., Holmes, N. D. and Croll, D. A. (2015) The importance of islands for the protection of biological and linguistic diversity. *Bioscience* **65**: 592–597.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M. and Rozen, S. G. (2012) Primer3-new capabilities and interfaces. *Nucleic Acids Res.* **40**: 1–12.

Weber, D., Stewart, B. S., Garza, J. C. and Lehman, N. (2000) An empirical genetic assessment of the severity of the northern elephant seal population bottleneck. *Curr. Biol.* **10**: 1287–1290.

Wood, K. A., Stillman, R. A. and Hilton G. M. (2018) Conservation in a changing world needs predictive models. *Anim. Conserv.* **21**: 87–88.