Online Resource 1:

Species-specific primers were designed based on a unique region of Cytochrome Oxidase I (*COI*) sequence obtained from specimens provided by the Head of the Forestry and National Parks Department, Mr Anthony Jeremiah, and Forest Ranger, Mr Doland Francis (Peters *et al.* 2022). The designed primers: LW4_F: GTTATACCAATCATGATTGGG and LW4_R: GTTAATGGCGGTTGTAATAAAG targeted a 261 bp region of *COI* and were tested against seven species, including 3 closely related species found on Grenada, to ensure amplification of Grenada Dove DNA only (Figure ESM1; Table ESM1). Optimised PCR cycling parameters were as follows: initial denaturation at 95 °C for 5 min, 45 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 60 s and a final extension at 72 °C for 5 min. All non-invasively collected samples were processed using this primer set. Demographic and biological information can be used to assist individual identification (Johnson *et al.* 2007a; Russello *et al.* 2015), therefore temporal and spatial information provided with the samples - site location, GPS information and sample collection dates – were used along with molecular sexing data in an effort to distinguish between different individuals (Online Resource 2).

Using standard sequence identification methods, sequenced samples were queried in NCBI's Basic Local Alignment Search Tool (BLAST®) nucleotide database to confirm species identification (Jarman *et al.* 2004; Ross *et al.* 2008; Ovaskainen *et al.* 2010; Zhuang *et al.* 2012; Price *et al.* 2015). A BLAST® search was also conducted for each sample against previously obtained Grenada Dove samples (Peters *et al.* 2022). The sequences obtained for this study were consistent with previously published sequences for *Leptotila* (*ND2*: >94.56% Per. Ident with 882 total score for *Leptotila plumbeiceps* Accession Number: HQ993544.1; *Cyt b* : >95.56% Per. Ident with 970 total score for *Leptotila cassini* Accession Number: HQ993505.1 (Johnson and Weckstein 2011)). This indicates the DNA fragments amplified in this study represent the intended target species, which is the only *Leptotila* resident on Grenada (Peters *et al.* 2022).

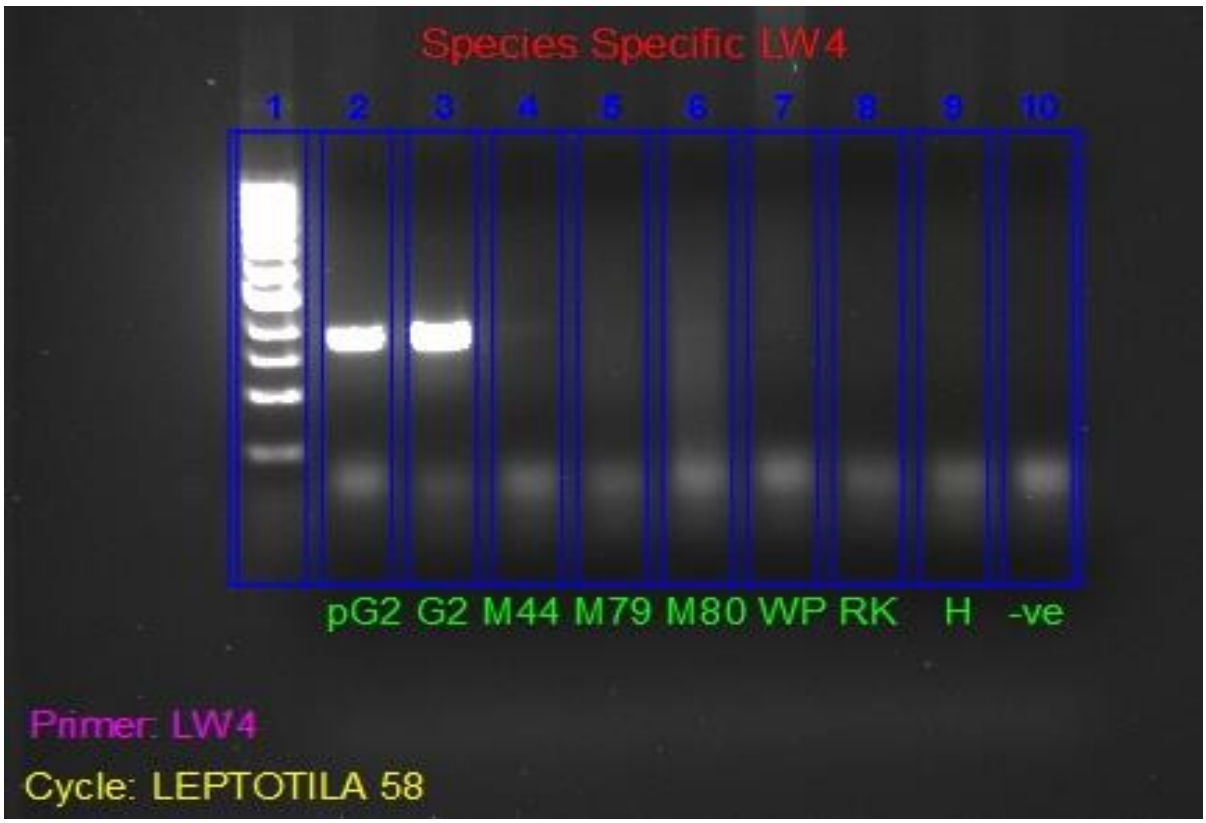

Figure ESM1: Gel (2% w/v agarose) image showing species specific primer optimisation displaying successful amplification of target species only with corresponding sample information shown in table ESM1.

Table ESM1: Species specific primer optimisation sample information for corresponding gel image above.

Lane	Sample	Sample Source	Species
2	pG2	Forestry and National Parks Department of Grenada	*Leptotila wellsi*
3	G2	Forestry and National Parks Department of Grenada	*Leptotila wellsi*
4	M44	Forestry and National Parks Department of Grenada	*Zenaida aurita*[1]*
5	M79	Forestry and National Parks Department of Grenada	*Patagioenas leucocephala*[2]*
6	M80	Forestry and National Parks Department of Grenada	*Petrophassa rufipennis *[3]*
7	WP	University of Chester	*Columba palumbus*
8	RK	University of Chester	*Milvus milvus*
9	H	University of Chester	*Homo sapiens*

*NCBI's Basic Local Alignment Search Tool (BLAST®) used to predict species identification from non-invasively collected samples

[1] *Zenaida aurita* - Genbank: accession number AF182704.1; total score 1681; Per. Ident 98.24%

[2] *Patagioenas leucocephala* - Genbank: accession number JQ175689.1; total score 989; Per. Ident 96.77%

[3] *Petrophassa rufipennis* - Genbank: accession number KU194386.1; total score 1203; Per. Ident 92.19%

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TITLE: Non-invasive sampling reveals low mitochondrial genetic diversity an island endemic species: the Critically Endangered Grenada Dove *Leptotila wellsi*

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