Ardeola 69(2), 2022, XX-XX DOI: 10.13157/arla.69.2.2022.sc1

Short Communications

OBTAINING DNA SAMPLES FROM SENSITIVE AND ENDANGERED BIRD SPECIES: A COMPARISON OF SALIVA AND BLOOD SAMPLES

OBTENCIÓN DE MUESTRAS DE ADN DE ESPECIES DE AVES SENSIBLES Y EN PELIGRO: COMPARACIÓN DE MUESTRAS DE SALIVA Y SANGRE

Christopher Cambrone[1, 2, 3] *, Sébastien Motreuil[2], Francis O. Reyes[3, 4], Miguel A. Landestroy[5], Frank Cézilly[1, 2] and Etienne Bezault[1, 2]

Summary.—Methods used to collect biological samples from birds for genetic analyses should allow high-quality DNA to be obtained in sufficient quantities, while limiting negative effects on sampled individuals. In this context, we assessed the potential use of saliva sampling (using buccal swabs) as an alternative to blood sampling (supposedly more stressful) in a near-threatened Caribbean-endemic, the White-crowned Pigeon *Patagioenas leucocephala*, a bird known to be highly sensitive to capture and handling, based on samples collected from 28 adults captured in the wild. We quantitatively and qualitatively compared DNA extracts, amplifications of two mitochondrial genes (~430 bp and 1040 bp), and molecular sexing between saliva and blood samples. As expected, blood samples provided larger amounts of DNA of heavy molecular weight than buccal swabs. However, buccal swabs were as reliable as blood samples as a source of genetic material to sequence mtDNA. On the other hand, buccal swab samples might require an improved PCR protocol to sex all individuals successfully. We discuss the use of buccal swabs vs. blood sampling as a way to obtain DNA in relation to research objectives and minimising stress and harmful effects.—Cambrone, C., Motreuil, S., Reyes, F.O., Landestroy, M.A.,

[1] UMR BOREA (MNHN, SU, UCN, CNRS-8067, IRD-207), Université des Antilles, Guadeloupe, France.

[2] UMR CNRS 6282 Biogéosciences, Université de Bourgogne-Franche Comté, Dijon, France.

[3] Caribaea Initiative, Département de Biologie, Université des Antilles - Campus de Fouillole, 97157 Pointe-à-Pitre, Guadeloupe, France.

[4] Dirección de Biodiversidad, Ministerio de Medio Ambiente y Recursos Naturales, Santo Domingo, República Dominicana.

[5] Escuela de Biología, Universidad Autónoma de Santo Domingo, Av. Alma Mater, Santo Domingo, República Dominicana.

* Corresponding author: christopher.cambrone@yahoo.com

Cézilly, F. & Bezault, E. (2022). Obtaining DNA samples from sensitive and endangered bird species: a comparison of saliva and blood samples. *Ardeola*, 69: XX-XX.

Key words: buccal swabs, mtDNA, non-destructive sampling, non-passerine, *Patagioenas leucocephala*, White-crowned Pigeon.

RESUMEN.—Los métodos utilizados para recolectar muestras biológicas de aves para análisis genéticos deberían permitir la obtención de ADN de alta calidad en cantidades suficientes, limitando al mismo tiempo los efectos negativos a los individuos muestreados. En este contexto, evaluamos el uso potencial del muestreo de saliva (usando hisopos bucales) como una alternativa al muestreo de sangre (supuestamente más estresante) en la paloma coronita *Patagioenas leucocephala*, una especie endémica del Caribe, casi amenazada, y conocida por ser altamente sensible a la captura y manejo. En el estudio se usaron muestras recolectadas de 28 aves adultas capturadas en la naturaleza. Comparamos cuantitativa y cualitativamente extractos de ADN, la amplificación de dos genes mitocondriales (~430 pb y 1040 pb) y el sexado molecular entre la saliva y la sangre. Como era de esperar, las muestras de sangre produjeron mayores cantidades de ADN y con mayor peso molecular que los hisopos bucales. Sin embargo, los hisopos bucales resultaron ser tan confiables como las muestras de sangre como fuente de material genético para secuenciar el ADNmt. Por otro lado, las muestras de frotis bucales pueden requerir un protocolo de PCR mejorado para sexar a todos los individuos. Discutimos el uso de hisopos bucales frente a la toma de muestras de sangre como una forma de obtener ADN en relación con los objetivos de la investigación y la minimización del estrés y los efectos nocivos.—Cambrone, C., Motreuil, S., Reyes, F.O., Landestroy, M.A., Cézilly, F. y Bezault, E. (2022). Obtención de muestras de ADN de especies de aves sensibles y en peligro: comparación de muestras de saliva y sangre. *Ardeola*, 69: XX-XX.

Palabras clave: ADN mitocondrial, aves no Paseriformes, hisopos bucales, muestreo no destructivo, paloma coronita, *Patagioenas leucocephala*.

INTRODUCTION

Securing reasonably large amounts of high-quality DNA from rare or threatened taxa is of prime importance in avian conservation biology. Indeed, individual DNA samples can be required for various investigations, such as sex determination in sexually monomorphic species (Underwood *et al*., 2002; Patiño *et al*., 2013; Niemc *et al*., 2018), parentage analyses and pedigree reconstruction (Pemberton, 2008; Le Gouar *et al*., 2011; Ferrie *et al*., 2013), assessing levels of inbreeding and gene flow among populations (Cortes-Rodriguez *et al*., 2019; Davidović *et al*., 2020; Cambrone *et al*., 2021; Li *et al*., 2021) or estimating effective population size (Olah *et al*., 2021). To that end, the sampling method should achieve an optimal balance between the necessity of acquiring sufficient amounts of high-quality DNA and the need for minimising invasiveness or harm to the individual, especially in the case of rare species or those particularly sensitive to capture and handling (Wilson & McMahon, 2006; McMahon *et al*., 2012; Zemanova, 2017, 2020).

Blood, usually obtained through puncturing the brachial vein, has been the most common tissue collected from bird species for genetic analyses (Owen, 2011). In addition, the presence of nucleated red blood cells in birds ensures the obtention of sufficient amounts of high-quality DNA. However, some concerns have been raised about the negative consequences of blood sampling on survival, breeding success, dispersal or behaviour (Sheldon *et al*., 2008; Brown & Brown, 2009; Voss *et al*., 2010; but see Angelier *et al*., 2011; Redmond & Murphy,

2011; Smith *et al*., 2017). Although plucked feathers have been used with some success as an alternative source of DNA in avian studies (Taberlet & Bouvet, 1991; Dubiec & Zagalska-Neubauer, 2006; Harvey *et al*., 2006), that method has also been criticised for having a negative impact on some species (McDonald & Griffith, 2011), while providing very limited genetic material.

More recently, buccal cell sampling has been proposed as a less invasive and alternative technique to blood and feather sampling (Handel *et al*., 2006; Vilstrup *et al*., 2018). The method has been used with some success to obtain DNA in a few avian species so far (Yannic *et al*., 2011; Wellbrock *et al*., 2012; Dai *et al*., 2015), although its ability to provide an adequate amount and quality of DNA and a high amplification success rate while maintaining low contamination risk and sequencing errors (Taberlet *et al*., 1999) deserves further consideration. Still, the method seems particularly suited for threatened species or those that are very sensitive to capture and handling (Zemanova, 2020). Also, compared to blood sampling, it is easier to implement (Vilstrup *et al*., 2018).

We assessed the relative performance of blood sampling and buccal swabs as sources of DNA in the Caribbean-endemic and near-threatened White-crowned Pigeon, *Patagioenas leucocephala* (BirdLife International, 2020). Although the species is exposed to strong hunting pressure and habitat destruction, reliable demographic and genetic data are scarce (Wiley, 1979; Strong *et al*., 1991, 1994; Strong & Bancroft, 1994; Rivera-Milán *et al*., 2016), hence the interest in obtaining DNA samples from that species. However, the White-crowned Pigeon is extremely sensitive to capture and handling (Meyer & Wilmers, 2007), sometimes displaying tonic immobility, an innate defensive state characterised by intense immobility, analgesia and lack of responsiveness (Hohtola, 1981; Mills & Faure, 1991; Gallup & Rager, 1996). A supposedly less stressful DNA sampling method, such as buccal cell sampling, could then be an appropriate alternative to blood sampling for that species. We therefore compared DNA extraction efficiency, amplification success and sequencing quality based on two mitochondrial genes of different sizes, D-Loop (~430 bp) and ND2 (~1040 bp), and assessed success in sex identification using each DNA source, as the White-crowned Pigeon is sexually monomorphic.

Methods

Capture and sampling

We captured 28 White-crowned Pigeons on an islet in Oviedo Lagoon, in the Dominican Republic (17.740575, −71.365890) in July 2019 by mounting three 3m-high mist nets of different lengths (3m, 6m and 12m). We continually monitored mist nets in order to minimise capture stress by promptly extracting captured pigeons from the nets. Immediately after, birds were placed into 50 × 50 cm opaque tents for at least ten minutes before ringing them and collecting blood and saliva samples. In order to minimise feather loss due to stress (Møller *et al*., 2006; Awasthy, 2010) and contact with wet hands, experimenters wore laboratory gloves during capture and handling. We collected blood from the brachial vein, as described by Owen (2011) and buccal cells using a swab, from each captured pigeon. Buccal swabs consisted of a foam tip on wood (VWR Int., United States, ref. 82030-594) that was gently rotated inside the mouth for ten seconds, taking care to avoid the tongue. The whole process, from capture to blood and saliva sampling lasted about thirteen minutes per bird. About 50-80µL of blood were collected and stored in absolute ethanol. Buccal swabs were air-dried and dry-stored in sterile collection tubes. Both samples were stored at room

temperature during the field period (ten days) and then at −20°C for up to 18 months until DNA extraction.

DNA extraction and amplification

DNA extraction was performed using the DNeasy Blood & Tissue commercial kit (Qiagen Inc., Valencia, CA, USA). For blood samples, we collected a piece of the blood clot weighing about 20.82 ± 9.15mg (mean ± S.D.) with a sterile spatula, since blood precipitates in contact with absolute ethanol. We amplified and sequenced mitochondrial genes D-loop (DLP, ~430 bp) and NADH dehydrogenase 2 (ND2, ~1040 bp). The same PCR protocol was used for DNA samples from both blood and buccal swabs for each mtDNA gene. To compare sex identification, we relied on universal primers 2550F and 2718R. According to sex identification performed on other columbid species (e.g., Monceau *et al*., 2013; Ayadi *et al*., 2016), two bands are expected for females and a single one for males (females being ZW and males ZZ; see detailed protocol in Supplementary Material, Appendix 1).

Statistical analyses

We relied on a two-tailed paired Wilcoxon signed-rank test to compare DNA concentrations obtained from blood and buccal samples. Using the two mtDNA genes, we compared amplification success, forward and reverse sequence quality, and sequencing success between the two tissue types. Amplification success was assessed as the percentage presence of correct band size for either blood or buccal swab samples based on agarose gel electrophoresis. The assessment of sequence quality relied on the Phred quality scores (Ewing & Green, 1998). Forward and reverse sequences were analysed independently, as sequencing performance may differ between primers. Sequencing success corresponded to the percentage of final sequence produced after the alignment of the forward and reverse sequences, and after manual correction for inconsistent base-calls using a reference sequence. We relied on Fisher Exact tests to assess to what extent the quality of sequenced bases, amplification success and sequencing success were dependent on the type of tissue used (see detailed protocol in Supplementary Material, Appendix 1). All statistical analyses were performed using R software 4.1.0 (R Core Team, 2021), with a significance level set at 0.05.

RESULTS

We successfully extracted DNA from both blood and buccal swab samples from every studied individual (N = 28). However, we extracted more DNA from blood than from buccal cells (Table 1). DNA quality was also higher in blood samples, with DNA fragments superior to 10kb present in all of them (Figure 1). The quality of DNA extracted from buccal swabs was more heterogeneous, with more fragmented DNA molecules. For nine buccal swab samples, nothing was visible on agarose gel due to the low DNA concentration and/or the presence of highly fragmented DNA molecules (7.31 ± 3.75ng/µL; mean ± S.D.). Five samples presented pale smears, suggesting that DNA extracts were very degraded and/or contained a low amount of DNA (12.12 ± 3.21ng/µL; mean ± S.D.). Eight samples had more marked smears with DNA molecules between 1kb to 10kb long, suggesting that DNA molecules were less degraded (13.22 ± 3.85ng/µL; mean ± S.D.). Finally, although also fragmented, six samples presented DNA molecules longer than 10kb (28.71 ± 10.12ng/µL; mean ± S.D.; Figure 1, Supplementary Material, Appendix 2, Figure B1).

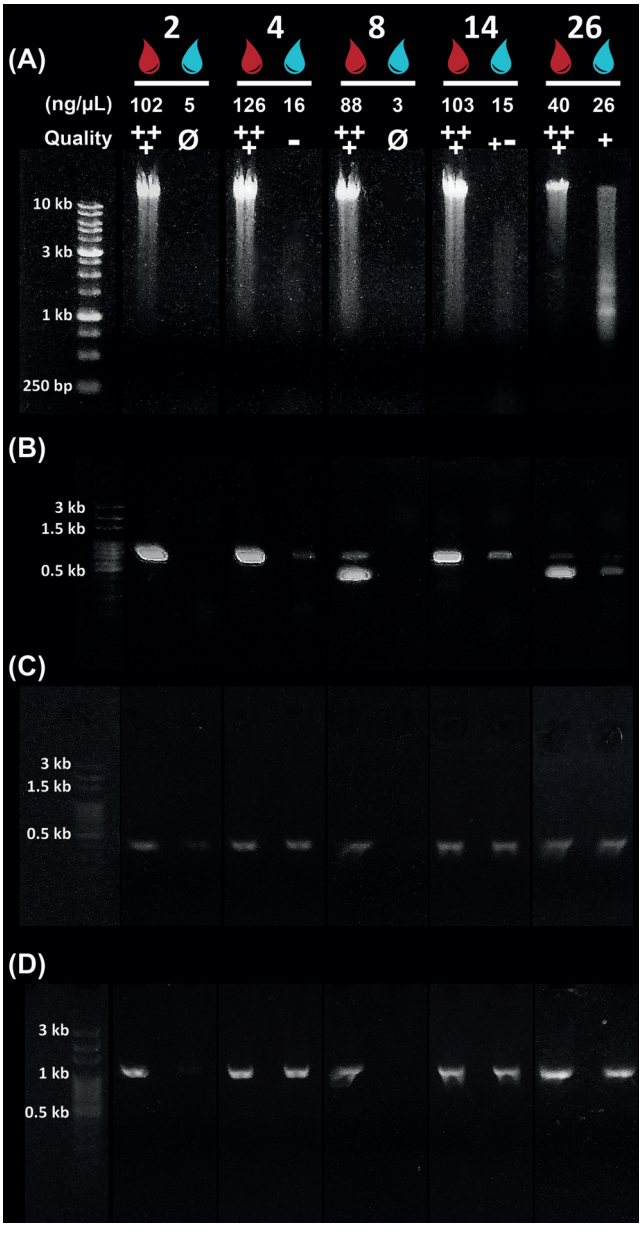
Although amplification failure only occurred with DNA extracted from buccal swabs, irrespective of the mtDNA gene used, amplification success was statistically not dependent on the sample source (Table 1). Moreover, we managed to improve the amplification of samples that failed during the one-shot PCR by conducting a second PCR

TABLE 1

Statistical summary comparing buccal swabs with blood samples. R and F indicate sequences obtained from reverse and forward primers, respectively. Percentages of HQ, MQ and LQ respectively are the mean percentages of bases of high, medium and low quality among each reverse and forward sequence, for each mtDNA gene. DNA concentrations correspond to the mean calculated from either all blood samples or all buccal samples. Dispersions around means were estimated through standard deviation. Outputs of electrophoresis gels used for scoring amplification successes are shown in Supplementary Material, Appendix 2, Figure B2 for D-loop gene (DLP) amplification, B6 for NADH dehydrogenase 2 gene (ND2) amplification and B7 for molecular sexing.
[Resumen estadístico para comparar los hisopos bucales con las muestras de sangre para su uso como muestra genética fiable. En esta tabla, R y F indican las secuencias obtenidas a partir de los cebadores inverso y directo, respectivamente. Los porcentajes de HQ, MQ y LQ, respectivamente, son el porcentaje medio de bases de alta, media y baja calidad entre cada secuencia inversa y directa, para cada uno de los genes de ADNmt. Las concentraciones de ADN corresponden a la media calculada a partir de todas las muestras de sangre o de todas las muestras bucales. La dispersión en torno a las medias se estimó mediante la desviación estándar. Los geles de electroforesis utilizados para puntuar los éxitos de amplificación se encuentran en el Apéndice 2 del Material Suplementario, Figura B2, para la amplificación del gen D-Loop (DLP), B6 para la amplificación del gen de la NADH deshidrogenasa 2 (ND2) y B7 para el sexado molecular, respectivamente.]

Steps	Blood	Buccal Swabs	Statistics
DNA concentration (ng/μL)	91.54 ± 27.49	14.19 ± 9.76	**Paired Wilcoxon test: V = 459, P < 0.001**
Amplification (success/fail)	DLP: 28/0	DLP: 27/1	Fisher exact test: NS McNemar test: $\chi^2 = 0$; NS
	ND2: 28/0	ND2: 25/3	Fisher exact test: NS McNemar test: $\chi^2 = 1.33$; NS
Sequence quality (% bases HQ/MQ/LQ)	DLP (F): 52/13/35	DLP (F): 67/6/27	Fisher exact test: NS
	DLP (R): 41/13/46	DLP (R): 66/5/29	**Fisher exact test: P < 0.01**
	ND2 (F): 72/9/19	ND2 (F): 64/13/23	Fisher exact test: NS
	ND2 (R): 52/16/32	ND2 (R): 61/14/25	Fisher exact test: NS
Sequencing success (success/fail)	DLP: 21/7	DLP: 21/7	Fisher exact test: NS McNemar test: $\chi^2 = 0$; NS
	ND2: 23/5	ND2: 20/8	Fisher exact test: NS McNemar test: $\chi^2 = 0.57$; NS
Molecular sexing (success/fail)	28/0	19/9	Fisher exact test, P < 0.001

FIG. 1.—Example of electrophoresis gel outputs based on five samples for (A) assessing DNA quality, (B) molecular sexing, and amplification of (C) D-loop and (D) ND2 mtDNA genes. Grey beads (red in the colour version of the figure) correspond to blood samples and white beads (blue in the colour version) to buccal swab samples. Entire electrophoresis gels are shown in Supplementary Material, Appendix 2, Figures B1, B2, B6 and B7. Symbols Key. **+++** DNA extracts of good quality, with many long DNA molecules (> 10kb); **+** DNA extracts contain long DNA molecules (> 10kb) but also many degraded molecules; **+–** DNA molecules shorter than 10kb and very degraded; **–** DNA molecules very degraded, shorter or equal to 3kb; **Ø** blank result. In the same line of "(ng/µL)", whole numbers correspond DNA concentration of samples.



[Ejemplo de los resultados de gel de electroforesis basados en cinco muestras para evaluar (A) la calidad del ADN, (B) el sexado molecular y la amplificación de (C) los genes de ADNmt D-loop y (D) ND2. Las cuentas grises (rojo en la versión en color de la figura) corresponden a muestras de sangre y las blancas (azul en la versión en color) a muestras de hisopos bucales. Los geles de electroforesis completos se encuentran en el Material Suplementario, Apéndice 2, Figuras B1, B2, B6 y B7. En cuanto a los símbolos utilizados para la calidad del ADN, (+++) significa que los extractos de ADN eran de buena calidad, con muchas moléculas de ADN largas (> 10kb); (+) significa que los extractos de ADN contienen moléculas de ADN largas (> 10kb), pero también tienen muchas moléculas degradadas; (+–) significa que las moléculas de ADN tienen un tamaño inferior a 10kb y están muy degradadas; (–) significa que las moléculas de ADN están muy degradadas, con tamaños de molécula inferiores o iguales a 3kb; (Ø) significa que no se veía nada en el gel de agarosa. En la misma línea de "(ng/µL)", los números enteros corresponden a la concentración del ADN de las muestras.]

increasing DNA quantity (30ng) and BSA concentration (1.50µg/µL). Regarding sequence quality, the percentages of sequenced bases/nucleotides of high, medium and low quality were dependent on sample type for the reverse sequence of the D-loop mtDNA gene (Table 1), with the percentage of high-quality bases being higher in sequences obtained from buccal swab samples and the percentage of medium and low-quality bases being lower compared to sequences obtained from blood samples. For the other sequences, no difference was found between sample types (Table 1). Sequencing success did not differ between blood and buccal samples, irrespective of the mtDNA gene considered (Table 1). When aligning final sequences produced from blood and buccal swab samples, sequences were identical.

Regarding sex identification, 100% (N = 28) of blood samples allowed sexing of individuals, whereas only 66% (18/28) of buccal samples did so (Fisher's exact test, $P < 0.001$). However, after optimising the PCR protocol for buccal samples, either by increasing DNA quantity (20ng to 60 ± 16ng) or by adding 4% of DMSO in PCR reactions (Supplementary Material, Appendix 2, Figure B2-B4), sexing rate reached 75% (21/28), which was still lower than for blood samples (Fisher exact test, $P < 0.05$). No mismatch was found between the two sample types for sex identification. In total, our samples consisted of 17 females and 11 males, of which 14 females and 7 males could be identified using buccal swabs. Although CHD1-Z (750 bp) was more difficult to amplify from buccal swab samples in females (heterozygous ZW) compared to CHD1-W (450 bp) (see Supplementary Material, Appendix 2, Figures B2-B5 and Tables B1-B2), the observed sex ratio was independent of the type of tissue used for DNA extraction (Fisher exact test, *NS*). However, low-quality DNA samples more frequently failed to sex individuals than higher-quality DNA samples (Fisher exact test, $P < 0.05$). In contrast, the concentration of DNA extract did not differ between buccal samples that failed and those that succeeded to sex individuals (Mann-Whitney-Wilcoxon test, $W = 69$, *N.S.*; (mean ± S.D.) = 13.84 ± 9.65ng/µL vs. 15.26 ± 10.78ng/µL, respectively).

Discussion

As expected, our results confirm that blood is a tissue of prime choice for genetic analyses, as it allows extracting large amounts of DNA of heavy molecular weight. However, although extraction from buccal swabs resulted in smaller amounts and a lower quality of DNA compared to blood, buccal cells appear to be a reliable source of DNA, allowing the sequencing of mtDNA genes up to 1040 bp. Interestingly, they even produced DLP sequences of higher quality than obtained from blood, probably because whole blood contains several inhibitors affecting DNA polymerase activity, as shown with human haemoglobin and immunoglobulin (Abu Al-Soud & Rådström, 2001; Sidstedt *et al.,* 2018), blood proteins found in all vertebrates (Hawkey *et al.,* 1991). Failure to observe any difference with the ND2 gene was probably related to the poorer DNA quality obtained with buccal cells, which may have limited amplifications of the longest sequences, thus hiding the effect of blood inhibitors. Although we did not assess microsatellite markers, other studies have showed that buccal samples can be used to amplify microsatellites (Yannic *et al*., 2011; Dai *et al*., 2015), even from saliva samples left behind by birds in their environment (Monge *et al*., 2020). In addition, the amount of DNA obtained from buccal swabs in this study was greater than what is usually obtained from plucked or shed feathers (e.g., Harvey *et al*., 2006; Peters *et al*., 2019; C. Cambrone, unpublished results), and of the same order of magnitude,

or greater, than usually obtained from buccal cell samples (Yannic *et al.,* 2011; Vilstrup *et al.,* 2018).

When considering molecular sexing, a significant difference was found between buccal and blood samples, with 75% and 100% successful identifications respectively. Furthermore, there was 100% consistency in sex identification from both blood and saliva for a given individual, although the success rate obtained with buccal cells accorded with results obtained by Asawakarn *et al.* (2018; success rate = 74%). Overall, the CHD1-W allele (450 bp) was relatively easier to amplify than the CHD1-Z allele (750 bp). This resulted in the absence of the Z-band on electrophoresis gels for most females when using buccal samples, and a less pronounced Z-band, compared to the W-band, with blood samples. Thus, most females were identified from the sole presence of CHD1-W fragment when using buccal swab samples, rather than their heterozygous ZW genotype, which does not cause any ambiguity since the W-chromosome is the sex determiner in birds (Fridolfsson & Ellegren, 1999). This difference of amplification might be explained by the lower quantity and quality of DNA samples obtained through buccal swabs, inducing stronger competition among annealing sites. The use of alternative primers, such as those developed by Griffiths *et al.* (1998) or Kahn *et al.* (1998) that produce smaller amplicons (Z = 282 bp, W = 287 bp, and Z = 224 bp, W = 252 bp, respectively), could improve the amplification of degraded DNA. Several studies have shown that these primers perform equally well when sexing birds using buccal samples (e.g., Bush *et al.*, 2005; Handel *et al.*, 2006; Wellbrock *et al.*, 2012; Yannic *et al.*, 2016), including *Columba* (Dijkstra *et al.*, 2010) and *Streptopelia* species, although for the latter, a combination of Kahn's forward and Griffiths' reverse primers seems to be more efficient (Secondi *et al.*, 2002; den Hartog *et al.*, 2010).

The harmful effects of blood sampling and feather plucking may vary according to the species and age of individuals. It may also depend, to a certain extent, on climatic conditions, with a higher risk of infection following vein puncturing in tropical or subtropical environments (Brown & Brown, 2006; Sheldon *et al.*, 2008; Voss *et al.*, 2010). On the other hand, recent information suggests that venepuncture in itself does not add stress to capture and handling (Bonnet *et al.*, 2020; Huber *et al.*, 2021). In any case, however, the method inherently increases handling time, potentially resulting in an increased level of stress (Wilson & McMahon, 2006; Duarte, 2013), especially with highly sensitive species such as the White-crowned Pigeon. In that respect, non-destructive buccal cell sampling may be a valuable alternative to blood sampling and feather plucking in the case of the White-crowned Pigeon, particularly when sampling chicks or juveniles. Although not free of discomfort, the use of buccal swabs may reduce accumulated stress, by reducing handling time and the number of steps needed to collect DNA samples. In addition, it also avoids the injury risk inherent to blood sampling.

From a practical point of view, the quantity and quality of DNA contained in buccal swabs may be an important issue in the future in relation to Next-Generation Sequencing (NGS) technologies. Such techniques offer new avenues for wildlife genetic studies (Hudson, 2008; Ekblom & Galindo, 2011; Kumar & Kocour, 2017; Carroll *et al.*, 2018) but require high-quality and quantity DNA (Kumar & Kocour, 2017; Carroll *et al.*, 2018). However, DNA extraction cannot always be performed rapidly nor may samples be frozen immediately upon collection under field conditions, either or both of which could limit subsequent genetic analysis significantly. It has been shown that DNA extracted from amphibian fresh buccal swab samples or that has been promptly frozen at −18°C

contains more DNA than samples stored at room temperature for nine weeks (Pidancier *et al*., 2003). The situation may be even worse when fieldwork lasts over several days in hot and moist field areas as humidity, warmth and sunlight exposure can increase DNA degradation (Baus *et al*., 2019). Given such constraints we opted for an easy and inexpensive sampling method to collect buccal cells that would be accessible to a majority of researchers, including those from developing countries who do not necessarily have access to laboratory grade buffers and/or long-term reliable low temperature storage equipment. In addition, transport of dry samples does not conflict with commercial airflight regulations, unlike samples kept in ethanol, a highly flammable product, which may not be carried either in checked-in or carry-on baggage. However, in the absence of studies comparing the effect of storage conditions of buccal swab samples, whether or not preserved in buffer solutions, we suggest that DNA extractions or sample storage at −18°C, should soon follow collection, whenever possible, in order to preserve DNA integrity and obtain maximum DNA yield. Relying on buffer storage is an efficient way to preserve DNA integrity (Seutin *et al.,* 1990; Kilpatrick, 2002) but may limit DNA yield after extraction, as buccal cells are likely to come loose from swabs and be suspended in the solution. As an alternative to buffer preservation, freezing samples becomes more and more feasible, even during isolated field work, thanks to the availability of portable freezers with rechargeable batteries that allow several hours of autonomy.

Additional information, such as infection with various pathogens known to infect columbid species (Lennon *et al*., 2013; Panella *et al*., 2013; Stockdale *et al*., 2015) could be obtained from saliva samples by using PCR techniques (quantitative, reverse-transcriptase or classical PCR). These include in particular the West Nile Virus (Komar *et al*., 2002), columbid herpesvirus (Phalen *et al*., 2017), avian influenza (Hall *et al*., 2013) or avian trichomoniasis (Lennon *et al*., 2013). The last of these is particularly relevant in the cases of the White-crowned Pigeon and other Caribbean columbids, such as the strongly invasive Eurasian Collared-dove, *Streptopelia decaocto*, a potential host of such pathogens (Lennon *et al*., 2013; Panella *et al*., 2013) suspected of transmitting *Trichomonas* spp. to the native avifauna in Saint Kitts and Nevis islands (Stimmelmayr *et al*., 2012). However, if buccal cells seem to be as effective as blood for classical genetic analyses, their use to investigate questions on avian parasite load, physiology, ecology and biology remains limited compared to blood. Indeed, given appropriate storage conditions, blood samples can provide information on hormonal levels (e.g., stress or reproductive hormones), metabolism, diet and movements (through using stable isotopes) and immunological parameters (Owen, 2011; Albano, 2012). Such data are also relevant for the conservation of species and/or populations but would be more difficult to obtain from saliva samples. For instance, a recent work showed that mesotocin (a hormone analogous to oxytocin in mammals and involved in avian social behaviour) can be quantified from saliva in Common Ravens *Corvus corax*, but with a low success rate (Stocker *et al*., 2021). Further technical improvements in collection and analysis methods may however allow the measurement of some hormones from bird saliva. However, collecting blood samples will remain necessary to investigate the prevalence of blood parasites (e.g. avian malaria), an important issue in ecology, population biology and conservation of bird species (LaPointe *et al*., 2012; Ricklefs *et al*., 2016). Therefore, the decision to rely on blood samples or to switch to buccal swabs as a way to collect biological samples should be made through assessing the balance between the need to minimise handling stress

and the benefits of extracting as much information as possible from samples, taking into account storage conditions and the interval between collection and DNA extraction. One possibility, where a large enough number of individuals are available for sampling in a population, could be to collect saliva from every individual handled systematically but to collect blood from only a randomly chosen subset of all captured individuals.

ACKNOWLEDGMENTS.—We thank the Ministry of Environment of the Dominican Republic (*Ministerio de Medio Ambiente y Recursos Naturales*) for permission to capture White-crowned Pigeons and collect biological samples. The study was funded by Caribaea Initiative. CC was funded by a joint-doctoral grant from the Conseil Régional de Guadeloupe and Caribaea Initiative. We thank two anonymous referees and the editor for helpful comments on a first version of the manuscript.

AUTHOR CONTRIBUTIONS.—CC, FC and EB conceived the project, design and experiments. CC, SM, MAL and FOR collected the samples. CC conducted the molecular analyses, CC, FC. and EB analysed the data, wrote, and edited the article.

REFERENCES

Abu Al-Soud, W. & Rådström, P. (2001). Purification and characterization of PCR-inhibitory components in blood cells. *Journal of Clinical Microbiology*, 39: 485-493.

Albano, N. (2012). Conservation physiology tools: their role in assessing habitat quality in birds. *Ardeola*, 59: 197-216.

Angelier, F., Weimerskirch, H. & Chastel, O. (2011). Capture and blood sampling do not affect foraging behaviour, breeding success and return rate of a large seabird: the black-browed albatross. *Polar Biology*, 34: 353-361.

Asawakarn, S., Teeranuwat, I., Watcharaprapapong, N., Siriwatchaiporn, N., Somsai, P., Kuldee, M., *et al*. (2018). Comparison of dried blood spot, buccal swab, cloacal swab and feces as DNA sources to identify avian sexes by PCR. *Thai Journal of Veterinary Medicine*, 48: 325-330.

Awasthy, M. (2010). 'Fright moult' in kereru (*Hemiphaga novaeseelandiae*) during capture results in loss of tail feathers. *Notornis*, 57: 37-38.

Ayadi, T., Hammouda, A., Kididi, S., Yahyaoui, M.H. & Selmi, S. (2016). Sexual size dimorphism and morphometric sexing in a North African population of Laughing Doves *Spilopelia senegalensis*. *Ostrich*, 87: 173-177.

Baus, I., Miño, C.I. & Monge, O. (2019). Current trends in the application of non-invasive genetic sampling to study Neotropical birds: Uses, goals, and conservation potential. *Avian Biology Research*, 12: 125-132.

BirdLife International (2020). *Patagioenas leucocephala*. *The IUCN Red List of Threatened Species 2020*.

Bonnet, X., Billy, G. & Lakušić, M. (2020). Puncture versus capture: which stresses animals the most? *Journal of Comparative Physiology B*, 190: 341-347.

Brown, M.B. & Brown, C.R. (2009). Blood sampling reduces annual survival in cliff swallows (*Petrochelidon pyrrhonota*). *The Auk*, 126: 853-861.

Bush, K.L., Vinsky, M.D., Aldridge, C.L. & Paszkowski, C.A. (2005). A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater Sage-Grouse (*Centrocercus uropihasianus*). *Conservation Genetics*, 6: 867-870.

Butkauskas, D., Švažas, S., Bea, A., Prakas, P., Olano, I., Grishanov, G., Mischenko, A., Kozulin, A., Stanevičius, V., Báldi, A., Huysentruyt, F., Vaitkuvienė, D. & Red'kin, Y. (2019). Designation of flyways and genetic structure of Woodpigeon *Columba palumbus* in Europe and Morocco. *European Journal of Wildlife Research*, 65: 91.

Butkauskas, D., Švažas, S., Sruoga, A., Bea, A. & Grishanov, G. (2008). Variability of haplotypes among different populations of Woodpigeon (*Columba Palumbus*) in Southern Europe and in the Eastern Baltic Region. *Acta Zoologica Lituanica*, 18: 77-82.

Cambrone, C., Cézilly, F., Wattier, R., Eraud, C. & Bezault, E. (2021). Levels of genetic differentiation and gene flow between four populations of the Scaly-naped Pigeon, *Patagioenas squamosa*: implications for conservation. *Studies on Neotropical Fauna and Environment* (early access).

Carroll, E.L., Bruford, M.W., DeWoody, J.A., Leroy, G., Strand, A., Waits, L. & Wang, J. (2018). Genetic and genomic monitoring with minimally invasive sampling methods. *Evolutionary Applications*, 11: 1094-1119.

Cortes-Rodriguez, N., Campana, M., Berry, L., Faegre, S., Derrickson, S., Ha, R., Dikow, R., Rutz, C. & Fleischer, R. (2019). Population genomics and structure of the critically endangered Mariana Crow (*Corvus kubaryi*). *Genes*, 10: 187.

Dai, Y., Lin, Q., Fang, W., Zhou, X. & Chen, X. (2015). Noninvasive and nondestructive sampling for avian microsatellite genotyping: a case study on the vulnerable Chinese Egret (*Egretta eulophotes*). *Avian Research*, 6: 24.

Davidović, S., Jelić, M., Marinković, S., Mihajlović, M., Tanasić, V., Hribšek, I., Sušić, G., Dragićević, M. & Stamenković-Radak, M. (2020). Genetic diversity of the Griffon Vulture population in Serbia and its importance for conservation efforts in the Balkans. *Scientific Reports*, 10: 20394.

den Hartog, P.M., den Boer-Visser, A.M. & Ten Cate, C. (2010). Unidirectional hybridization and introgression in an avian contact zone: evidence from genetic markers, morphology, and comparisons with laboratory-raised F1 hybrids. *The Auk*, 127: 605-616.

Dijkstra, C., Riedstra, B., Dekker, A., Goerlich, V.C., Daan, S. & Groothuis, T.G.G. (2010). An adaptive annual rhythm in the sex of first pigeon eggs. *Behavioral Ecology and Sociobiology*, 64: 1393-1402.

Duarte, L.M.G. (2013). Impact of capture and handling on wild birds - an introduction and summary. *PhD Thesis, Cardiff University, UK*1-35, 165-173.

Dubiec, A. & Zagalska-Neubauer, M. (2006). Molecular techniques for sex identification in birds. *Biological Letters*, 43: 3-12.

Ekblom, R. & Galindo, J. (2011). Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity*, 107: 1-15.

Ewing, B. & Green, P. (1998). Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Research*, 8: 186-194.

Ferrie, G.M., Cohen, O.R., Schutz, P., Leighty, K.A., Plasse, C., Bettinger, T.L. & Hoffman, E.A. (2013). Identifying parentage using molecular markers: Improving accuracy of studbook records for a captive flock of marabou storks (*Leptoptilus crumeniferus*). *Zoo Biology*, 32: 556-564.

Fridolfsson, A.-K. & Ellegren, H. (1999). A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology*, 30: 116-121.

Gallup, G.G. & Rager, D.R. (1996). Tonic immobility as a model of extreme states of behavioral inhibition. In *Motor activity and movement disorders*, pp. 57-80.

Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J.G. (1998). A DNA test to sex most birds. *Molecular Ecology*, 7: 1071-1075.

Hall, J.S., Krauss, S., Franson, J.C., TeSlaa, J.L., Nashold, S.W., Stallknecht, D.E., Webby, R.J. & Webster, R.G. (2013). Avian influenza in shorebirds: experimental infection of ruddy turnstones (*Arenaria interpres*) with avian influenza virus. *Influenza and Other Respiratory Viruses*, 7: 85-92.

Handel, C.M., Pajot, L.M., Talbot, S.L. & Sage, G.K. (2006). Use of buccal swabs for sampling DNA from nestling and adult birds. *Wildlife Society Bulletin*, 34: 1094-1100.

Harrisson, K.A., Pavlova, A., Telonis-Scott, M. & Sunnucks, P. (2014). Using genomics to characterize evolutionary potential for conservation of wild populations. *Evolutionary Applications*, 7: 1008-1025.

Harvey, M.G., Bonter, D.N., Stenzler, L.M. & Lovette, I.J. (2006). A comparison of plucked feathers versus blood samples as DNA sources for molecular sexing. *Journal of Field Ornithology*, 77: 136-140.

Hawkey, C.M., Bennett, P.M., Gascoyne, S.C., Hart, M.G. & Kirkwood, J.K. (1991). Erythrocyte size, number and haemoglobin content in vertebrates. *British Journal of Haematology*, 77: 392-397.

Hohtola, E. (1981). Tonic immobility and shivering in birds: Evolutionary implications. *Physiology & Behavior*, 27: 475-480.

Holderegger, R., Kamm, U. & Gugerli, F. (2006). Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landscape Ecology*, 21: 797-807.

Huber, N., Mahr, K., Tóth, Z., Szarka, E.Z., Çınar, Y.U., Salmón, P. & Lendvai, Á.Z. (2021). The stressed bird in the hand: Influence of sampling design on the physiological stress response in a free-living songbird. *Physiology & Behavior*, 238: 113488.

Hudson, M.E. (2008). Sequencing breakthroughs for genomic ecology and evolutionary biology. *Molecular Ecology Resources*, 8: 3-17.

Johnson, K.P. & Sorenson, M.D. (1998). Comparing Molecular Evolution in Two Mitochondrial Protein Coding Genes (Cytochromeband ND2) in the Dabbling Ducks (Tribe: *Anatini*). *Molecular Phylogenetics and Evolution*, 10: 82-94.

Kahn, N.W., St. John, J. & Quinn, T.W. (1998). Chromosome-specific intron size differences in the avian CHD gene provide an efficient method for sex identification in birds. *The Auk*, 115: 1074-1078.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28: 1647-1649.

Kilpatrick, C.W. (2002). Noncryogenic preservation of mammalian tissues for DNA extraction: an assessment of storage methods. *Biochemical Genetics*, 40: 53-62.

Komar, N., Lanciotti, R., Bowen, R., Langevin, S. & Bunning, M. (2002). Detection of West Nile Virus in oral and cloacal swabs collected from bird carcasses, 8: 741-742.

Kumar, G. & Kocour, M. (2017). Applications of next-generation sequencing in fisheries research: A review. *Fisheries Research*, 186: 11-22.

LaPointe, D.A., Atkinson, C.T. & Samuel, M.D. (2012). Ecology and conservation biology of avian malaria. *Annals of the New York Academy of Sciences*, 1249: 211-226.

Le Gouar, P., Sulawa, J., Henriquet, S., Tessier, C. & Sarrazin, F. (2011). Low evidence for extra-pair fertilizations in two reintroduced populations of Griffon Vulture (*Gyps fulvus*). *Journal of Ornithology*, 152: 359-364.

Lennon, R.J., Dunn, J.C., Stockdale, J.E., Goodman, S.J., Morris, A.J. & Hamer, K.C. (2013). Trichomonad parasite infection in four species of *Columbidae* in the UK. *Parasitology*, 140: 1368-1376.

Li, S., Li, D., Zhang, L., Shang, W., Qin, B. & Jiang, Y. (2021). High levels of genetic diversity and an absence of genetic structure among breeding populations of the endangered Rufous-backed Bunting in inner Mongolia, China: implications for conservation. *Avian Research*, 12: 7.

Liu, Y., Liu, S., Yeh, C.-F., Zhang, N., Chen, G., Que, P., *et al*. (2018). The first set of universal nuclear protein-coding loci markers for avian phylogenetic and population genetic studies. *Scientific Reports*, 8: 15723.

Mataragka, A., Balaskas, C., Sotirakoglou, K. & Ikonomopoulos, J. (2020). Comparative evaluation of the performance of the PCR assays commonly used for the determination of sex in avian species. *Journal of King Saud University - Science*, 32: 228-234.

McDonald, P.G. & Griffith, S.C. (2011). To pluck or not to pluck: the hidden ethical and scientific costs of relying on feathers as a primary source of DNA. *Journal of Avian Biology*, 42: 197-203.

McKay, J.K. & Latta, R.G. (2002). Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution*, 17: 285-291.

McMahon, C.R., Hindell, M.A. & Harcourt, R.G. (2012). Publish or perish: why it's important to publicise how, and if, research activities affect animals. *Wildlife Research*, 39: 375-377.

Meyer, K. & Wilmers, T. (2007). Foraging habitats, winter residency, survival, and philopatry of adult White-crowned Pigeons in the lower Florida Keys. In *Final report, Florida Fish and Wildlife Conservation Commission*.

Mills, A.D. & Faure, J.-M. (1991). Divergent selection for duration of tonic immobility and

social reinstatement behavior in Japanese quail (*Coturnix coturnix japonica*) chicks. *Journal of Comparative Psychology*, 105: 25-38.

Møller, A.P., Nielsen, J.T. & Erritzøe, J. (2006). Losing the last feather: feather loss as an anti-predator adaptation in birds. *Behavioral Ecology*, 17: 1046-1056.

Monceau, K., Cézilly, F., Moreau, J., Motreuil, S. & Wattier, R. (2013). Colonisation and diversification of the Zenaida Dove (*Zenaida aurita*) in the Antilles: phylogeography, contemporary gene flow and morphological divergence. *PLOS ONE*, 8: e82189.

Monge, O., Dumas, D. & Baus, I. (2020). Environmental DNA from avian residual saliva in fruits and its potential uses in population genetics. *Conservation Genetics Resources*, 12: 131-139.

Niemc, A., Remisiewicz, M., Avni, J. & Underhill, L.G. (2018). Sexual dimorphism in adult little stints (*Calidris minuta*) revealed by DNA sexing and discriminant analysis. *PeerJ*, 6: e5367.

Olah, G., Stojanovic, D., Webb, M.H., Waples, R.S. & Heinsohn, R. (2021). Comparison of three techniques for genetic estimation of effective population size in a critically endangered parrot. *Animal Conservation*, 24: 491-498.

Owen, J.C. (2011). Collecting, processing, and storing avian blood: a review. *Journal of Field Ornithology*, 82: 339-354.

Panella, N.A., Young, G. & Komar, N. (2013). Experimental infection of Eurasian collared Dove (*Streptopelia decaocto*) with West Nile virus. *Journal of Vector Ecology*, 38: 210-214.

Patiño, L., Cruz, M., Martínez, P. & Cedeño-Escobar, V. (2013). Using PCR-RFLP for sexing of the endangered Galápagos Petrel (*Pterodroma phaeopygia*). *Genetics and Molecular Research*, 12: 4760-4767.

Pemberton, J. (2008). Wild pedigrees: the way forward. *Proceedings of the Royal Society B: Biological Sciences*, 275: 613-621.

Peters, C., Nelson, H., Rusk, B. & Muir, A. (2019). A novel method to optimise the utility of underused moulted plumulaceous feather samples for genetic analysis in bird conservation. *Conservation Genetics Resources,* 12: 457-467.

Phalen, D.N., Alvarado, C., Grillo, V., Mason, P., Dobson, E. & Holz, P. (2017). Prevalence of columbid herpesvirus infection in feral pigeons from new South Wales and Victoria, Australia, with spillover into a wild Powerful Owl (*Ninox struena*). *Journal of Wildlife Diseases*, 53: 543-551.

Pidancier, N., Miquel, C. & Maud, C. (2003). Buccal swabs as a non-destructive tissue sampling method for DNA analysis in amphibians. *Herpetological Journal*, 13: 175-178.

R Core Team (2021). R: A Language and Environment for Statistical Computing. Vienna, Austria. https://www.r-project.org/.

Redmond, L.J. & Murphy, M.T. (2011). Multistate mark-recapture analysis reveals no effect of blood sampling on survival and recapture of eastern kingbirds (*Tyrannus tyrannus*). *The Auk*, 128: 514-521.

Ricklefs, R.E., Soares, L., Ellis, V.A. & Latta, S.C. (2016). Haemosporidian parasites and avian host population abundance in the Lesser Antilles. *Journal of Biogeography*, 43: 1277-1286.

Rivera-Milán, F.F., Boomer, G.S. & Martínez, A.J. (2016). Sustainability assessment of plain pigeons and white-crowned pigeons illegally hunted in Puerto Rico. *The Condor*, 118: 300-308.

Secondi, J., Ten Cate, C. & De Bakker, M. (2002). Female responses to male coos in the Collared Dove *Streptopelia decaocto*. *Behaviour*, 139: 1287-1302.

Seki, S.-I., Takano, H., Kawakami, K., Kotaka, N., Endo, A. & Takehara, K. (2007). Distribution and genetic structure of the Japanese Wood Pigeon (*Columba janthina*) endemic to the islands of East Asia. *Conservation Genetics*, 8: 1109-1121.

Seutin, G., White, B.N. & Boag, P.T. (1991). Preaservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, 69: 82-90.

Sheldon, L.D., Chin, E.H., Gill, S.A., Schmaltz, G., Newman, A.E.M. & Soma, K.K. (2008). Effects of blood collection on wild birds: an update. *Journal of Avian Biology*, 39: 369-378.

Sidstedt, M., Hedman, J., Romsos, E.L., Waitara, L., Wadsö, L., Steffen, C.R., *et al*. (2018). Inhibition mechanisms of hemoglobin, immuno-

globulin G, and whole blood in digital and real-time PCR. *Analytical and Bioanalytical Chemistry*, 410: 2569-2583.

Smith, R.J., Benson, T.J. & Carey, M. (2017). Blood sampling does not negatively affect apparent survival, recapture probability, or within-season site fidelity in field sparrows (*Spizella pusilla*). *The Auk*, 134: 240-246.

Stimmelmayr, R., Stefani, L.M., Thrall, M.A., Landers, K., Revan, F., Miller, A., Beckstead, R. & Gerhold, R. (2012). Trichomonosis in free-ranging eurasian collared doves (*Streptopelia decaocto*) and african collared dove hybrids (*Streptopelia risorii*) in the Caribbean and description of ITS-1 region genotypes. *Avian Diseases*, 56: 441-445.

Stockdale, J.E., Dunn, J.C., Goodman, S.J., Morris, A.J., Sheehan, D.K., Grice, P.V. & Hamer, K.C. (2015). The protozoan parasite *Trichomonas gallinae* causes adult and nestling mortality in a declining population of european turtle doves, Streptopelia turtur. *Parasitology*, 142: 490-498.

Stocker, M., Prosl, J., Vanhooland, L.-C., Horn, L., Bugnyar, T., Canoine, V. & Massen, J.J.M. (2021). Measuring salivary mesotocin in birds - Seasonal differences in ravens' peripheral mesotocin levels. *Hormones and Behavior*, 134: 105015.

Strong, A.M. & Bancroft, G.T. (1994). Post-fledging dispersal of white-crowned pigeons: implications for conservation of deciduous seasonal forests in the Florida Keys. *Conservation Biology*, 8: 770-779.

Strong, A.M., Sawicki, R.J. & Bancroft, G.T. (1991). Effects of predator presence on the nesting distribution of white-crowned pigeons in Florida Bay. *Wilson Bull*., 103: 415-425.

Strong, A.M., Sawicki, R.J. & Bancroft, G.T. (1994). Estimating White-Crowned Pigeon population size from flight-line counts. *The Journal of Wildlife Management*, 58: 156.

Taberlet, P. & Bouvet, J. (1991). A single plucked feather as a source of DNA for bird genetic studies. *The Auk*, 108: 959-960.

Taberlet, P., Waits, L.P. & Luikart, G. (1999). Noninvasive genetic sampling: look before you leap. *Trends in Ecology & Evolution*, 14: 323-327.

Underwood, R.M., Crockett, R.J., Roth, R.R., Keeler Jr., C.L. & Parcells, M.S. (2002). A comparison of flow cytometry and the polymerase chain reaction as sexing techniques for the Wood Thrush. *Journal of Field Ornithology*, 73: 239-245.

Vilstrup, J.T., Mullins, T.D., Miller, M.P., McDearman, W., Walters, J.R. & Haig, S.M. (2018). A simplified field protocol for genetic sampling of birds using buccal swabs. *The Wilson Journal of Ornithology*, 130: 326-334.

Voss, M., Shutler, D. & Werner, J. (2010). A hard look at blood sampling of birds. *The Auk*, 127: 704-708.

Wellbrock, A.H.J., Bauch, C., Rozman, J. & Witte, K. (2012). Buccal swabs as a reliable source of DNA for sexing young and adult common swifts (*Apus apus*). *Journal of Ornithology*, 153: 991-994.

Wiley, J.W. (1979). The White-crowned Pigeon in Puerto Rico: status, distribution, and movements. *The Journal of Wildlife Management*, 43: 402-413.

Wilson, R. & McMahon, C. (2006). Measuring devices on wild animals: what constitutes acceptable practices? *Frontiers in Ecology and the Environment*, 4: 147-154.

Yannic, G., Broquet, T., Strøm, H., Aebischer, A., Dufresnes, C., Gavrilo, M.V., *et al*. (2016). Genetic and morphological sex identification methods reveal a male-biased sex ratio in the Ivory Gull *Pagophila eburnea*. *Journal of Ornithology*, 157: 861-873.

Yannic, G., Sermier, R., Aebischer, A., Gavrilo, M.V., Gilg, O., Miljeteig, C., Sabard, B., Strøm, H., Pouivé, E. & Broquet, T. (2011). Description of microsatellite markers and genotyping performances using feathers and buccal swabs for the Ivory Gull (*Pagophila eburnea*). *Molecular Ecology Resources*, 11: 877-889.

Zemanova, M.A. (2017). More training in animal ethics needed for european biologists. *BioScience*, 67: 301-305.

Zemanova, M.A. (2020). Towards more compassionate wildlife research through the 3Rs principles: moving from invasive to non-invasive methods. *Wildlife Biology*, 2020.

Supplementary Electronic Material

Additional supporting information may be found in the online version of this article. See the volumen 69(2) on www.ardeola.org

[Información adicional sobre este artículo en su versión en línea en www.ardeola.org, volumen 69(2).]

Appendix 1: Detailed protocol for DNA extraction and amplification, and statistical analyses. *[Protocolo detallado para la extracción y amplificación del ADN, y de los análisis estadísticos.]*

Figure A1. Additional steps to extract remaining lysis solution from swab tips.
[Pasos adicionales para extraer el remanente de la disolución de los hisopos bucales.]

Appendix 2: Assessment of DNA quality and concentration, and molecular sexing.
[Evaluación de la calidad y concentración del ADN, y del sexado molecular.]

Figures B1-B7. Electrophoresis gel outputs used to visually assess DNA quality, identify sex, and gene amplifications.
[Geles de electroforesis para evaluar visualmente la calidad del ADN, la identificación del sexo y la amplificación de los genes.]

Table B1. Summary of DNA quality score, DNA concentration and molecular sexing for blood samples.
[Resumen de la calidad y concentración del ADN, y del sexado molecular para las muestras de sangre.]

Table B2. Summary of DNA quality score, DNA concentration and molecular sexing for buccal swab samples.
[Resumen de la calidad y concentración del ADN, y del sexado molecular para las muestras de sangre.]

Submitted: October 22, 2021
Major revision: December 14, 2021
Second version arrived: February 22, 2022
Accepted: April 07, 2022

Editor: Alice Cibois