The mitochondrial genome and phylogenetic characteristics of the Thick-billed Green-Pigeon, *Treron curvirostra*: the first sequence for the genus

Nan Xu¹, Jiayu Ding¹, Ziting Que¹, Wei Xu¹, Wentao Ye¹, Hongyi Liu¹

¹ College of Biology and the Environment, Nanjing Forestry University, Nanjing 210037, China

Corresponding author: Hongyi Liu (hongyi_liu@njfu.edu.cn)

Academic editor: G. Sangster  |  Received 30 October 2020  |  Accepted 17 February 2021  |  Published 2 June 2021

http://zoobank.org/E1EEC61A-4A09-41E2-A6A6-2B66F0D0B868

Citation: Xu N, Ding J, Que Z, Xu W, Ye W, Liu H (2021) The mitochondrial genome and phylogenetic characteristics of the Thick-billed Green-Pigeon, *Treron curvirostra*: the first sequence for the genus. ZooKeys 1041: 167–182. https://doi.org/10.3897/zookeys.1041.60150

Abstract

Members of the genus *Treron* (Columbidae) are widely distributed in southern Asia and the Indo-Malayan Region but their relationships are poorly understood. Better knowledge of the systematic status of this genus may help studies of historical biogeography and taxonomy. The complete mitochondrial genome of *T. curvirostra* was characterized, a first for the genus. It is 17,414 base pairs in length, containing two rRNAs, 22 tRNAs, 13 protein coding genes (PCGs), and one D-loop with a primary structure that is similar to that found in most members of Columbidae. Most PCGs start with the common ATG codon but are terminated by different codons. The highest value of the Ka/Ks ratio within 13 PCGs was found in ATP8 with 0.1937, suggesting that PCGs of the mitochondrial genome tend to be conservative in Columbidae. Moreover, the phylogenetic relationships within Columbidae, which was based on sequences of 13 PCGs, showed that (*T. curvirostra* + *Hemiphaga novaeseelandiae*) were clustered in one clade, suggesting a potentially close relationship between *Treron* and *Hemiphaga*. However, the monophyly of the subfamilies of Columbidae recognized by the Interagency Taxonomic Information System could not be corroborated. Hence, the position of the genus *Treron* in the classification of Columbidae may have to be revised.

Keywords

Columbidae, genome sequencing, Ka/Ks ratio, mitochondrial DNA, phylogenetic tree

* Authors contributed equally to this work.

Copyright Nan Xu et al. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Introduction

Mitochondrial DNA sequences can be reliable markers for studying the origin and phylogenetic relationships of species owing to its fast evolution rate, simple structure, light molecular weight, and maternal inheritance (Nabholz et al. 2016; Martins et al. 2019). Mitochondrial genomes of birds have a closed loop structure with lengths of 15,500–23,000 base pairs (bp) (Sammler et al. 2011; Xu et al. 2019; Wang et al. 2020). They typically contain 13 protein coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and one D-loop (Bensch and Härlid 2000; Sun et al. 2020), while some species were found to have duplicate regions (Eberhard and Wright 2016).

The pigeons and doves (family Columbidae) are widely distributed on all continents except Antarctica, ranging from tropical to temperate regions (Gibbs et al. 2001). The number of subfamilies of Columbidae differs among taxonomic authorities. Dickinson and Remsen (2013) recognize three subfamilies (Columbinae, Peristerinae, and Raphinae), whereas the Interagency Taxonomic Information System (ITIS) recognizes five subfamilies (Columbinae, Didunculinae, Gourinae, Otidiphabinae, and Treroninae), as well as 49 genera and more than 300 extant species (Integrated Taxonomic Information System 2020).

All species of green-pigeons (Treron) are listed as second-class national protected animals under China’s Catalog of Wildlife of the Key State Protection. Most species in the genus are declining (Birdlife International 2018); however, only a few genetic resources are available for the genus Treron (e.g., Sorenson et al. 2003; Pereira et al. 2007; Hackett et al. 2008; Price et al. 2014; Claramunt and Cracraft 2015).

The Thick-billed Green-Pigeon Treron curvirostra (Gmelin, 1789) is mainly distributed in virgin, evergreen, broad-leaved, and secondary forests of the tropical and subtropical hilly zone in Southeast Asia and South Asia (Gibbs et al. 2001). Like most species of Columbidae, T. curvirostra feeds on seeds and fruits (Korzun et al. 2008). Members of this species have a medium-sized body and a colorful plumage (Korzun et al. 2008) distinguished by their grey head and green neck. The lower body is yellowish green, while the wing is nearly black, with a yellow feather margin and a distinct yellow wing spot. The central tail feathers are green, while the remaining feathers are gray with black secondary end spots (Korzun et al. 2008; Nair 2010). At present, only few studies have focused on T. curvirostra: Nair (2010) discussed the zoogeography.

To understand the systematic position of the genus Treron among Columbidae, we sequenced and characterized the first complete mitochondrial genome sequence of T. curvirostra. We compared the complete mitochondrial genome of T. curvirostra with that of 33 other pigeons and doves and determined its genetic structural characteristics. In addition, we used 13 protein-coding genes (PCGs) to reconstruct a phylogenetic tree, which we use to infer the taxonomic position of the species and illuminate the phylogenetic relationships among species of Columbidae.
Materials and methods

Sample collection and DNA extraction

This study was authorized by Nanjing Forestry University. The youngest tail feathers of a male Thick-billed Green-Pigeon *T. curvirostra* were collected from an individual rescued from a net that was used to prevent birds from stealing fruit at the Xieyang peak of Dali City, Yunnan Province, China. The bird was identified as *T. curvirostra* based on its morphological characters (Gibbs et al. 2001). After sample collection, the bird was released. The tail feather samples were transported to the Laboratory of Animal Molecular Evolution at the Nanjing Forestry University and stored at -80 °C. The tubules were cut and the pulp was removed for genomic DNA extraction using the FastPure Cell/Tissue Isolation Mini kit (Vazyme Biotechnology Co., Ltd., Nanjing, China) and stored at -20 °C for later use.

PCR amplification and sequencing

Primers were designed based on the mitochondrial gene sequences of *Streptopelia decaocto*, *Hemiphaga novaeseelandiae*, and *Columba hodgsonii* (GenBank accession numbers KY827036, EU725864, and MN919176, respectively) using DNASTAR software (DNASTAR, USA; Burland 2000). Primer sequences are listed in Table 1. The PCR reaction volume was 25 μL, which included 1 μL of template DNA, 12.5 μL of the 2×Rapid Taq Master Mix (Vazyme Biotech Co., Ltd, Nanjing, China), 1 μL per primer, and 9.5 μL double-distilled (dd)H₂O. The PCR reaction procedure consisted of a pre-denaturation at 95 °C for 3 min, a denaturation at 95 °C for 15 s, an annealing at 50 °C to 60 °C for 15 s, which was adjusted according to the primers’ own conditions, an extension at 72 °C for 2 min, cycling 35 times, and a final extension at 72 °C for

| Table 1. Primers used for amplification of the *T. curvirostra* mitogenome. |
|---------------------------------|-----------------|-----------------|-----------------|
| Fragment | Region | Primer pair | Primer sequence (5’-3’) |
| DG 1 | COI-COII | DG 1F | CACTCAGCCATCTTACCT |
| | | DG 1R | ACAGATTCTGACCATGTC |
| DG 2 | COII-ND4 | DG 2F | CCAATCCGCATCAGTC |
| | | DG 2R | GGTTCCTCTACGCTGTA |
| DG 3 | ND4-ND5 | DG 3F | CAGCCTTCTAATGGCA |
| | | DG 3R | GTAGGGCGGAGACCTGAG |
| DG 4 | ND5-Cyt b | DG 4F | ACAGGGCGGAAGAAGC |
| | | DG 4R | TAGAAGATACCTGCTG |
| DG 5 | Cyt b-12S rRNA | DG 5F | GCAGGCTCTACCATACCC |
| | | DG 5R | GTTAAATCTGCTGAGTACC |
| DG 6 | 12S rRNA-16S rRNA | DG 6F | GCTGCCAGTACAGGCC |
| | | DG 6R | TTGGGCTCTGGTACTGTA |
| DG 7 | 16S rRNA-ND2 | DG 7F | CAGTGGGCCGCGACGCTGAG |
| | | DG 7R | AGATGGGAGGAGATGAGGC |
| DG 8 | ND2-COI | DG 8F | GCAGCAGCAATCATG |
| | | DG 8R | ATAGATTTGTCATCTCC |
5 min. The PCR products were detected by a 1% agarose gel electrophoresis, and then sent to Tsingke Biotech Co., Ltd. (Nanjing, China), where the original primers were used for the bidirectional sequencing.

**Sequence analysis**

By comparing and identifying the DNA sequence of each mitochondrial gene in other pigeon families, the range and location of *T. curvirostra'*s mitochondrial genes were annotated. Hence, the complete mitochondrial genome sequence was used to predict the transcriptional direction of each gene component using the Improved de novo Metazoan Mitochondrial Genome Annotation (MITOS) platform (Bernt et al. 2013). The annotated mitochondrial genome sequence of *T. curvirostra* was submitted to GenBank (accession number MT535857). The mitochondrial ring structure was plotted, and 22 tRNA clover two-dimensional structures were predicted using programs, such as the comparative genomics (CG) View Server and the tRNAscan-Se (Stothard and Wishart 2005; Lowe and Chan 2016). Composition skew was calculated according to the following formulae: AT-skew = (A-T)/(A+T) and GC-skew = (G-C)/(G+C) (Perna and Kocher 1995). Moreover, the relative synonymous codon usage (RSCU) frequency and the ratio of the number of nonsynonymous substitutions per nonsynonymous site to the number of synonymous substitutions per synonymous site (Ka/Ks) of 13 PCGs of Columbidae were calculated using MEGA7 (Kumar et al. 2016), while the RSCU comparison graph was drawn by PhyloSuite (Zhang et al. 2020).

**Phylogenetic analysis**

We used a concatenated set of base sequences of the 13 PCGs from 34 pigeons and doves to investigate the phylogenetic position of *T. curvirostra* (Table 2). Yellow-throated Sandgrouse (*Pterocles gutturalis* Smith, 1836) was used as an outgroup. All operations were performed in the PhyloSuite software package (Zhang et al. 2020). The sequences were aligned in batches using MAFFT software (Katoh et al. 2002). ModelFinder was used to partition the codons and identify the best substitution model for the phylogenetic analyses (Kalyaanamoorthy et al. 2017). Phylogenetic trees were constructed with Bayesian inference (BI) and maximum-likelihood (ML) (Yang 1994; Huelsenbeck and Ronquist 2001). The best substitution model of BI was selected according to codon 1, 2 and 3, while the model of ML was determined by the automatic partitioning (Table 3). For the BI tree, Markov chains were run for one million generations and were sampled every 100 generations. The majority-rule consensus trees were estimated by combining the results from duplicated analyses, while discarding the first 25% of generations. Besides, we checked for nuclear copies of mitochondrial sequences (numts) and possible chimerism (Sangster et al. 2016; Sangster and Luksenburg 2020).
Results and discussion

Mitochondrial genome structure and organization

The mitochondrial genome of the Thick-billed Green-Pigeon was found to be 17,414 bp in length, which agrees with the length of most of the other sequenced species of pigeons and doves (Table 4, Table 5, Table 6) (Pereira et al. 2007; Zhang et al. 2015). In addition, the base composition of T. curvirostra was found to be $A = 30.32\%$, $G = 13.61\%$, $T = 24.83\%$, and $C = 31.24\%$, where the $A+T$ content (55.15%) was higher than the $G+C$ content (44.85%) and is similar to other birds in Columbidae (Table 5 and Table 6) (Huang et al. 2016; Jang et al. 2016). Moreover, the genome had a closed circular ring structure, containing 22 tRNAs, 2 rRNAs, 13 PCGs, and one D-loop. The ND6 gene and the other 8 tRNAs (tRNA-Gln, tRNA-Ala, tRNA-
Table 3. The best substitution models for Bayesian inference (BI) and maximum-likelihood (ML) analyses.

| Gene | Strand | Position | Anticodon | Size (bp) | Start codon | Intergenic length |
|------|--------|----------|-----------|-----------|-------------|------------------|
| tRNA-Phe | H | 1–68 | GAA | 68 | ATG | AGA |
| 12S rRNA | H | 69–1041 | | 973 | | 0 |
| tRNA-Val | H | 1042–1114 | UAC | 73 | | 0 |
| 16S rRNA | H | 1115–2703 | | 1589 | | 0 |
| tRNA-Leu | H | 2704–2777 | UAA | 74 | | 12 |
| ND1 | H | 2790–3755 | 966 | ATG | AGA |
| ND2 | H | 3989–5027 | 1039 | ATG | T |
| tRNA-Ile | H | 3773–3844 | GAU | 72 | | 5 |
| tRNA-Gln | L | 3850–3920 | UUG | 71 | | 0 |
| tRNA-Met | H | 3921–3988 | CAU | 68 | | 0 |
| tRNA-Trp | H | 5028–5098 | UCA | 71 | | 0 |
| tRNA-Ala | L | 5100–5168 | UGC | 69 | | 2 |
| tRNA-Asn | L | 5171–5243 | GUU | 73 | | 2 |
| tRNA-Cys | L | 5246–5313 | GCA | 68 | | 0 |
| tRNA-Tyr | L | 5314–5384 | GUA | 71 | | 1 |
| COI | H | 5386–6936 | 1551 | ATG | AGG |
| tRNA-Ser | L | 6937–7001 | UGA | 65 | | 2 |
| tRNA-Asp | H | 7004–7072 | GUC | 69 | | 1 |
| COII | H | 7074–7757 | 684 | ATG | TAA |
| tRNA-Lys | H | 7759–7828 | UUU | 70 | | 1 |
| ATP8 | H | 7830–7997 | 168 | ATG | TAA |
| ATP6 | H | 7988–8671 | 684 | ATG | TAA |
| COIII | H | 8671–9454 | 784 | ATG | T |
| tRNA-Gly | H | 9455–9523 | UCC | 69 | | 0 |
| ND3 | H | 9524–9875 | 352 | ATT | TAA |
| tRNA-Arg | H | 9877–9945 | UCG | 69 | | 1 |
| ND4L | H | 9947–10243 | 297 | ATG | TAA |
| ND4 | H | 10237–11614 | 1378 | ATG | T |
| tRNA-His | H | 11615–11683 | GUG | 69 | | 0 |
| tRNA-Ser | H | 11684–11749 | GCU | 66 | | 0 |
| tRNA-Leu | H | 11750–11819 | UAG | 70 | | 0 |
| ND5 | H | 11820–13637 | 1818 | ATG | AGA |
| Cyt b | H | 13646–14788 | 1143 | ATG | TAA |
| tRNA-Thr | H | 14789–14856 | UGU | 68 | | 6 |
| tRNA-Pro | L | 14863–14932 | UGG | 70 | | 4 |
| ND6 | L | 14937–15458 | 522 | ATG | TAG |
| tRNA-Glu | L | 15462–15532 | UUC | 71 | | 0 |
| D-loop | L | 15553–17414 | 1862 | | | 0 |

Table 4. Mitochondrial genetic composition of *T. curvirostra*.
Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser (UGA), tRNA-Pro, and tRNA-Glu) were transcribed from the light (L)-strand, while the other genes were transcribed from the heavy (H)-strand (Fig. 1, Table 4). In addition, two pairs of overlapping regions among the ATP6/COIII and ND4L/ND4 were found, with an overlapping region of ATP6/COIII being one bp and the overlapping region of ND4L/ND4 being seven bp. Furthermore, 18 intergenic spacers were observed between the mitochondrial regions with lengths between -7 and 17 bp. Among all these intergenic spacers, the shortest was -7 bp (found between ND4L and ND4), while the longest was 17 bp (found between ND1 and tRNA-Ile).

The PCGs

The total length of the PCGs was 11,386 bp, which is consistent with the average length of PCGs found in Columbidae (Table 5). The base composition of PCGs was $A = 29.46\%$, $G = 12.23\%$, $T = 24.56\%$, and $C = 33.76\%$, while the A+T content (54.01%) was slightly higher than the G+C content (45.99%). The AT-skew of $T.\ curvirostra$ was positive, while the GC-skew was negative (Table 5). Furthermore, the PCG regions of $T.\ curvirostra$ contained genes coding for cytochrome b (Cytb), two
ATPases (ATP6 and ATP8), three cytochrome c oxidases (COI, COII, and COIII), and seven NADH dehydrogenases (ND1-6 and ND4L). With the exception of ND3 (which had ATT as its start codon), all the other PCGs had ATG as a start codon. Six PCGs had the complete stop codon of TAA, while four PCGs had the other complete stop codons of AGA (ND1 and ND5), AGG (COI), and TAG (ND6). ND2, ND4, and COIII had the incomplete stop codon of T (Table 4). The RSCU of *T. curvirostra* is illustrated in Fig. 2, where Leu1 had the highest concentration and Cys had the lowest. In addition, Met only had AUG, while the other seven regions had four codons. With *T. curvirostra* as a baseline, the Ka/Ks ratio (Hurst 2002) of the 13 PCGs in 17 species of doves were all less than 1, with the highest Ka/Ks ratio (0.1937) in ATP8 and the lowest ratio (0.0243) in COI (Fig. 3). Hence, it seems that evolution tended to be conservative and maintained the generated protein (Hanada et al. 2007).

**Figure 1.** Circular map of the *T. curvirostra* mitochondrial genome.
Figure 2. Codon distribution and relative synonymous codon usage in *T. curvirostra* mitogenome.

Figure 3. The ratio of the number of nonsynonymous substitutions per nonsynonymous site to the number of synonymous substitutions per synonymous site of 13 PCGs among 17 species of pigeons and doves. *T. curvirostra* was set as a baseline.
Transfer RNAs, ribosomal RNAs, and the D-loop

The mitogenome of *T. curvirostra* contained 22 tRNAs with lengths ranging from 65 bp (tRNA-Ser (UGA)) to 74 bp (tRNA-Leu (UAA)), which is similar to that in the mitogenomes of other pigeons and doves (Zhang et al. 2015). Moreover, the total length of the tRNAs was 1,534 bp, with an A+T content of 57.50%, a G+C content of 42.50%, an AT-skew of 0.1247, and a GC-skew of -0.2025 (Table 5). Among all the secondary structures of the 22 tRNA genes from the *T. curvirostra* mitochondrial genome, with the exception of tRNA-Ser (GCU), all had a typical cloverleaf structure (Fig. 4).

The total size of the two rRNAs was 2,562 bp, with an A+T content of 53.94%, an AT-skew of 0.2142, and a GC-skew of -0.1729 (Table 5). The 12S rRNA was 973 bp in length and was located between tRNA-Phe and tRNA-Val, while the 16S rRNA was 1,589 bp in length and was located between tRNA-Val and tRNA-Leu (UAA).

A D-loop was found between tRNA-Glu and tRNA-Phe, and was 1,862 bp in length, with an A+T content of 61.76%, an AT-skew of -0.0139, and a GC-skew of -0.3764 (Table 5). Duplication and rearrangement of the avian mitochondrial genomes is common, but *T. curvirostra* had only one D-loop, which is similar to that present in other known mitogenomes of Columbidae (Pacheco et al. 2011; Eberhard and Wright 2016; Bruxaux et al. 2018).

Phylogenetic analysis

Although the topology of ML tree and BI tree were similar to each other, they differed with respect to the phylogenetic position of *T. curvirostra*. *Treron curvirostra* clustered with *Hemiphaga novaeseelandiae* (Gmelin, 1789) in the BI tree, whereas it did not cluster with any species in the ML tree (Fig. 5). Therefore, we tested for the presence of the numts and chimerism. All these tests were negative, indicating the validity of *T. curvirostra* mitogenome. The phylogenetic trees also highlighted the stable relationships among the same genera within Columbidae, which was consistent with previous studies from analyses of mitochondrial and nuclear genes (Kan et al. 2010; Pacheco et al. 2011; Hung et al. 2013; Mlíkovský 2016; Soares et al. 2016; Kretschmer et al. 2020; Liu et al. 2020) (Fig. 5). However, the phylogenetic analysis did not support the arrangement of pigeons into five subfamilies (Columbinae, Didunculinae, Gourinae, Otidiphabinae, and Treroninae) as recognized by ITIS. *Caloenas*, *Geopelia*, and *Tragula terrestris* (which were placed in Columbinae by ITIS) clustered with species from other subfamilies in our phylogenies (Fig. 5). The most likely cause might be that the original classification system was based mainly on patterns of overall similarity in morphology which may not accurately reflect phylogenetic relationships. Similar contradictions between overall similarity and phylogeny have also been found in other groups of birds, including terns (Bridge et al. 2005), rails (Sangster et al. 2015), nightjars (Han et al. 2010), eagles (Lerner and Mindell 2005), laughing thrushes (Luo et al. 2008), and chats and flycatchers (Sangster et al. 2010). Our results indicate that
Figure 4. Secondary structure of 22 tRNA genes from the *T. curvirostra* mitochondrial genome.
the subfamily classification of Columbidae may not accurately reflect historical relationships and may need to be revised. However, the poor branch support of basal clades of Columbidae precludes such a revision at present. Clearly, future attempts to resolve the phylogeny of Columbidae with confidence should include a suitable set of nuclear markers.

Acknowledgements

The authors declare no competing interest exists. This study was supported by the National Natural Science Foundation of China (No. 31800453), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and the Innovation and Entrepreneurship Training Program for College Students of China (202010298035Z).
References

Bensch S, Härlid A (2000) Mitochondrial genomic rearrangements in songbirds. Molecular Biology and Evolution 17: 107–113. https://doi.org/10.1093/oxfordjournals.molbev.a026223

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF (2013) MITOS: improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69: 313–319. https://doi.org/10.1016/j.ympev.2012.08.023

BirdLife International (2018) Treron curvirostra. The IUCN Red List of Threatened Species 2018: e.T22691160A130177198. https://doi.org/10.2305/IUCN.UK.2018-2.RLTS.T22691160A130177198.en [accessed 18 January 2021]

Bridge ES, Jones AW, Baker AJ (2005) A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. Molecular Phylogenetics and Evolution 35: 459–469. https://doi.org/10.1016/j.ympev.2004.12.010

Bruxaux J, Gabrielli M, Ashari H, Prŷs-Jones R, Joseph L, Milá B, Besnard G, Thébaud C (2018) Recovering the evolutionary history of crowned pigeons (Columbidae: Goura): Implications for the biogeography and conservation of New Guinean lowland birds. Molecular Phylogenetics and Evolution 120: 248–258. https://doi.org/10.1016/j.ympev.2017.11.022

Burland TG (2000) DNASTAR’s Lasergene sequence analysis software. Methods in Molecular Biology 132: 71–91. https://doi.org/10.1385/1-59259-192-2:71

Claramunt S, Cracraft J (2015) A new time tree reveals Earth history’s imprint on the evolution of modern birds. Science Advances 1: e1501005. https://doi.org/10.1126/sciadv.1501005

Eberhard JR, Wright TF (2016) Rearrangement and evolution of mitochondrial genomes in parrots. Molecular Phylogenetics and Evolution 94: 34–46. https://doi.org/10.1016/j.ympev.2015.08.011

Gibbs D, Barnes E, Cox J (2001) Pigeons and doves: a guide to the pigeons and doves of the world. Pica Press, Robertsbridge.

Hackett SJ, Kimball RT, Reddy S, Bowie RCK, Braun EL, Braun MJ, Chojnowski JL, Cox WA, Han K-L, Harshman J, Huddleston CJ, Marks BD, Miglia KJ, Moore WS, Sheldon FH, Steadman DW, Witt CC, Yuri T (2008). A phylogenomic study of birds reveals their evolutionary history. Science 320: 1763–1768. https://doi.org/10.1126/science.1157704

Han K-L, Robbins MB, Braun MJ (2010) A multi-gene estimate of phylogeny in the nightjars and nighthawks (Caprimulgidae). Molecular Phylogenetics and Evolution 55: 443–453. https://doi.org/10.1016/j.ympev.2010.01.023

Hanada K, Shiu SH, Li WH (2007) The nonsynonymous/synonymous substitution rate ratio versus the radical/conservative replacement rate ratio in the evolution of mammalian genes. Molecular Biology and Evolution 24: 2235–2241. https://doi.org/10.1093/molbev/msm152

Huang ZH, Tu FY, Liu XH (2016) Determination of the complete mitogenome of Spotted Dove, Spilopelia chinensis (Columbiformes: Columbidae). Mitochondrial DNA Part A 27: 4224–4225. https://doi.org/10.3109/19401736.2015.1022750

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754
Hung CM, Lin RC, Chu JH, Yeh CF, Yao CJ, Li SH (2013) The de novo assembly of mitochondrial genomes of the extinct Passenger Pigeon (*Ectopistes migratorius*) with next generation sequencing. PLoS ONE 8: e56301. https://doi.org/10.1371/journal.pone.0056301

Hurst LD (2002) The Ka/Ks ratio: diagnosing the form of sequence evolution. Trends in Genetics 18: 486–487. https://doi.org/10.1016/S0168-9525(02)02722-1

Integrated Taxonomic Information System [ITIS] (2020) Columbidae. Taxonomic Serial No.: 177061. http://www.itis.gov

Jang KH, Ryu SH, Kang SG, Hwang UW (2016) Complete mitochondrial genome of the Japanese Wood Pigeon, *Columba japonica* (Columbiformes, Columbidae). Mitochondrial DNA Part A 27: 2165–2166. https://doi.org/10.3109/19401736.2014.982608

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) Modelfinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Kan XZ, Li XF, Zhang LQ, Chen L, Qian CJ, Zhang XW, Wang L (2010) Characterization of the complete mitochondrial genome of the Rock Pigeon, *Columba livia* (Columbiformes: Columbidae). Genetics and Molecular Research 9: 1234–1249. https://doi.org/10.4238/vol9-2gmrr853

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066. https://doi.org/10.1093/nar/gkf436

Korzun LP, Erard C, Gasc JP, Dzerzhinsky FJ (2008) Bill and hyoid apparatus of pigeons (Columbidae) and sandgrouse (Pteroclididae): a common adaptation to vegetarian feeding? Comptes Rendus Biologies 331: 64–87. https://doi.org/10.1016/j.crvi.2007.10.003

Kretschmer R, Furo IO, Gomes A JB, Kiamim LG, Gunsik RJ, Del Valle Garnero A, Pereira JC, Ferguson-Smith MA, Corrêa de Oliveira EH, Griffin DK, Freitas TRO, O’Connor RE (2020) A comprehensive cytogenetic analysis of several members of the family Columbidae (Aves, Columbiformes). Genes 11(6): e632. https://doi.org/10.3390/genes11060632

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lerner HRL, Mindell DP (2005) Phylogeny of eagles, Old World vultures, and other Accipitridae based on nuclear and mitochondrial DNA. Molecular Phylogenetics and Evolution 37: 327–346. https://doi.org/10.1016/j.ympev.2005.04.010

Liu HY, Sun CH, Zhu Y, Zhang QZ (2020) Complete mitogenomic and phylogenetic characteristics of the Speckled Wood-pigeon (*Columba hodgsonii*). Molecular Biology Reports 47: 3567–3576. https://doi.org/10.1007/s11033-020-05448-w

Lowe TM, Chan PP (2016) tRNAscan–SE on–line: search and contextual analysis of transfer RNA genes. Nucleic Acids Research 44: W54–W57. https://doi.org/10.1093/nar/gkw413

Luo X, Qu YH, Han LX, Li SH, Lei FM (2008) A phylogenetic analysis of laughing thrushes (Timaliidae: Garrulax) and allies based on mitochondrial and nuclear DNA sequences. Zoologica Scripta 38: 9–22. https://doi.org/10.1111/j.1463-6409.2008.00355.x

Martins G, Balbino E, Marques A, Almeida C (2019) Complete mitochondrial genomes of the *Spondias tuberosa* Arr. Cam and *Spondias mombin* L. reveal highly repetitive DNA sequences. Gene 720: 144026. https://doi.org/10.1016/j.gene.2019.144026
Mitochondrial genome of the thick-billed green-pigeon

Mlíkovský J (2016) The type species of the genus Geotrygon Gosse, 1847 (Aves: Columbidae). Zootaxa 4126: 138–140. https://doi.org/10.11646/zootaxa.4126.1.8

Nabholz B, Lanfear R, Fuchs J (2016) Body mass-corrected molecular rate for bird mitochondrial DNA. Molecular Ecology 25: 4438–4449. https://doi.org/10.1111/mec.13780

Nair MV (2010) Thick-billed Green-Pigeon Treron curvirostra in Similipal Hills, Orissa: an addition to the avifauna of peninsular India. Indian Birds 6: 19–20. http://www.indianbirds.in/pdfs/Nair_ThickbilledGreenPigeon.pdf

Pacheco MA, Battistuzzi FU, Lentino M, Aguilar RF, Kumar S, Escalante AA (2011) Evolution of modern birds revealed by mitogenomics: timing the radiation and origin of major orders. Molecular Biology and Evolution 28: 1927–1942. https://doi.org/10.1093/molbev/msr014

Pereira SL, Johnson KP, Clayton DH, Baker AJ (2007) Mitochondrial and nuclear DNA sequences support a Cretaceous origin of Columbiformes and a dispersal-driven radiation in the Paleogene. Systematic Biology 56: 656–672. https://doi.org/10.1080/10635150701549672

Perna NT, Kocher TD (1995) Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. Journal of Molecular Evolution 41: 353–358. https://doi.org/10.1007/BF01215182

Reddy S, Kimball RT, Pandey A, Hosner PA, Braun MJ, Hackett SJ, Han KL, Harshman J, Huddleston CJ, Kingston S, Marks BD, Miglia KJ, Moore WS, Sheldon FH, Witt CC, Yuri T, Braun EL (2017) Why do phylogenomic data sets yield conflicting trees? Data type influences the avian tree of life more than taxon sampling. Systematic Biology 66: 857–879. https://doi.org/10.1093/sysbio/syx041

Sammler S, Bleidorn C, Tiedemann R (2011) Full mitochondrial genome sequences of two endemic Philippine hornbill species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. BMC Genomics 12: e35. https://doi.org/10.1186/1471-2164-12-35

Sangster G, Alström P, Forsmark E, Olsson U (2010) Multi-locus phylogenetic analysis of Old World chats and flycatchers reveals extensive paraphyly at family, subfamily and genus level (Aves: Muscicapidae). Molecular Phylogenetics and Evolution 57: 380–392. https://doi.org/10.1016/j.ympev.2010.07.008

Sangster G, García-R JC, Trewick SA (2015) A new genus for the Lesser Moorhen Gallinula angulata Sundevall, 1850 (Aves, Rallidae). European Journal of Taxonomy 153: 1–8. https://doi.org/10.5852/ejt.2015.153

Sangster G, Luksenburg JA (2020) Chimeric mitochondrial genomes: a hazard for phylogenetics and environmental DNA identification of fishes. Authorea (Online). https://doi.org/10.22541/au.160205226.65255244/v1

Sangster G, Roselaar CS, Irestedt M, Ericson PGP (2016) Sillem’s Mountain Finch Leucosticte sillemi is a valid species of rosefinch (Carpodacus, Fringillidae). Ibis 158: 184–189. https://doi.org/10.1111/ibi.12323

Soares AER, Novak BJ, Haile J, Heupink TH, Fjeldså J, Gilbert MTP, Poinar H, Church GM, Shapiro B (2016) Complete mitochondrial genomes of living and extinct pigeons revise the timing of the columbiform radiation. BMC Evolutionary Biology 16: e230. https://doi.org/10.1186/s12862-016-0800-3

Sorenson MD, Oneal E, García-Moreno J, Mindell DP (2003) More taxa, more characters: the Hoatzin problem is still unresolved. Molecular Biology and Evolution 20: 1484–1498. https://doi.org/10.1093/molbev/msg157
Stothard P, Wishart DS (2005) Circular genome visualization and exploration using CGView. Bioinformatics 21: 537–539. https://doi.org/10.1093/bioinformatics/bti054

Sun CH, Liu HY, Lu CH (2020) Five new mitogenomes of Phylloscopus (Passeriformes, Phylloscopidae): Sequence, structure, and phylogenetic analyses. International Journal of Biological Macromolecules 146: 638–647. https://doi.org/10.1016/j.ijbiomac.2019.12.253

Wang E, Zhang D, Braun MS, Hotz-Wagenblatt A, Pärt T, Arlt D, Schmaljohann H, Bairlein F, Lei F, Wink M (2020) Can mitogenomes of the Northern Wheatear (Oenanthe oenanthe) reconstruct its phylogeography and reveal the origin of migrant birds? Scientific Reports 10: e9290. https://doi.org/10.1038/s41598-020-66287-0

Xu N, Zhang QZ, Chen R, Liu HY (2019) The complete mitogenome of Red-collared Lorikeet (Trichoglossus rubritorquis) and its phylogenetic analysis. Mitochondrial DNA Part B 4: 3116–3117. https://doi.org/10.1080/23802359.2019.1667917

Yang Z (1994) Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. Journal of Molecular Evolution 39: 306–314. https://doi.org/10.1007/BF00160154

Zhang D, Gao FL, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20: 348–355. https://doi.org/10.1111/1755-0998.13096

Zhang RH, Xu MJ, Wang CL, Xu T, Wei D, Liu BJ, Wang GH (2015) The complete mitochondrial genome of the Fancy Pigeon, Columba livia (Columbiformes: Columbidae). Mitochondrial DNA 26: 162–163. https://doi.org/10.3109/19401736.2014.1003851