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The complete mitochondrial genome and phylogenetic position of the critically endangered Trinidad Piping Guan, *Pipile pipile* synonym *Aburria pipile* (Aves: Galliformes).

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The complete mitochondrial genome of the critically endangered Trinidad Piping Guan, *Pipile pipile* (Jacquin 1784) synonym *Aburria pipile* was sequenced for the first time in this study. The genome is 16,665bp in length with overall base compositions of 30.1, 23.7, 32.3 and 13.9% for A, T, C and G respectively. Structurally the *P.pipile* mitogenome is comparable to that of other Galliformes, thereby demonstrating typical avian gene organisation. The mitogenome was subsequently used to produce a revised phylogenetic placement of *P. pipile* within the Galliforme order, positioning the *Pipile* genus basal within the Cracidae family. It is further envisaged that this novel genomic data will contribute to a wider understanding of genetic relationships within the genus *Pipile* and the analysis of the evolutionary relationships of the Galliforme order in a wider avian context.

Keywords: mitochondrial; *Pipile*; *Aburria*; Pawi; *Cracidae*; Galliformes

Mitogenome Announcement

The Trinidad Piping Guan *Pipile pipile* is a critically endangered Cracid (Galliforme) endemic to the island of Trinidad, with an estimated population of less than 200 individuals (Hayes, Sanasie and Samad, 2009a). The population has been in ongoing decline largely due to anthropogenic pressures (Hayes et al., 2009b, Hayes, Sanasie and Samad, 2009a). The IUCN and BirdLife International recognise Trinidad Piping Guan as a species of concern, classified as Critically Endangered, protected under Appendix I of the Convention on the International Trade in Endangered Species (BirdLife International, 2015, CITES, 2015, IUCN, 2015). This mitochondrial genome represents the novel use of genetic data sourced from contemporary Trinidad Piping Guans from the wild on the island of Trinidad.

Samples were sourced from wild Trinidad Piping Guans (*P. pipile*) in the Matura Forest Reserve, Northern Range, Trinidad. *P. pipile* mitochondrial genomes were amplified from the feathers of live birds and liver tissue from a non-viable embryo.

The complete mitochondrial genome of *P. pipile*, sequenced for both Heavy and Light strands, was 16,665bp±2bp in length (Genbank: KU221051, KU221052, KU221053), 13 protein-coding genes, 2 rRNAs and 22 tRNAs were identified demonstrating organisation consistent with Galliforme mito-genomes. Base composition of the three complete genomes demonstrate an A+T bias (53.8%), where overall base composition of the genome were as follows; A (30.1%), T (23.7%), C (32.3%) and G (13.9%).

All compositional features of the genome were consistent between samples, with length variation occurring due to indels in the hypervariable sections of the Control Region. The majority of genes are encoded on the Heavy strand, however the ND6 gene and eight tRNA genes are Light strand encoded, as in other Galliformes. The majority of genes are contiguous within the sequence however gene overlaps occur in four locations; tRNA^{Gln} and tRNA^{Met}, tRNA^{Cys} and tRNA^{Tyr}, ATP8 and ATP6, and ND4L and ND4. Additionally non-coding intergenic spacers of variable length (1-13nts) occur between some genes. The additional non-translated nucleotide at position 174 of the ND3 gene was observed consistent with the observation of this additional nucleotide in other Galliformes (Mindell, Sorenson and Dimcheff, 1998).

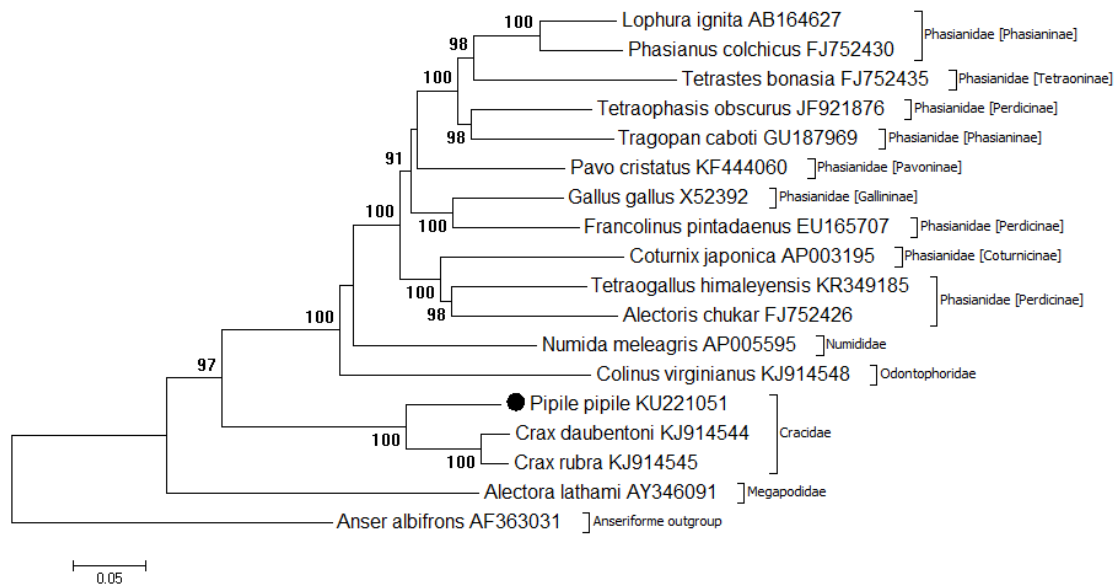


Figure 1. Phylogenetic relationships of *Pipile pipile* within the Galliforme order, inferred from whole mitochondrial genomes. Phylogenetic tree constructed using complete mitogenomes from Genbank in MEGA 7.0 using the Maximum Likelihood method with 1000 bootstrap replicates. Genbank accession numbers given after the species name, bootstrap support given at each node.

Phylogenetic analysis of the mitochondrial genome of *P. pipile* and other Galliforme species is shown in Figure 1, constructed using MEGA7.0 (Kumar, Stecher & Tamura, 2016). The *Pipile* genus resolves basally within the Cracidae order of the Galliforme phylogenetic tree, with a high degree of bootstrap support (100%). Position of the Cracidae family within the Galliforme tree is consistent with initial mito-genome analysis (*Crax* sp.) in previous research (Meiklejohn et al., 2014). Basal divergence of the *Pipile* genus in relation to the *Crax* is consistent with individual mitochondrial gene analysis of Crowe et al. (2006), however full resolution of the Cracidae family is restricted by a lack of complete mitochondrial genome information for Cracid species. The complete mito-genome of the Trinidad Piping Guan, *Pipile pipile*, is used for the first time in phylogenetic analysis, providing essential molecular data and evolutionary information for further analysis of both the *Pipile* genus and the Cracidae family.

Declaration of Interest – The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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