

Eocene Diversification of Crown Group Rails (Aves: Gruiformes: Rallidae)

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Central to our understanding of the timing of bird evolution is debate about an apparent conflict between fossil and molecular data. A deep age for high-level taxa within Neoaves is evident from molecular analyses but much remains to be learned about the age of diversification in modern bird families and their evolutionary ecology. In order to better

entirely unknown but most of the putatively “primitive” species, as well as several distinctive genera, inhabit forests of the Old World tropics [42]. Fewer genera are found in the New World, and most of these have been interpreted as being derived from an Old World stem [42]. Some genera (e.g. *Rallus* and *Fulica*) appear to

Sampling

The data set compiled new assembled mitochondrial genomes of six species within Rallidae and one species within Heliornithidae plus five published rail mitochondrial genomes. To maximize lineage diversity we selected species using available geographic ecological and phylogenetic information. We include three widespread and flying representatives associated with wetland and grassland areas: *Fulica atra* (common coot; KF644582), *Gallinula chloropus* (common moorhen; HQ896036), and *Porphyrio porphyrio* (purple swampen; KF701062). *Coturnicops exquisitus* (Swinhoe's rail; NC012143) found in wetlands and *Rallina eurizonoides* (slaty-legged crane; NC012142) inhabiting forests are both volant species present in Asia. *Gallirallus philippensis* (banded rail; KF701061) is distributed in Asia and Oceania, and *Eulabeornis castaneoventris* (chestnut rail; KF644583) in Oceania and both are flying species that occupy wetlands. *Gallirallus okinawae* (Okinawa rail; NC012140), is found endemic to wet forest on Okinawa island in the Japanese archipelago. *Gallirallus australis* (weka; KF701060) and *Porphyrio hochstetteri* (takahe; EF532934) endemic to New Zealand, and *Lewinia muelleri* (Auckland rail; KF644584) is endemic to the subantarctic Auckland Islands. *Gallirallus australis*, *P. hochstetteri* and *L. muelleri* live in mixed forest and grassland habitats. *Gallirallus okinawae*, *G. australis* and *P. hochstetteri* are absolutely flightless while *L. muelleri* is reported to fly well but infrequently.

mtDNA genomes

Sample tissue details can be found in Table S1. Taking into account the already available mtDNA genomes of rails in GenBank we chose these species because of their geographical range in the Southern Hemisphere (e.g. *E. castaneoventris* and *L. muelleri*) and the relationships among genera. It has been inferred from molecular phylogenetics that Grues (suborder comprising Rallidae, Gruidae, Heliornithidae, Aramididae, Psophiidae and Aptornithidae) has a palaeo-austral signature [46,49] but the fossil record is mainly found in the Northern Hemisphere. Although the intrafamilial relationships in Rallidae are mostly unknown, we sought to include representatives of different and more distant genera in the family following Olson [42]. *Heliornis fulica* (sungrebe) was included as a close outgroup [36,52]. We used a modified phenol-chloroform procedure [53] involving digestion in CTAB buffer for genomic DNA extraction. Genome DNA extractions were verified by gel electrophoresis and quantified using Qubit 2.0. An estimated 2–10 ng of each DNA was subjected to Whole Genome Amplification (WGA) via next generation sequencing (NGS) using the Illumina HiSeq platform (Beijing Genomics Institute, BGI) with 100 bp paired-end reads. Library preparation for sequencing was as described by Shendure and Ji [54] and Mardis [55].

Se uence uality, mapping and assembly

For quality control of the fastq files we used FastQC v0.10.1 (<http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc>) which helps to identify clusters with a low signal and low-quality base calls based on score value chastity ≥ 0.6 . Contigs were created using de novo assembler Velvet v1.1.06 [56] which has been developed for assembly of short read using a Brujin graph algorithm. We conducted assemblies of the paired reads using multiple hash lengths ($k = 43, 53, 63, 73, 83$) and assembled the contigs obtained from the best kmer lengths (generally around 73). All the assemblies were performed on a server with 72 cores and 144 Gb access memory. Sequences were mapped using Geneious v6.0.5 [57] with reference to the previously published mtDNA genomes of Okinawa rail, GenBank accession number NC012140

[58] and common moorhen, GenBank accession number NC015236 [59], and visualized in Tablet v1.11.08.10 [60]. New mtDNA genomes were submitted to GenBank (Table S1).

Phylogenetic analyses

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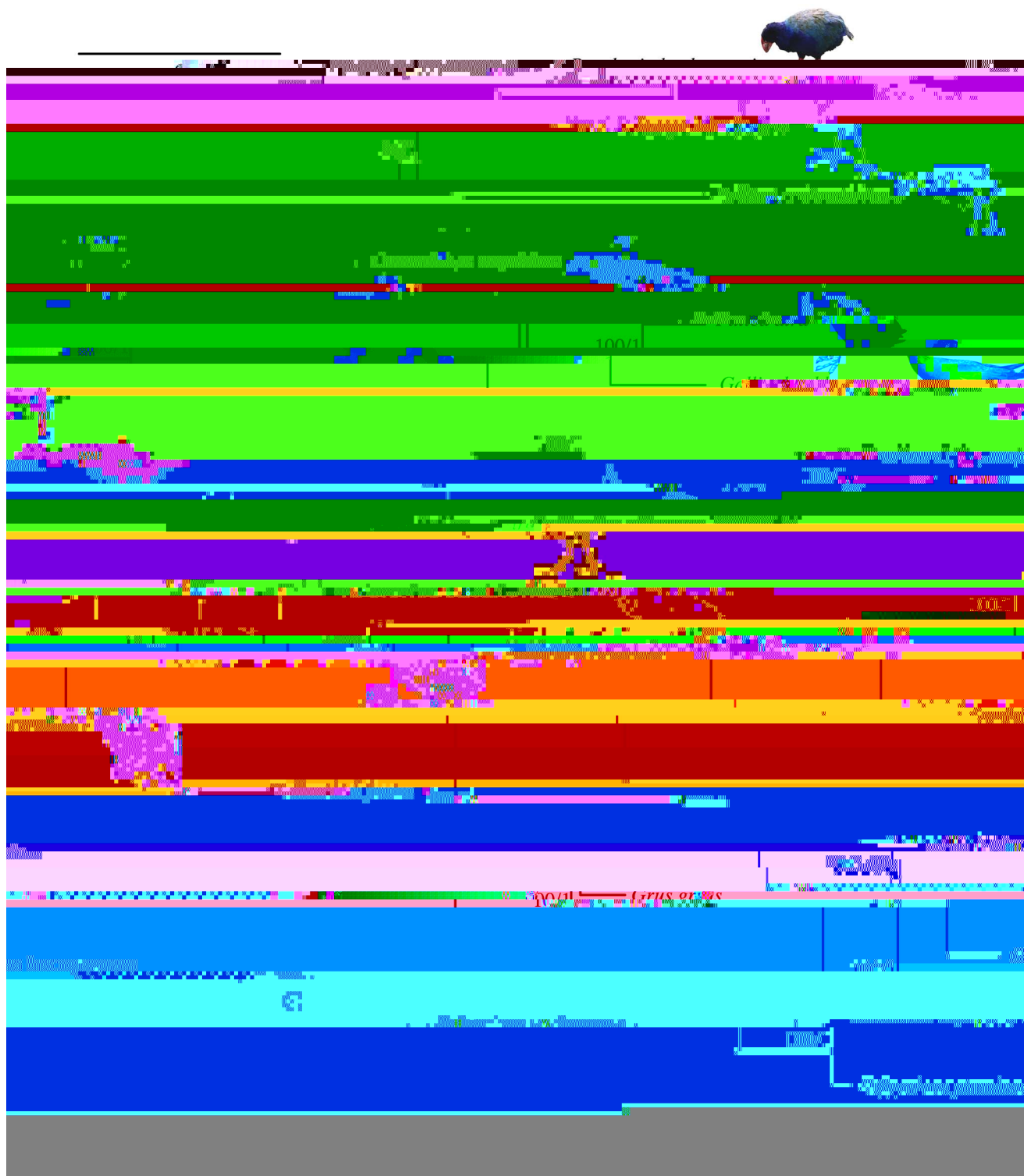


Fig 1. Phylogenetic tree of the Gruidae family. Species outside of the Gruidae are not shown. Bootstrap support over 70% and Bayesian posterior probabilities over 0.9 are indicated in each branch. Letters below the nodes refer to the families within the Order Gruiformes included in this study (A = Rallidae, B = Heliothrididae, C = Gruidae). doi:10.1371/journal.pone.0109635.g002

Fossils of cranes (Gruidae) have been reported from the middle Eocene in Europe [88–90], and the earliest sungrebe fossil record is from the middle Miocene (14 Mya) in North America [91]. Two Paleogene Ralloidea fossils designated as Messelornis and

Walkbeckornis are consistent with our estimated age of Ralloidea around 52 (60–44) Mya [15,17]. The estimated time in our study for the common ancestor of living rallids is about 7 million years older than existing fossils assigned to the crown group. However,

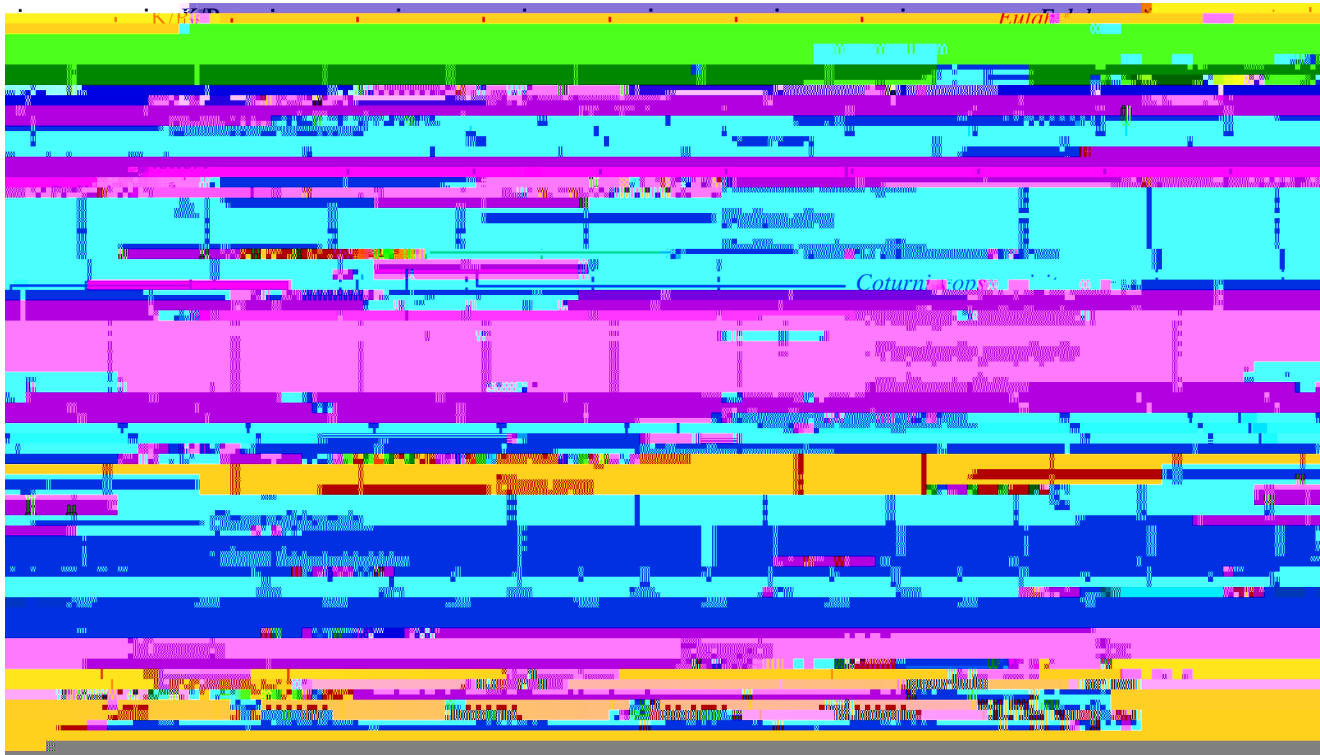


Figure 4. Phylogenetic tree showing relationships between various bird lineages, with color-coded clades and numerous taxonomic labels.

our lower interval value is consistent with European fossils within *Belgirallus* from the late Eocene–early Oligocene that have been suggested as representing the earliest Rallidae [92–94]. Recent examination of the humerus, coracoid and tarsometatarsus led to the proposal that *Belgirallus* belongs to stem group Ralloidea closely related to Palaeoramides from the late Oligocene–early Miocene [52]. Nevertheless, great caution is needed in attribution of stem/crown group fossils when the availability of suitable comparisons is limited, systematics of the group is uncertain, and morphological characters can mislead phylogeny [35,95–97]. The current absence of suitable fossils from the Eocene does not demonstrate that a common ancestor of living Rallidae did not exist at that time, and indeed some have been tentatively attributed to the family. *Palaeorallus*, *Eocrex* or *Fulcaletornis* from the Early Eocene in North America [94,98] or rail-like taxa of the genus *Songzia* from the Early Eocene in China [99,100] might represent extinct crown group rails, but their placement must remain equivocal [15,88] because of the fragmentary nature of the specimens. The fossils at least hint that extinct species that share common ancestry with the living lineages in our analysis might have existed. As with the continuing discovery of Cretaceous bird fossils [12,97,101,102], it is likely that better rail and *Grues* specimens will be forthcoming.

Crown age of bird lineages

Studies of the origin and diversification of crown bird lineages provide insights into the rates and modes of ecological speciation. Comparisons of data from studies of birds makes it very clear that

stem and crown group ages are not correlated, which is expected where speciation and extinction rates are uneven over time. For instance, several studies using complete mtDNA genomes or gene sequences show a relatively recent diversification of passerine and non-passerine bird lineages [32,87,103–105], with most crown lineages appearing during the Neogene (Figure 4), while taxa based on fossils assigned as part of stem groups are much older or younger than molecular date estimations [15,34,88,106–108]. However, assessment of radiations in birds must be characterized by their geographical settings [9] because the spatial context of family level diversification is highly variable. For example, extant honeycreepers (family Fringillidae) in Hawaii [103] and whistlers (family Pachycephalidae) in the Indo-Pacific [105] represent recent insular radiations apparently responding to local ecological opportunities and climatic variations. Within the Palaeognaths, the extinct New Zealand moa radiation is classified in three families [32]. Available evidence indicates Pliocene diversification within Dinornithiformes; even if treated as a single New Zealand family, the moa clade shows a shallow insular radiation (Figure 4).

Insular lineages appear to have relatively shallow crown ages even though some archipelagos are comparatively old [109–111], whereas lineages that achieved wider distributions have deeper ages. For instance, widespread parrots (family Cacatuidae) [104], cranes (family Gruidae) [87] and rails (family Rallidae) have substantially deeper history (Figure 4). This indicates that a larger spatial range might increase the probability of lineage survival. The remarkable capacity of the rails to colonise and adapt to a wide variety of habitats perhaps favoured the retention of lineages through time. Rails show a fantastic capacity and propensity for

range expansion and local adaptation with instances of supertramp species, such as *P. porphyrio* and *G. philippensis* [112,113], which have colonized remote archipelagos in the Pacific [25]. However, the group has mainly retained a sedentary-ground walking ecology. Many lineages within Rallidae are not specialised to narrow marginal habitats but have proved resilient throughout the globe in diverse conditions. It seems likely that the temporal resilience of Rallidae and other cosmopolitan bird lineages has been guided by spatial and ecological plasticity. Further analysis with additional sampling will help reveal to what degree historical

biogeographic signal has been retained in the current lineage distribution.

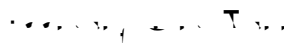


Figure S1 Chronogram showing all species analysed in this study. Divergence times are based on analysis of complete

constraints used to estimate divergence times are shown as red bars where a = calibration fossil of Galloanserae with a minimum age of 66 Mya and maximum age of 86.5 Mya, and b = calibration fossil of Sphenisciformes with an age range from 61.5 Mya to 65.5 Mya.

(TIF)

Table S1 Taxa, Family and Order, museum voucher numbers, type of tissue, specimen sampling locality, GenBank accession numbers, and original source of data of the mtDNA genomes included in this study. N/A = Not Available. Acronyms for museums are: ANWC = Australian National Wildlife Collection, Australia; MZUSP = Museu de Zoologia da Universidade de São Paulo (Brazil).

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