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

Juan C. García-R, Gillian C. Gibb, Steve A. Trewick
Phoenix Lab, Ecology Group, Institute of Agriculture and Environment, Massey University, Palmerston North, New Zealand

Abstract
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 higher 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 understand the timing and pattern of diversification within the family Rallidae we used a relaxed molecular clock, fossil calibrations, and complete mitochondrial genomes from a range of rallid species analysed in a Bayesian framework. The estimated time of origin of Rallidae is Eocene, about 40.5 Mya, with evidence of intrafamiliar diversification from the Late Eocene to the Miocene. This timing is older than previously suggested for crown group Rallidae, but fossil calibrations, extent of taxon sampling and substantial sequence data give it credence. We note that fossils of Eocene age tentatively assigned to Rallidae are consistent with our findings. Compared to available studies of other bird lineages, the rail clade is old and supports an inference of deep ancestry of ground-dwelling habits among Neoaves.

Introduction
Hypotheses favouring Cenozoic diversification of modern bird orders after the Cretaceous-Palaeogene (K–Pg) mass extinction, inferred from the scarcity of Cretaceous fossils [1–3], have been rejected by analyses indicating the origin of several lineages during the Cretaceous [4–9]. This conclusion derives from calibration of molecular clocks [10,11], using ever increasing genetic data, and is supported by a rising number of Cretaceous fossils [12–14]. Although there is some uncertainty about phylogenetic placement, most adequate, confident and diagnostic neognath fossil material found from the early Palaeogene suggests an extensive diversification of neognaths during the late Cretaceous [15–17]. Analyses using mitochondrial and nuclear data largely agree in terms of the age of the lineages leading to the main orders of modern birds [18–21], but see 22]. Studies are nevertheless sensitive to calibration and dating tools [23], and a persistent difficulty is the influence of missing lineages on inferences regarding the timing of modern diversity [24,25].

In particular, studies that use data from single species to represent clades suffer from uncertain node age and placement [26,27], and are not informative about crown group ages. For example, recent analysis of genetic data for Palaeognathae have shed light on the ancestry of moa (Dinornithiformes) by supporting the flying South American tinamous as sister to moa within the Struthioniformes [28,29]. This finding supports a hypothesis of flying ancestors for modern day flightless Struthioniformes [30,31] contrary to the assumed flightless ancestry of the entire group. Separate studies support a recent radiation of moa [32,33] with their origin occurring only 5 million years ago (Pliocene). This estimated time leaves considerable disparity in the assumption that the common ancestor of species of moa was itself flightless [34]. Similarly, raptors or birds of prey are not monophyletic although they share a primary reliance on carnivory, either by scavenging or by capture of prey, and a number of associated functional niches [25,35–37]. These convergent lifestyle specializations of falcons, hawks and eagles indicate a possible early group of raptors (late-Cretaceous) from which a variety of other carnivore groups have adapted to more aquatic lifestyles [35,37].

This stem versus crown age problem is common to many phylogenetic studies seeking to identify the timing of diversification within Neoaves, whether associated with location, ecology or taxonomy. Mitochondrial genes and genomes have proved valuable in avian evolutionary studies providing the means to address questions about the placement and recognition of clades within the avian phylogeny and their likely time of diversification [8,11,20,37–40]. These studies have mainly focused at the order level, whilst studies at family level are scarce. Recent analysis of mitochondrial DNA (mtDNA) genomes suggests that at least 30 major orders of Neoaves originated in the Cretaceous and survive to the present, but no representatives of families within Gruiformes were included [8].

The rail family (Aves: Rallidae) is globally distributed and extant species occupy niches associated with terrestrial and freshwater habitats. Among these predominantly ground dwelling and foraging birds are a high frequency of flightless species [41,42]. The ecology and geographic origins of modern rails are almost...
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 have specialized and radiated in the Americas before reinvading the Old World [42]. There are 135–143 currently recognized species within 33–40 genera [43–45], only four of which (Porzana, Porphyrio, Gallinula, and Fulica) have a worldwide distribution (Figure 1). Porzana, as currently recognized, is the most speciose genus in the family (11.9% of rails are currently classified as Porzana), followed by Gallirallus (11.2%), Fulica (7.7%), and Gallinula, Laterallus, Rallus, Amadorornis and Sarothrura (6.3% each). Molecular analysis shows that Sarothrura belongs to a separate lineage from Rallidae, Sarotheruridae, and more closely related to the family Helornithidae [36,46–48], but current taxonomy and bird lists still retain this lineage within Rallidae [43,44]. Most of the diversity of Porzana and Gallirallus is found in Asia and Oceania (Figure 1). Asia also contains three endemic genera (Aramidopsis, Habroptila and Gallicrex) as does Oceania (Nesolopetus, Eulabeornis and Megacrex). All of those genera except Nesolopetus are monotypic. Africa has 16 endemic species in seven endemic genera (including Atlantisia in the South Atlantic islands), 54 species occur in America, including 27 in eight endemic genera and six of the nine Rallus species, while only nine species occur naturally in Europe.

Spatial, morphological and current phylogenetic information suggests that rails may be are old within the Neoaves. Analyses indicate a deep placement of the lineage but the inference is based on limited species representatives and short DNA sequences, which requires better resolution [9,36,46,49–51]. In the present study, we use complete mitochondrial genomes to assess temporal diversification within Rallidae. We further compare our estimates with those available for other extant bird lineages in order to shed light on biogeographic/spatial patterns operating in diversification within Neoaves.

Materials and Methods

Ethics statement

Museum tissue samples representing *Eulabeornis castaneoven-tris* (Australian National Wildlife Collection, ANWC50493), *Fulica atra* (Australian National Wildlife Collection, ANWC50980), *Gallirallus philippensis* (Australian National Wildlife Collection, ANWC32326) and *Helornis fulica* (Museu de Zoologia da Universidade de São Paulo, MZUSP79862) were imported into New Zealand under Massey University guidelines for importation of nonviable animal specimens. Tissue samples representing *Gallirallus australis* and *Porphyrio porphyrio* were collected from road kill animals by the Department of Conservation NZ staff without repository institution, and *Lewinia muelleri* blood sample was taken from a wild caught specimen, which was released at the site of capture, under a permit from the Department of Conservation NZ and ethics committee approval from Department of Conservation Institutional Animal Care and Use Committee (IACUC). This research does not require ethics committee approval as no animal was sacrificed, and there was no animal husbandry, experimentation or welfare concerns. Mitochondrial DNA genomes have been submitted to GenBank: accession numbers KF644581–84; KF701060–62.
Sampling

The data set compiled new assembled mitochondrial genomes of six species within Rallidae and one species within Helornithidae 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; HQ986093), and Porphyrio martinicus (purple swamphen; KF701062). Coturniceus exquisitus (Swinhoe’s rail; NC012143) found in wetlands and Gallinula chloropus (Swinhoe's rail; NC012143) inhabiting forests are both volant species present in Asia, Gallirallus philippensis (banded rail; KF701061) is distributed in Asia and Oceania, and Enabeornis castaneovenustus (chestnut rail; KF644583) in Oceania and both are flying species that occupy wetlands. Gallirallus okinavae (Okinawa rail; NC012140), is found endemic to wet forest on Okinawa island in the Japanese archipelago. Gallirallus australis (weka; KF701060) and Porphyrio hochstetteri (akaka; 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 okinavae, 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. castaneovenustus and L. muelleri) and the relationships among genera. It has been inferred from molecular phylogenetics that Grues (suborder comprising Rallidae, Gruidae, Helornithidae, Aramiidae, Psophiidae and Aptornithidae) has a palaeo-austral signature [46,49] but the fossil record is mainly found in the Northern Hemisphere. Although the infrageneric relationships in Rallidae are mostly unknown, we sought to include representatives of different and more distant genera in the family following Olson [42]. Helornis fulica (sungrebe) was included as a close outgroup [36,52]. We used a modified phenol-chloroform procedure [33] 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. 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. A library preparation for sequencing was as described by Shendure and Ji [54] and Mardis [55].

Sequence quality, 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 Bruijn 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 NC013236 [59], and visualized in Tablet v1.11.08.10 [60]. New mtDNA genomes were submitted to GenBank (Table S1).

Phylogenetic analyses

For phylogenetic analyses, mtDNA genomes of additional Neognathae species were downloaded from GenBank. These lineages were from closely related groups to Rallidae (e.g. Gruidae, Otididae, Cuculidae) and provide appropriate context for dating analyses (Table S1). Galloanseraeae species were used as a known outgroup to all these taxa [36]. Several studies [36,40,61] have shown that the kagu (Rhynochetos jubatus) is not, despite some morphological and behavioural similarities, grouped within the Gruiformes; therefore this species was not included in the analyses.

Alignment of the mitochondrial sequences was performed with Geneious v6.0.5 [57] using manual adjustment. Each gene alignment was checked prior to phylogenetic analysis. We partitioned the aligned genomes into protein-coding genes, tRNAs, rRNAs, and noncoding fragments (including the origin of replication and the hypervariable region) [10]. We further partitioned the protein-coding genes based on amino acid sequences, into stems and loops data for rRNAs [62,63] and cloverleaf pattern for tRNAs, which correspond to RNA secondary structures of those genes. Protein-coding genes were aligned manually based on the deduced amino acid sequences. The alignments of RNA and rRNA genes were corrected by excluding ambiguous positions, such as loops and indels. Stop codons and ambiguous alignments next to gaps (conserved amino acid and RNA stems defined the inclusion boundaries of ambiguous regions next to gaps) were excluded from the alignment. The Control Region and NADH6 were excluded from the analyses due to alignment instability and heterogeneous base composition which can confound phylogenetic inferences.

The total length of the analysed mitogenomic dataset was 13,768 nucleotides which included the following partition scheme [10]: 1) first-codon position of the 12 protein-coding genes and 2) second-codon position of the 12 protein-coding genes, 3) Ry–coding at the third-codon position of the 12 protein-coding genes, 4) loops of the tRNAs and rRNA combined, 5) stems of the tRNAs and rRNA combined.

We performed all subsequent analyses with this partition strategy. Phylogenies were inferred using Bayesian Markov Chain Monte Carlo (MCMC) as implemented in MrBayes [64] with 20 million generations sampled every 2000 generations and a general time reversible model with gamma distribution (GTR+Γ) model of evolution. The model was estimated in ModelTest v3.7 using the Akaike Information Criterion [65]. Convergence and diagnostics of the Markov process were evaluated by the reliability of parameter estimates across generations using Tracer v1.6 (http://tree.bio.ed.ac.uk/software/tracer/). A burn in of 10% gave optimal results. We obtained Effective Sample Sizes (ESS) above 200 for all parameters. Maximum Likelihood (ML) with rapid bootstrapping was implemented in RAxML using GTR+Γ. Analyses were performed via the Cipres portal [66] and trees were viewed in FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) and SplitsTree v4.12.8 [67].

Time of divergences

Divergence times were estimated using a Lognormal relaxed Bayesian clock implemented in BEAST v1.7.5 [68]. A lognormal distribution was chosen because this shape accommodates greater flexibility regarding a cladogenetic event [69,70]. For calibration constraints we used Galloanserinae [71,72] with a normal distribution of 66–86 Mya (95% range) and the stem sphenisciform
Origin and evolution of rails

Previous studies have addressed the question of the origin of the Gruiformes [46,49] and the biogeography and evolution of some lineages of rails have been recently considered [47,50,53,86]. Nevertheless, identification of the basal split and diversification of the rails has remained uncertain because of differences in the estimated dates and approaches used [46,49–51].

We find the width of the 95% HPD intervals for Heliornithidae-Rallidae clade divergence and the crown-group Rallidae in our analysis overlap with interval age estimations reported from nuclear genes by Fain [46], Houde [49], and Brown [51]. However, the mean estimates of Heliornithidae-Rallidae divergence and basal Rallidae in the present analysis were much older than mean estimations in those studies (about twice the age). The observed tendency of analyses using mtDNA to overestimate node age compared to nuclear markers might be the source of this discrepancy [22], and it has been inferred that mtDNA data tends to result in overestimated ages of shallower nodes in particular [22,70]. To minimise any potential overestimation of “shallower” nodes (compared to the nodes used to calibrate the tree) we applied several recommended strategies: 1) a partition scheme including RY–coding at the third-codon position; 2) relaxation of the molecular clock without assuming rate correlation among branches and; 3) variation across sites with GTR model and gamma distribution. We find that our estimations of “deeper” node ages (e.g. Rallioidea-Gruioidea divergence) are in fact very similar to those reported using nuclear data [49]. Although it appears that “shallower” node ages might be overestimated in our analysis (node A of the Rallidae lineage and node B of the Heliornithidae-Rallidae divergence in Figure 3), we have similar estimates for Gruinacea-Baleariniae (node C in Figure 3) to other studies using nuclear [46] and mitochondrial data [57] and different calibration constraints. This suggests there is no systemic tendency for overestimation of late Eocene nodes. More probably, disparity with previous molecular analysis relates to differences in calibration constraints and taxon sampling of our study and others. For instance, Fain et al. [46] used as constraints external to Grues a lower (30 Mya) and upper (45 Mya) bound of the alcid-larid split.
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,
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 *Palaeoaramides* 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 *Fulicaletornis* 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 [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 *Fulicaletornis* 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 [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 *Fulicaletornis* 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 [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 *Fulicaletornis* 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 [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 *Fulicaletornis* 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 [35,95–97].

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 Psittacidae) [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

**Figure 3.** Chronogram based on analysis of complete mitochondrial genomes with a Lognormal relaxed–clock Bayesian analysis using BEAST. Age constraints were established by calibration fossils of Galloanserae with a minimum age of 66 Mya and maximum age of 86.5 Mya and Sphenisciformes with an age range from 61.5 Mya to 65.5 Mya. For each node the estimate time of divergence is indicate and the green bar represents the 95% HPD intervals of node ages. The time scale is in millions of years ago (Mya) and geological eras, periods and epochs are indicate where Pli is Pliocene and Ple is Pleistocene. A complete figure including all species analysed in this study is found in supplementary Figure S1. Bootstrap support, Bayesian posterior probabilities and letters referring families within order Gruiformes are the same as in Figure 2.

doi:10.1371/journal.pone.0109635.g003
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.

**Supporting Information**

Figure S1  **Chronogram showing all species analysed in this study.** Divergence times are based on analysis of complete mitochondrial genomes with a relaxed-clock Bayesian analysis using BEAST. Bootstrap support over 70% and Bayesian posterior probabilities over 0.9 are indicated on each branch. Calibration
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.

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.

Acknowledgments
We are grateful to the following individuals and institutions for providing samples: Leo Joseph and Robert Palmer (Australian National Wildlife Collection), Kath Walker and Graeme Elliot (Department of Conservation, New Zealand), and Luis Fabio Silveira (Museu de Zoologia da Universidade de São Paulo, Brazil). Thanks to Isabel Castro and members of the Phoenix lab (http://evolves.massey.ac.nz/) for comments on various stages of this manuscript. We would also like to thank Bennet McComish who helped assemble the mitochondrial genomes of Enaleboris castaneovestris and Fulica atra. We are grateful to Lorenzo Rook, Vanessa De Pietri and two anonymous reviewers who provided helpful comments that greatly improved the several incarnations of this manuscript. Images courtesy of Ramon Möller Jensen, Colin O’Donnell, Eddy Smith, Trevor Collins, Delforge Gilles, Pete Morris, Yann Muzika, Dubi Shapiro, Alan and Elaine Wilson. JCG-R was supported by a Massey Doctoral Scholarship and a New Zealand International Doctoral Scholarship. JCG-R would like to thank to Ana Maria Soto and Tane Garcia. PhD examiners Allian Baker, Adrian Paterson and David Penny provided thoughtful and constructive comments. This work partially fulfills the requirements for the Doctorate degree of JCG-R at the Massey University.

Author Contributions
Conceived and designed the experiments: JCG-R, GCG, SAT. Performed the experiments: JCG-R. Analyzed the data: JCG-R, GCG. Contributed to the writing of the manuscript: JCG-R, GCG. SAT.

References
1. Olsen SL (1989) Aspects of the global avian dynamics during the Cenozoic. Proceedings of the 19th International Ornithological Congress. 2023–2029.
2. Feduccia A (1995) Explosive evolution in Tertiary birds and mammals. Science 267: 637–638.
3. Feduccia A (2003) Big bang for tertiary birds? Trends Ecol Evol 18: 172–176.
4. Hedges SB, Parker PH, Syble CG, Kumar S (1996) Continental breakup and the ordinal diversification of birds and mammals. Nature 381: 226–229.
5. Cooper A, Penny D (1997) Mass survival of birds across the Cretaceous–Tertiary boundary: molecular evidence. Science 275: 1109–1113.
6. Cracraft J (2001) Avian evolution, Gondwanan biogeography and the Cretaceous –Tertiary mass extinction event. Proc R Soc B 268: 459–469.
7. Smith VS, Ford T, Johnson KP, Johnson PC, Yoshizawa K, et al. (2011) Multiple lineages of lace pass through the K-Pg boundary. Biol Lett 7: 782–783.
8. Parcheco MA, Battistuzzi FU, Lentino M, Aguilar RF, Kumar S, et al. (2011) Evolution of modern birds revealed by mitogenomes: Timing the radiation and origin of major orders. Mol Biol Evol 28: 1927–1942.
9. Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO (2012) The global diversity of birds in space and time. Nature 491: 444–448.
10. Harrison GL, McLenachan PA, Phillips MJ, Slack KE, Cooper A, et al. (2004) Four new avian mitochondrial genomes help get to basic evolutionary questions in the Late Cretaceous. Mol Biol Evol 21: 974–983.
11. Brown JW, Rest JS, Garcia-Moreno J, Sorensen MD, Mindell DP (2008) Strong mitochondrial DNA support for a Cretaceous origin of modern avian lineages. BMC Biol 6: 1–18.
12. Clarke JA, Tambusi CP, Noriega JI, Erickson GM, Ketcham RA (2005) Definitive fossil evidence for the extant avian radiation in the Cretaceous. Nature 433: 305–308.
13. Agnolin FL, Novas FE, Lio G (2006) Neornithine bird coracoid from the Upper Cretaceous of Patagonia. Ameghiniana 43: 245–248.
14. Agnolin F, Novas F (2012) A carpometacarpus from the upper cretaceous of Patagonia sheds light on the Ornithurine bird radiation. Pala eontologische Zeitschrift 86: 85–89.
15. Mayr G (2009) Paleogene fossil birds. Berlin, Germany: Springer. 262 p.
16. Mayr G, Scourfield RF (2014) First diagnostic non-sphenisciform bird from the early Paleocene of New Zealand. J R Soc NZ 44: 48–56.
17. Mayr G (2014) The origins of crown group birds: molecules and fossils. Palaeontology 57: 231–242.
18. Paton T, Hadlforth O, Baker AJ (2002) Complete mitochondrial DNA genome sequences show that modern birds are not descended from transitional shorebirds. Proc R Soc B 269: 839–846.
19. Paton TA, Baker AJ, Grout JG, Barrowclough GF (2003) RAG-1 sequences resolve phylogenetic relationships within Charadriiformes birds. Mol Phylogenet Evol 29: 264–278.
20. Pereira SL, Baker AJ (2006) A mitogenomic timescale for birds detects variable phylogenetic rates of molecular evolution and refutes the standard molecular clock. Mol Biol Evol 23: 1731–1740.
21. Hadlforth O, Baker AJ (2012) Multiple nuclear genes and retrotransposons support cicatrance and dispersal of the paleognaths, and an Early Cretaceous origin of modern birds. Proc R Soc B.
22. Kerpl TA, Ware JL, Lamm KS (2014) Flying rocks and flying clocks: disparity in fossil molecular dates for birds. Proc R Soc B 281.
23. Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Mol Biol Evol 22: 1561–1568.
24. Barradough TG, Nee S (2001) Phylogenetics and speciation. Trends Ecol Evol 16: 391–399.
25. Trewick SA, Gibb GC (2010) Vicars, tramps and assembly of the New Zealand avifauna: A review of molecular phylogenetic evidence. Isis 152: 226–253.
26. Hendy MD, Penny D (1999) A framework for the quantitative study of evolutionary trees. Syst Biol 38: 297–309.
27. Holland BR, Penny D, Hendy MD (2003) Outgroup misplacement and phylogenetic inaccuracies associated with a molecular clock. Syst Biol 52: 229–238.
28. Phillips MJ, Gibb GC, Crisp EA, Penny D (2010) Pigeous and moa flock together: Mitochondrial genome sequence analysis reveals independent losses of flight among ratites. Syst Biol 59: 90–107.
29. Baker AJ, Hadlforth O, McPherson JD, Cloutier A (2014) Genomic support for a Moa-Tinamou clade and adaptive morphological convergence in flightless ratites. Mol Biol Evol.
30. Houde P, Olsen SL (1981) Paleognathous carinate birds from the Early Tertiary of North America. Science 214: 1236–1237.
31. Houde P (1986) Ostrich ancestors found in the Northern Hemisphere suggest new hypothesis of ratite origins. Nature 324: 563–565.
32. Bunce M, Worth TY, Phillips MJ, Holdaway RN, Willerslev E, et al. (2009) The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. Proc Nat Acad Sci USA 106: 20646–20651.
33. Hadlforth O, Baker AJ (2001) Complete mitochondrial DNA genome sequences of extinct birds: ratite phylogenetics and the vicariance biogeography hypothesis. Proc R Soc B 268: 939–945.
34. Tennyson A, Worthy T, Jones G, Scourfield P, Hand S (2010) Moa’s ark: Moecine fossils reveal the great antiquity of moa (Aves: Dinornithiformes) in Zealandia. Records of the Australian Museum 62: 105–114.
35. Livezey BC, Zusi RL (2007) Higher-order phylogeny of modern birds (Theropoda, Aves : Neornithes) based on comparative anatomy. II. Analysis and discussion. Zool J Linn Soc 149: 1–95.
36. Hackett SJ, Kimball RT, Reddy S, Bowie RCK, Braun EL, et al. (2008) A phylogenomic study of birds reveals their evolutionary history. Science 320: 1763–1768.
37. Gibb GC, Karda lsky O, Kimball RT, Braun EL, Penny D (2007) Mitochondrial genomes and avian phylogeny: Complex characters and resolvability without explosive radiations. Mol Biol Evol 24: 269–280.
38. Slack KE, Jones CM, Ando T, Harrison GL, Fordyce RE, et al. (2006) Early pigeon fossils, plus mitochondrial genomes, calibrate avian evolution. Mol Biol Evol 23: 1144–1155.
39. Pratt RC, Gibb GC, Morgan-Richards M, Phillips MJ, Hendy MD, et al. (2009) Toward resolving deep neoaves phylogeny: Data, signal enhancement, and priors. Mol Biol Evol 26: 315–326.
40. Morgan-Richards M, Twere SA, Bartosch-Harid A, Kardal sky O, Phillips MJ, et al. (2008) Bird evolution: Testing the Metaves clade with six new mitochondrial genomes. BMC Evol Biol 8: 1–12.
41. Livezey BC (2005) Evolution of flightlessness in rails (Gruiformes: Railidae): Phylogenetic, ecomorphological, and ontogenetic perspectives, Monographs O, editor. Washington, D.C.: The American Ornithologist’ Union. 1–654 p.
42. Olson SL (1973) A classification of the Railidae. Wilson Bull 85: 381–416.
43. Telford RM, Brown AL (1996) Rails: A guide to the rails, crakes, gallinules and coots of the world. New Haven, Connecticut: Yale University Press. 600 p.
44. Clements JF, Schulenberg TS, Bill MJ, Sullivan BL, Wood CL, et al. (2012) The Clements checklist of birds of the world: Version 6.7. Available: http://
56. Zerbino DR, Birney E (2008) Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18: 821–829.

55. Lindow BEK, Dyke GJ (2006) Bird evolution in the Eocene: climate change in Europe and a Danish fossil fauna. Biol Rev 81: 483–499.

54. Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotech 26: 1148–1155.

53. Slikas B, Olson SL, Fleischer RC (2002) Rapid, independent evolution of flightlessness in four species of Pacific Island rails (Rallidae): An analysis based on mitochondrial sequence data. J Avian Biol 33: 5–14.

52. Ericson PGP, Anderson CL, Britton T, Elzanowski A, Johansson US, et al. (2010) Complete mitochondrial genome sequences and the phylogeny of cranes (Gruiformes: Gruidae). The Auk 127: 440–452.

51. Brown JW, Payne RB, Mindell DP (2007) Nuclear DNA does not reconcile ‘rocks’ and ‘clocks’ in Neornithes: A comment on Ericson et al. Biol Lett 3: 257–260.

50. Ericson PGP, Kijne JWM, Johansson US, et al. (2010) Complete mitochondrial genome sequences and the phylogeny of the enigmatic, rail-like avian taxon Songzia Hou, 1990 (Songziidae) from the early Oligocene of France. The Condor 108: 717–720.

49. Houde P (2009) Cranes, rails, and allies (Gruiformes). In: Hedges SB, Kumar S, editors. The Timetree of Life. Oxford University Press. 440–444.

48. Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23: 254–267.

47. Ho SYW, Phillips MJ (2009) Accounting for calibration uncertainty in molecular clocks. In: Hedges SB, Kumar S, editors. The timetree of life: Oxford University Press. 35–86.

46. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for the inference of large phylogenetic trees. New Orleans: Proceedings of the Gateway Computing Environments Workshop (GCE). 1–8.

45. Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotech 26: 1148–1155.

44. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for the inference of large phylogenetic trees. New Orleans: Proceedings of the Gateway Computing Environments Workshop (GCE). 1–8.

43. Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: A new method to directly infer the distribution of time-to-root and branch lengths from DNA sequences. Mol Biol Evol 13: 935–952.

42. Mayr G, Smith R (2001) Ducks, rails, and limicoline waders (Aves: Anseriformes, Gruiformes, Charadriiformes) and its biogeographical significance. Cladistics 17: 71–81.

41. Olson SL (2003) First fossil record of a finfoot (Aves: Heliornithidae) and its biogeographical significance. J Paleontol 77: 1340–1344.

40. Mayr G, Smith R (2001) Ducks, rails, and limicoline waders (Aves: Anseriformes, Gruiformes, Charadriiformes) and its biogeographical significance. Cladistics 17: 71–81.

39. Olson SL, Brower VJR, Stidham TA (2012) Evolutionary rates of mitochondrial cytochrome b genes correspond to diversification rates and to contemporary species richness in birds and reptiles. Roy Soc B.

38. Mayr G (2001) The Paleogene fossil record of birds in Europe. Biol Rev 76: 315–342.

37. Mayr G, Smith R (2001) Ducks, rails, and limicoline waders (Aves: Anseriformes, Gruiformes, Charadriiformes) and its biogeographical significance. Cladistics 17: 71–81.

36. Ho SYW, Phillips MJ (2009) Accounting for calibration uncertainty in molecular clocks. In: Hedges SB, Kumar S, editors. The timetree of life: Oxford University Press. 35–86.

35. Mayr G, Smith R (2001) Ducks, rails, and limicoline waders (Aves: Anseriformes, Gruiformes, Charadriiformes) and its biogeographical significance. Cladistics 17: 71–81.

34. Ho SYW, Phillips MJ (2009) Accounting for calibration uncertainty in molecular clocks. In: Hedges SB, Kumar S, editors. The timetree of life: Oxford University Press. 35–86.

33. Brown J, van Tuinen M (2011) Evolving perceptions on the antiquity of the modern avian tree. In: Dyke GJ, Kaiser G, editors. Living Dinosaurs: The World: A monograph of the family Rallidae: David R. Godine. 339–379.

32. Mayr G, Smith R (2001) Ducks, rails, and limicoline waders (Aves: Anseriformes, Gruiformes, Charadriiformes) and its biogeographical significance. Cladistics 17: 71–81.

31. Ho SYW, Phillips MJ (2009) Accounting for calibration uncertainty in molecular clocks. In: Hedges SB, Kumar S, editors. The timetree of life: Oxford University Press. 35–86.

30. Huelsenbeck JP, Ronquist F (2004) MrBayes: Bayesian phylogenetic inference via Markov chain Monte Carlo sampling. Bioinformatics 19: 157–158.

29. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 157–158.

28. Ericson PGP, Kijne JWM, Johansson US, et al. (2010) Complete mitochondrial genome sequences and the phylogeny of the enigmatic, rail-like avian taxon Songzia Hou, 1990 (Songziidae) from the early Eocene of China. Auk 127: 440–452.

27. Mayr G (2005) The Paleogene fossil record of birds in Europe. Biol Rev 80: 593–619.

26. Ho SYW, Phillips MJ (2009) Accounting for calibration uncertainty in molecular clocks. In: Hedges SB, Kumar S, editors. The timetree of life: Oxford University Press. 35–86.

25. Fordyce RE, Jones CM (1996) A new method to directly infer the distribution of time-to-root and branch lengths from DNA sequences. Mol Biol Evol 13: 935–952.

24. Fordyce RE, Jones CM (1996) Penguin history and new fossil material from New Zealand. In: Davis LS, Darby JT, editors. Penguin Biology. San Diego, CA, USA: Academic Press. 306–324.

23. Fordyce RE, Jones CM (1996) Penguin history and new fossil material from New Zealand. In: Davis LS, Darby JT, editors. Penguin Biology. San Diego, CA, USA: Academic Press. 306–324.

22. Fordyce RE, Jones CM (1996) Penguin history and new fossil material from New Zealand. In: Davis LS, Darby JT, editors. Penguin Biology. San Diego, CA, USA: Academic Press. 306–324.

21. Fordyce RE, Jones CM (1996) Penguin history and new fossil material from New Zealand. In: Davis LS, Darby JT, editors. Penguin Biology. San Diego, CA, USA: Academic Press. 306–324.

20. Fordyce RE, Jones CM (1996) Penguin history and new fossil material from New Zealand. In: Davis LS, Darby JT, editors. Penguin Biology. San Diego, CA, USA: Academic Press. 306–324.
110. Johnson NK, Marten JA, Ralph CJ (1989) Genetic evidence for the origin and relationships of hawaiian honeycreepers (Aves: Fringillidae). The Condor 91: 379–396.

111. Fleischer RC, McIntosh CE, Tarr CL (1998) Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Mol Ecol 7: 533–545.

112. Diamond J (1974) Colonization of exploded volcanic islands by birds: The supertramp strategy. Science 184: 803–806.

113. Mayr E, Diamond J (2001) The birds of northern Melanesia: Speciation, ecology and biogeography. New York: Oxford University Press. 548 p.