Original Research Article

DNA barcodes for estimation of species phylogeny in some Rails (Gruiformes: Rallidae)

ABSTRACT

Genetic datasets and evolutionary information for rails are scarce, and genetic studies for them are very sparse and insufficient compared to their widespread use in different zones. Therefore, the phylogenetic lineages among five species of rails belonging to the family Rallidae from Egypt were estimated and evaluated using DNA barcoding, for the first time. In the present study, mitochondrial and nuclear DNA sequence data were analyzed for these rails. Based on 16S rRNA and RAG-1 sequences, phylogenetic trees were constructed using the Maximum Evolution method, revealing that there were two separate genetic clusters, one cluster for *Rallusaquaticus, Crexcrex, Porzanaparva*and*Porzanapusilla* and the second cluster for*Porzanaporzana*. *Crexcrex* and*Rallusaquaticus* were laid in one clade and formed a sister group. Moreover, *Porzanaparva and Porzanapusilla* formed another sister clade. This study demonstrated the ability of 16S rRNA and RAG-1 genes to identify rails, as well as deducing the genetic relationship between them. The present work is considered a contribution to enriching the genetic relationships among the studied species.

Keywords: Porzana; birds; genus; taxa; markers.

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1. INTRODUCTION

The diversity of taxa contained in the Gruiformes is referred by the relatively high number of monotypic families known, the allocation of its species among ten orders by Stresemann [1] and Wolters [2], and a frequent view of the group as a possibly artificial aggregation [3- 9].
The Rallidae, exceed all other gruiform families in included difference in body mass and most environmental parameters, containing habitat, reproduction, diet and migratory habit. Rallids reside in a difference of environments, including freshwater and salt-water marshes, mangroves, tropical forests and grasslands [10]. There are 135–143 currently identified species within 33–40 genera [11-13], only four ofwhich (*Porzana, Porphyrio*, *Gallinula* and *Fulica*) have a widely distribution. Rails have one of the most widely geographical distributions of any family of vertebrates. *Porzana*, as currently known, contains the greatest number of species [14], and is the most speciose genus in Rallids (11.9% of rails are currently classified as *Porzana*).
“The utility of nucleotide sequence variations in a single gene to estimate evolutionary relationships was first widely applied” by Carl Woese [15]. “He

found that sequence divergences in a conserved gene, ribosomal RNA, could be used to deduce phylogenetic lineages. Sequence comparisons of rRNA from many different organisms led initially to identification of the Archaea, and then to a reconstructing of the tree of life. Lately, the polymerase chain reaction has allowed sequence variety in any gene to be tested. Genes that promote slowly, like rRNA, often do not differ among closely related organisms, but they are substantial in regaining archaic relationships" [16]. Otherwise, genes that promote rapidly may overwrite the traces of ancient affinities, but regularly detect differences between closely related species.
"As the most widespread and species-rich group of terrestrial vertebrates, birds show great verity in their phenotypes, ecology, habitats and behaviors" [17]. So far, a great effort has been devoted to resolve the phylogenetic lineages from higher taxonomic classes [18-20] to sister species [21-25]. "One nuclear exon that has confirmed beneficial in phylogenetic studies in birds is the recombination-activating gene 1(RAG-1) which has been used to explore relationships at the ordinal level and at the family level in passerines" [26, 27]. "The gene 16S rRNA (mitochondrial DNA gene) was used for phylogeny and taxonomy; and ultimately, the foundation of large public-domain datasets" [28, 29]. Different characteristics of the 16S rRNA gene make it the "Ultimate molecular chronometer", and the most prevalent housekeeping genetic tool; and a beneficial purpose for phylogeny. Here we investigate the performance of a fragment of the 16S ribosomal RNA gene (16S) in mtDNA barcoding of rails. As a contribution to the discussion about phylogenetic relationships, comparative constructed trees of 16S as mtDNA gene and RAG-1 as nuclear gene was investigatedin rails.
Clear attempts to reconstruct phylogenetic relationships within the Rallidae have been founded on a variety of evidence, and comprise the intuitive estimation by Olson [30], phenetic assessments established on DNA hybridization by Sibley and Ahlquist [31] and Sibley et al., [32], and limited reconstructions by Houde et al., [9] and Trewick [33] founded on sequence data. These analyses, however, have resulted basically divergent, only weakly confirmed groupings, and the phylogenetic relationships of many genera of Rallidae have not been investigated from any methodological perspective.
In this study, the goal firstly, study phylogenetic relationship of some Rallidae through genetic markers. Secondly, to test the resolving power of these markers, it was built a phylogenetic tree and estimated genetic relationship among studied birds depending on DNA sequences.

2. MATERIALS AND METHODS

2.1. Experimental birds

We followed guidelines of the Committee for experimental animals during this study. This study was carried out for five species: water rail *Rallusaquaticus*(Linnaeus, 1758), spotted crake *Porzanaporzana* (Linnaeus, 1766), little crake *Porzanaparva* (Scopoli, 1769), Baillon's crake *Porzanapusilla* (Pallas, 1776) and Corn crake *Crexcrex* (Linnaeus, 1758) were obtained from local hunters in Egypt. Twenty specimens are representing five species of Rallidae were sampled in the present study.

2.2. DNA extraction andPCR amplification

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Liver tissues were obtained from the different studied species, taken immediately and frozen at - 80ºC. DNA was extracted using a GeneJET™ kit Genomic DNA Kit#K0721.

Mitochondrial and nuclear DNA fragments were PCR amplified using various combinations of primers RAG-1 gene F50-5′ CTG ATC TGG TAA CCC CAG TGA AAT CC -3′ and R51-5′ GAC CCT CTT TCT GCT ATG AGG GGG C -3′ [34]. And 16S rRNA gene F8-5′ CGG TCT GAA CTC AGA TCA CGT A-3′ and R9-5′ CGA CCT GGA TTT CTC CGG TCTG-3′ [35].

Each PCR products were separated on 1.5% agarose gels, bands were visualized by ethidium bromide staining and viewed with an ultraviolet light source. The amplified products were purified using aGeneJET™ kit (Thermo K0701) according to the manufacturer's protocols. Sequencing was performed using an ABI 3730xl DNA sequencer.

2.3. Alignment and sequence properties

The resulting sequences were confirmed as being derived from studied species DNA using the GenBank Blast algorithm. The DNADynamo software version 1.459 was used for editing the sequences and they were aligned using Clustal W. Finally, the phylogenetic analyses used were Maximum Evolution in MEGA 6.0 software [36].

3. RESULTS

RAG-1 and 16S genes sequences generated in this work were corresponded with data stocked in National Centre of Biotechnological Information (NCBI) by nucleotide BLAST. The sample identified as 754 bp in length and exhibited similarity with RAG-1 gene of the studied species from GenBank database. The sequences of *Rallusaquaticus*, *Porzanaporzana* and *Porzanaparva* were compared with records deposited in Gen Bank, the results revealed entirely similar records (Accession numbers are KC613966, KC613961, KC613960) respectively while, *Porzanapusilla* and *Crexcrex* have no records deposited in Gen Bank.

In case of 16S rRNA gene, sample recognized as 652 bp in length and exhibited similarity with GenBank database. *Rallusaquaticus, Porzanaporzana, Porzanaparva, Porzanapusilla* and *Crexcrex* sequences were compared with records placed in GenBank, the results revealed entirely similar records (Accession numbers are KC614027, KC614023, KC614022, KC614021, KC613986) respectively.

RAG-1 and 16S rRNA genes were found significant differences in base composition biases between some of the studied taxa. For RAG-1 gene, the average nucleotide frequencies are 30.7% (A), 22.5% (T/U), 22.7% (C), and 24.1% (G). The percent composition of nucleotide varied from 29.5% to 33.8% (A), 19.1% to 24.4% (T), 20.4% to 28.3% (C), and 18.7% to 25.8% (G), which infers that studied birds, are rich in C, A and G while poor in T (Table I).

Table I. Percentage composition of nucleotides A, T, G, C, AT and GC in Rallidae species.

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Species	A% (16S/RAG-1)	T% (16S/RAG-1)	G% (16S/RAG-1)	C% (16S/RAG-1)	AT% (16S/RAG-1)	CG% (16S/RAG-1)
Crexcrex	36.2/30.0	21.5/22.7	16.8/25.8	25.5/21.5	57.7/52.7	42.3/47.3
Porzanaparva	36.7/29.5	20.8/24.4	16.9/25.7	25.5/20.4	57.5/53.9	42.4/46.1
Porzanaporzana	35.7/33.8	22.1/19.1	15.5/18.7	26.7/28.3	57.8/52.9	42.2/47.0
Porzanapusilla	37.7/30.3	20.7/23.3	15.1/25.3	26.5/21.1	58.4/53.6	41.6/46.4
Rallusaquaticus	37.3/29.8	20.7/23.3	15.9/25.7	26.0/21.2	58.0/53.1	41.9/46.9
Mean	**36.7/30.7**	**21.1/22.5**	**16.3/24.1**	**25.9/22.7**	**57.8/53.2**	**42.1/46.7**

The transition/transversion rate ratios are k1 = 6.436 (purines) and k2 = 6.929 (pyrimidines). The content of pyrimidine was barely higher than purine in the studied birds. Nucleotide frequencies for 16S rRNA are 36.7% (A), 21.1% (T/U), 25.9% (C), and 16.3% (G). and percent composition of nucleotide ranged from 35.7% to 37.7% (A), 20.7% to 22.1% (T), 25.5% to 26.7% (C), and 15.1% to 16.9% (G), which indicates that studied birds, are rich in T, A and C while poor in G (Table I). The transition/transversion rate ratios are k1 = 0.983 (purines) and k2 = 3.918 (pyrimidines). The content of purine was lower than pyrimidine in the studied birds.

Phylogenetic tree was constructed depending on RAG-1 sequences of the studied species of Rallidae using Maximum Evolution method (Fig. 1) reveals that there are two separate genetic clusters, one cluster for *Rallusaquaticus*, *Crexcrex, Porzanaparva* and *Porzanapusilla*, the second cluster includes *Porzanaporzana. Crexcrex*and *Rallusaquaticus* were laid in one clade and they are formed a sister group. Indeed, *Porzanaparva* and *Porzanapusilla* formed another sister clade. According to the RAG-1 dataset, haplotypes of the same species were always placed together in phylogenetic reconstruction.

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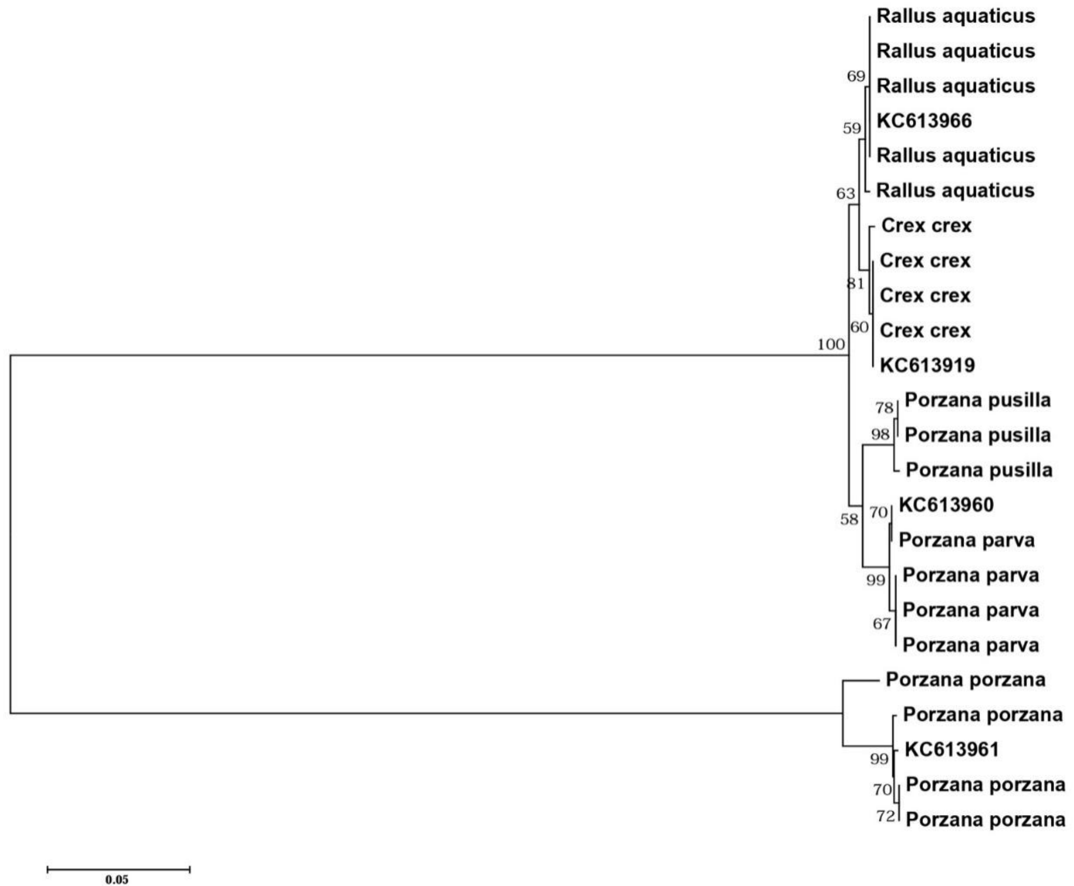

Fig.1. Maximum evolutionary tree analyses based on the RAG-1 gene from 24 barcode sequences from 5 rallids. The values at nodes represent the bootstrap confidence level (1000 replicates). Specimen's number denotes the accession number of GenBank.

Moreover, phylogenetic analysis performed in the current study using the Maximum Evolution method revealed that *Crexcrex* and *Rallusaquaticus* are closely genetic to each other (0.017), *Porzanaparva* and *Porzanapusilla* formed another sister clade on the other hand, and *Porzanaporzana* have the farthest genetic distance (0.624) (Table II).

Table II. Total genetic distance between the studied rails' species. 16S rRNA distances are below diagonal; RAG-1 distances are above diagonal.

	Crexcrex	*Porzanaparva*	*Porzanaporzana*	*Porzanapusilla*	*Rallusaquaticus*
Crexcrex	0	0.027	0.624	0.030	0.017
Porzanaparva	0.113	0	0.612	0.024	0.021
Porzanaporzana	0.218	0.202	0	0.621	0.624
Porzanapusilla	0.101	0.056	0.211	0	0.021
Rallusaquaticus	0.081	0.117	0.226	0.105	0

According to 16S rRNA dataset, haplotypes of the same species were always placed together in phylogenetic reconstruction. The phylogenetic tree of the studied Rallids species which was divided into two main clades, the first clade includes *Rallusaquaticus, Crexcrex, Porzanaparva* and *Porzanapusilla*, the second cluster includes *Porzanaporzana. Crexcrex* and *Rallusaquaticus*

were laid in one cluster and they are formed a sister group. Moreover, *Porzanaparva* and Porzana*pusilla* formed another sister clade (Fig. 2).

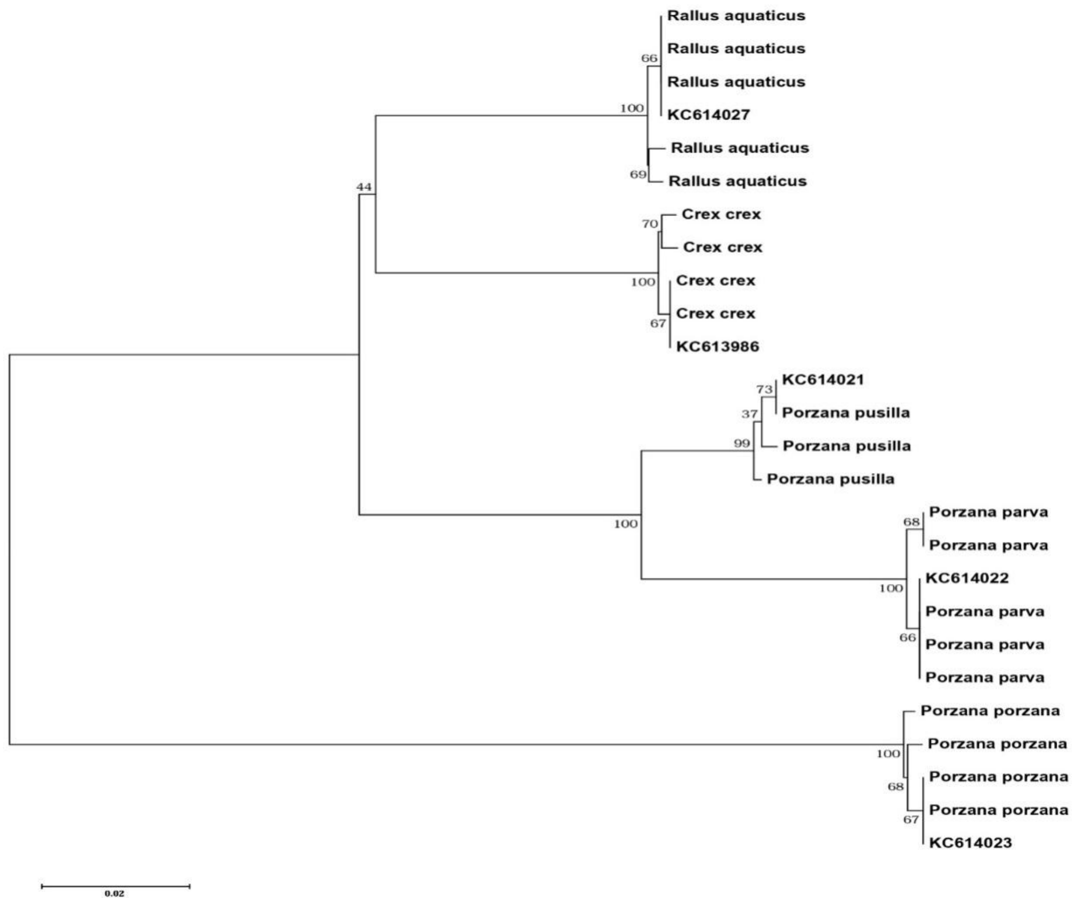

Fig.2. Maximum Evolution phylogenetic tree based on 16S gene sequences of the studied rail species. Values at nodes represent the bootstrap confidence level (1000 replicates). Specimen's number refers to the accession number of GenBank.

Phylogenetic analysis performed in the current study using the Maximum Evolution method revealed that *Crexcrex* and *Rallusaquaticus* are closely genetic to each other (0.081) on the other hand, *Porzanaporzana* have the farthest genetic distance (0.218, 0.226) respectively (Table II).

4. DISCUSSION

Previous studies have referred to the question of the originality of the Gruiformes [37, 38] and the biogeography and evolution of some relationships of rails have been recently believed [33, 39-41]."Nevertheless, recognition of the basal split and differentiation of the rails has remained uncertain because of diversity in the investigated dates and approaches used" [37, 38, 42, 43].

"The rails (Aves:Rallidae) are a fabulous group of birds with many discreet species that are difficult to observe and others that have managed to reside very isolated islands despitetheir apparent poor ability to fly" [44-47]. "Rails are a widespread family of birds extensively distributed throughout insular and continental settings and only absent in polar regions, waterless

deserts, and mountains above the snow line. There is a great interest in rail diversity, evolution, and biogeography which resulted in studies of their relationships using morphology and PCR-based Sanger approaches" [10, 39]. However, phylogenetic inferences for rails have confirmed difficult with morphology [10], and despite great effort with molecules [39-41, 48, 49], their relationships and diversification dynamics still poorly explained, with disagreements concerning key relationships due to few markers and species availability. Recently, one study used "mitogenomes to discover their basal relationships and diversification, but sampling was too limited for understanding deep evolutionary patterns as they lacked good taxon representation at the genus level" [14]. "The integration of state-of-the-art molecular techniques and analyses, together with a broad taxonomic sampling, can result new insights into bird interrelationships and divergence. However, their evolutionary significance, the relationships among many rail lineages stay unresolved as does the general timescale of rail evolution" [14].

"Utilizing of DNA barcoding and the molecular markers is for the time being essential since it represents a beneficial method for studying taxonomy and phylogenetic lineages among species. This technology has been taken as good tools for recognizing species as well as in archaeological remains and museum samples due to of degradation nature and fragmentation of ancient DNA" [50].

"The recombination activase gene-1 (RAG-1) uses as a phylogenetic marker for understanding relatively deep relationships has been investigated in birds, mammals and squamates"[26,51, 52]. "This gene is also useful for resolving phylogenetic relationships among birds and for resulting schisms between the models underlying the main technique used in phylogenetic analysis"[26,51, 52] .

The recombination activase gene-1 (RAG-1) sequences were used to estimate genetic relationship between *Rallusaquaticus, Porzanaporzana, Porzanaparva, Porzanapusilla* and *Crexcrex*. These results show a level of genetic differentiation among three genera of Rallidae. Maximum Evolution tree constructed on RAG-1 sequences divided the studied Rallidae into two separate genetic groups, one for genus *Porzanaporzana*and the other for; *Porzanaparva, Porzanapusilla, Crexcrex* and *Rallusaquaticus* members due to dark colour of *Porzanaporzana*underparts striped with white and black dorsum spotted with white while, other species have no these characters.

The result showed that the genus *Rallus* and genus Crex were supported as a sister group to each other. Also, species of genus *Porzana* were laid as a sister cluster except *Porzanaporzana* grouped as monophyletic clade to all studied species, that is explained by their shared gray underparts, olive and black dorsum striped with white, and buffy juvenile plumage of the sister group while, *Porzanaporzana* has a dark gray underparts striped with white and black dorsum spotted with white. The phylogenetic tree based on each of RAG-1 and 16S rRNA gave similar results and revealed the relationship between the studied rails.

5. CONCLUSION

In the present study, five rails have been identified and, studied the phylogenetic and taxonomic relationships between them in Egypt for the first

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time using DNA barcoding. This study revealed that DNA barcoding based on 16S rRNA gene (mtDNA gene) and RAG-1 gene (Nuclear gene) were demonstrated as a powerful and useful molecular marker in identifying, and estimating the phylogenetic relationship among studied rails species.

Ethical Approval:

All animal experiments involved in this study were approved by the Laboratory Animal Welfare and Animal Experimental Ethical Committee of IACUC Cairo University.

REFERENCES

1. Stresemann E. Aves. In Handbuch der Zoologie, vol. 7, no. 2 (ed.W.KÏkenthal&T.Krumbach). Berlin:W. de Gruyter; 1927-1934.
2. Wolters HE. Die Vogelarten der Erde, part 1. Hamburg: Paul Parey; 1975.
3. Lowe PR. On the relations of the Gruimorphae to the Charadriimorphae and Rallimorphae, with special reference to the taxonomic position of Rostratulidae, Jacanidae, and Burhinidae (Oedicnemidaeolim); with a suggested new order (Telmatomorphae). Ibis. 1931;73:491-534.
4. Verheyen R. Contribution au demembrement de l'ordoartificiel des Gruiformes (Peters 1934). I. Les Ralliformes. Bulletin de l'Institut royal des sciences naturelles de Belgique. 1957a;33:1-44.
5. Verheyen R. Contribution au demembrement de l'ordoartificiel des Gruiformes (Peters 1934). II. Les Cariamiformes. Bulletin de l'Institut royal des sciences naturelles de Belgique. 1957b;33:1-7.
6. Verheyen R. Contribution au demembrement de l'ordoartificiel des Gruiformes (Peters 1934). III. Les Jacaniformes. Bulletin de l'Institut royal des sciences naturelles de Belgique. 1957c;33:1-19.
7. Verheyen R. Contribution au demebrement de l'ordoartificiel des Gruiformes (Peters 1934). IV. Les Turniciformes. Bulletin de l'Institut royal des sciences naturelles de Belgique.1958;34:1-18.
8. Olson SL. The fossil record of birds. In Avian biology, vol. 8 (ed. D. S. Farner, J. R. King & K. C. Parkes). NewYork: Academic Press. 1985; pp. 79-238.
9. Houde P, Cooper A, Leslie E, Strand AE. Montaṅo GA. Phylogeny and evolution of 12S rDNA in Gruiformes (Aves). In Avian molecular evolution and systematics (ed. D. P. Mindell). San Diego: Academic Press. 1997; pp. 121-158.
10. Livezey CB. A phylogenetic analysis of the Gruiformes (Aves) based on morphological characters, with an emphasis on the rails (Rallidae). Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 1998;353(1378):2077–2151.
11. Taylor B. Rails: A guide to the rails, crakes, gallinules and coots of the world. New Haven, Connecticut. Yale University Press. 1998; pp. 600.
12. Clements JF, Schulenberg TS, Iliff MJ, Sullivan BL, Wood CL. The Clements checklist of birds of the world: Version 6.7. 2012; Available: http:// www.birds.cornell.edu/clementschecklist/downloadable-clements-checklist. Accessed: Jul 23, 2013.
13. Lepage D. Avibase - The world bird database. Bird Studies Canada: Avibase. Available: http://avibase.bsc-eoc.org/. Accessed: Jul 4, 2013.

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14. García-R JC, Gibb GC, Trewick SA. Eocene diversification of crown group rails (Aves: Gruiformes: Rallidae). PloS One 2014a;9(10):e109635.
15. Woese CR, Fox GE. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proceedings of the National Academy of Sciences of the United States of America. 1977;74(11:088–5090.
16. Woese CR. Interpreting the universal phylogenetic tree. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(15):8392–8396.
17. Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO. The global diversity of birds in space and time. Nature. 2012;491(7424):444–448.
18. Hackett SJ, Kimball RT, Reddy S, Bowie RC, Braun EL, Braun MJ, Chojnowski JL, Cox WA, Han KL, Harshman J, Huddleston CJ, Marks BD, Miglia KJ, Moore WS, Sheldon FH, Steadman DW, Witt CC, Yuri T. A phylogenomic study of birds reveals their evolutionary history. Science. 2008;320(5884):1763–1768.
19. 19.Berv JS, Prum RO. A comprehensive multilocus phylogeny of the Neotropicalcotingas (Cotingidae, Aves) with a comparative evolutionary analysis of breeding system and plumage dimorphism and a revised phylogenetic classification.Molecular Phylogenetics and Evolution. 2014;81:120–136.
20. Jonsson KA, Fabre PH, Kennedy JD, Holt BG, Borregaard MK, Rahbek C, Fjeldså J. A supermatrix phylogeny of corvoid passerine birds (Aves: Corvides). Molecular Phylogenetics and Evolution. 2016;94(Pt A):87–94.
21. Yeung CK, Lin RC, Lei F, Robson C, Hung M, Liang W, Zhou F, Han L, Li SH, Yang X. Beyond a morphological paradox: complicated phylogenetic relationships of the parrotbills (Paradoxornithidae, Aves). Molecular Phylogenetics and Evolution. 2011;61(1):192–202.
22. Backström N, Saetre GP, Ellegren H. Inferring the demographic history of European Ficedula flycatcher populations. BMC Evolutionary Biology. 2013;13:2.
23. Chu JH, Wegmann D, Yeh CF, Lin RC, Yang XJ, Lei FM, Yao CT, Zou FS, Li SH. Inferring the geographic mode of speciation by contrasting autosomal and sex-linked genetic diversity.Molecular Biology and Evolution. 2013;30(11):2519–2530.
24. Dong F, Li SH, Zou FS, Lei FM, Liang W, Yang JX, Yang XJ. Molecular systematics and plumage coloration evolution of an enigmatic babbler (*Pomatorhinusruficollis*) in East Asia.Molecular Phylogenetics and Evolution. 2014;70:76–83.
25. Wang N, Liang B, Wang J, Yeh C, Liu Y, Liang W, Yao C, Li S. Incipient speciation with gene flow on a continental island: Species delimitation of the Hainan Hwamei (Leucodioptroncanorumowstoni, Passeriformes, Aves).Molecular Phylogenetics and Evolution. 2016;102,62-73.
26. Groth JG, Barrowclough GF. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene.Molecular Phylogenetics and Evolution.1999;12(2):115–123.
27. Barker FK, Barrowclough GF, Groth JG. A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. Proceedings of the Royal Society B: Biological Sciences. 2002;269(1488):295–308.
28. Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ, Woese CR. The Ribosomal Database Project (RDP). Nucleic Acids Research. 1996;24(1):82–85.
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29. Van de Peer Y, Jansen J, Rijk PD, Wachter R. Database on the structure of small ribosomal subunit RNA. Nucleic Acids Research. 1997;25 1,111-116.
30. Olson SL. A classification of the Rallidae. The Wilson Bulletin. 1973;85:381-416.
31. Sibley CG, Ahlquist JE. Phylogeny and classification of birds: a study in molecular evolution. New Haven, CT: Yale University Press; 1990.
32. Sibley CG, Ahlquist J, DeBenedictis P. The Phylogenetic Relationships of the Rails, Based on DNA Comparisons. Journal of the Yamashina Institute for Ornithology. 1993;25,1-11.
33. Trewick SA. Flightlessness and phylogeny amongst endemic rails (Aves: Rallidae) of the New Zealand region. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 1997;352(1352):429–446.
34. Irestedt M, Johansson US, Parsons T, Ericson PG. Phylogeny of major lineages of suboscines (Passeriformes) analysed by nuclear DNA sequence data. Journal of Avian Biology. 2001;32:15-25.
35. Garcia-R JC, Gibb GC, Trewick SA. Deep global evolutionary radiation in birds: diversification and trait evolution in the cosmopolitan bird family Rallidae. Molecular Phylogenetics and Evolution. 2014b;81:96–108.
36. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.Molecular Phylogenetics and Evolution. 2013;30(12):2725–2729.
37. Fain MG, Krajewski C, Houde P. Phylogeny of "core Gruiformes" (Aves: Grues) and resolution of the Limpkin-Sungrebe problem.Molecular Phylogenetics and Evolution. 2007;43(2):515–529.
38. Houde P. Cranes, rails, and allies (Gruiformes). In: Hedges SB, Kumar S, editors. The Timetree of life. Oxford University Press. 2009; pp. 440-444.
39. Slikas B, Olson S, Fleischer R. Rapid, independent evolution of flightlessness in four species of Pacific Island rails (Rallidae): an analysis based on mitochondrial sequence data. Journal of Avian Biology. 2002;33:5-14.
40. Kirchman J. Speciation of Flightless Rails on Islands: A DNA-Based Phylogeny of the Typical Rails of the Pacific. The Auk. 2012;129:56–69.
41. Ruan L, Wang Y, Hu J, Ouyang Y. Polyphyletic origin of the genus *Amaurornis* inferred from molecular phylogenetic analysis of rails. Biochemical Genetics. 2012;50(11-12):959–966.
42. Ericson PG, Anderson CL, Britton T, Elzanowski A, Johansson US, Källersjö M, Ohlson JI, Parsons TJ, Zuccon D, Mayr G. Diversification of Neoaves: integration of molecular sequence data and fossils. Biology Letters. 2006;2(4):543–547.
43. Brown JW, Payne RB, Mindell DP. Nuclear DNA does not reconcile 'rocks' and 'clocks' in Neoaves: a comment on Ericson et al. Biology Letters. 2007;3(3):257–261.
44. Garcia-R JC, Trewick S. Dispersal and speciation in purple swamphens (Rallidae: Porphyrio). The Auk. 2015;132:140–155.
45. Garcia-R JC, Elliott G, Walker K, Castro I, Trewick SA. Trans-equatorial range of a land bird lineage (Aves: Rallidae) from tropical forests to subantarctic grasslands. Journal of Avian Biology. 2016;47:219-226.
46. Garcia-R JC, Joseph L, Adcock G, Reid J, Trewick SA. Interisland gene flow among populations of the buff-banded rail (Aves: Rallidae) and its implications for insular endemism in Oceania.Journal of Avian Biology. 2017;48:679-690.
47. Garcia-R JC, González-Orozco CE, Trewick S. Contrasting patterns of diversification in a bird family (Aves: GruiformesRallidae) are revealed by
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analysis of geospatial distribution of species and phylogenetic diversity. Ecography.2019;42:500–510.
48. Chen P, Han Y, Zhu C, Gao B, Ruan L. Complete mitochondrial genome of *Porzanafusca* and *Porzanapusilla* and phylogenetic relationship of 16 Rallidae species. Genetica. 2017;145(6):559–573.
49. Gong J, Zhao R, Huang Q, Sun X, Huang L, Jing M. Two mitogenomes in Gruiformes (*Amaurornisakool*/*A. phoenicurus*) and the phylogenetic placement of Rallidae. Genes and Genomics. 2017;39:987-995.
50. Murphy W, Eizirik E, Johnson W, Zhang Y, Ryder O, O'Brien S. Molecular phylogenetics and the origins of placental mammals. Nature. 2001;409:614-618.
51. Pereira F, Carneiro J, Amorim A. Identification of species with DNA-based technology: current progress and challenges. Recent Patents on DNA and Gene Sequences. 2008;2(3):187-99.
52. Townsend T, Alegre R, Kelley S, Wiens J, Reeder T. Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles.Molecular Phylogenetics and Evolution. 2008;47(1):129-42.

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