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Phylogenetic Investigation of *Megapodius cumingii* from Kabetan, Central Sulawesi, Based on the RDP1 Gene

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Abstract: This study investigated the phylogenetic relationships of *Megapodius cumingii* using bioinformatic analyses. Blood samples were collected from the Kabetan Island population, followed by DNA extraction using the QIAamp DNA Blood Mini Kit, PCR amplification with Takara Ex Taq™ and RDP1.U1/RDP1.L1 primers, and sequencing with BigDye Terminator v3.1 ABI PRISM 3100. Chromatograms were converted into nucleotide sequences using BioEdit, and sequence similarity was assessed using NCBI BLAST. Multiple sequence alignment, model selection, nucleotide composition analysis, and phylogenetic reconstruction were performed in MEGA 12. The RDP1 gene of *M. cumingii* from Kabetan showed a consistent nucleotide composition with high GC content (55.27%) and high similarity to *M. cumingii* sequence KF834037.1, with substitutions dominated by transitions (A↔G, T↔C). Maximum likelihood analysis under the T92+G model (1.03) revealed three major clades, with clades 1 and 2 strongly supported (bootstrap = 100). The Kabetan population formed a monophyletic subclade with KF834037.1 (bootstrap = 86) and showed a very short branch length (~0.05 substitutions/site), indicating low intraspecific divergence, with *Megapodius tenimberensis* as the closest sister taxon. These findings support the utility of RDP1 for phylogenetic resolution within Megapodiidae and provide a preliminary basis for conservation planning and population connectivity assessment of *M. cumingii* in Wallacea.

Keywords: *Megapodius cumingii*; Megapodiidae; phylogenetics; RDP1 gene

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INTRODUCTION

The family Megapodiidae, within the order Galliformes, has been a major focus of molecular data-based phylogenetic research over the past decade (Kimball et al., 2021; Stenhouse et al., 2022; Zhao et al., 2024). The wide range of conservation statuses within this family, from Least Concern to Endangered, together with their distinctive egg-incubation strategy that relies on environmental heat, underscores the urgency of continued research on this group (Radley et al., 2018, 2024; Samad et al., 2022; Yuda & Saputra, 2021). One member of this family is *Megapodius cumingii* (Philippine scrubfowl), whose distribution extends across parts of Wallacea, including Kabetan Island in Central Sulawesi. Given the importance of clarifying phylogenetic relationships within Megapodiidae, with particular attention to *Megapodius cumingii* from Kabetan, the selection of an informative genetic marker is crucial for reliable phylogenetic reconstruction.

One promising genetic marker for resolving the phylogeny of *Megapodius cumingii* from Kabetan is intron 1 of the rhodopsin gene (RDP1). Outside the family Megapodiidae, RDP1 has been widely used to reconstruct phylogenetic relationships across diverse bird groups. For example, Cox et al. (2007) showed that RDP1, together with eight other nuclear genes, provided strong support for placing the family Odontophoridae as the basal lineage within a Phasianidae clade that excludes guineafowl. Lovette et al. (2010) also demonstrated that the inclusion of RDP1 along with six other introns produced a stable phylogenetic tree topology and provided a robust basis for revising the systematics of the family Parulidae. Furthermore, Lovette et al. (2012) included RDP1 as one of the genetic markers that helped reconstruct and clarify the phylogeny of the family Mimidae. Collectively, these findings demonstrate that RDP1 is a valid and informative genetic marker for avian taxa.

RDP1 has also emerged as a highly useful marker for elucidating phylogenetic relationships within the family Megapodiidae. Birks & Edwards (2002) showed that RDP1 effectively reconstructed megapode phylogeny and yielded a robust topology at the genus level. This finding was reinforced by Budiarsa et al. (2010), who reported that RDP1 was capable of generating a phylogenetic tree with similarly high topological stability at the genus level for *Macrocephalon maleo*. In addition, Harris et al. (2014) integrated RDP1 with 15 other nuclear genes to examine biogeography, species trees, and the evolution of nesting behavior in Megapodiidae. These studies further support the relevance of RDP1 for phylogenetic analysis within Megapodiidae; however, they have not provided specific information on the phylogeny of *Megapodius cumingii* from Kabetan.

Based on these findings, a comprehensive phylogenetic investigation using the RDP1 gene is needed, given the limited scientific reports on the phylogeny of *Megapodius cumingii* from Kabetan. Therefore, this study aims to use the RDP1 gene as a genetic marker to reconstruct the phylogeny of *Megapodius cumingii* from Kabetan within the family Megapodiidae. These data are important for assessing population connectivity and establishing conservation priorities in insular landscapes characterized by geographic isolation, particularly because *Megapodius cumingii* from Kabetan represents a population inhabiting small islands within the Wallacea region.

METHOD

Sample collection and laboratory procedures

This study employed an exploratory descriptive design. Blood samples from two *Megapodius cumingii* individuals were collected from Kabetan Island, Central Sulawesi, Indonesia. DNA extraction was performed using the QIAamp DNA Blood Mini Kit. DNA amplification was subsequently carried out by PCR using Takara Ex Taq™ and the primers RDP1.U1 (forward) (5′-GTAACAGGGTGCTACATGA-3′) and RDP1 L1 (reverse) (5′-ACAGACACCACATATCGTT-3′), following Birks & Edwards (2002). Sequencing was then conducted using the BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3100 Avant Genetic Analyzer.

Data analysis

The sequencing chromatograms were converted into nucleotide sequences using BioEdit (Mahadani et al., 2016). BLAST analysis was then performed through the NCBI website (Wang & Gribskov, 2025). All subsequent data analyses were conducted in MEGA 12 (Kumar et al., 2024), including multiple alignment using ClustalW, selection of the best-fit DNA model, nucleotide composition analysis, substitution matrix estimation, and phylogenetic tree reconstruction. The phylogenetic analysis included 15 RDP1 sequences representing members of the family Megapodiidae obtained from NCBI (Table 1), together with the two *Megapodius cumingii* sequences from Kabetan Island.

Table 1. RDP1 sequence data for 15 species of the family Megapodiidae obtained from NCBI

No.	Accession code	Species	Sample origin	Reference
1	KF 834037.1	*Megapodius cumingii*	Tangkoko Dua Saudara Nature Reserve, Indonesia	Harris et al. (2014)
2	KF 834038.1	*Megapodius decollatus*	Crater Mountain, Papua New Guinea	Harris et al. (2014)
3	KF 834048.1	*Megapodius laperouse*	Mariana Islands, Micronesia	Harris et al. (2014)
4	AF 394652.1	*Megapodius eremita*	Solomon Islands, Papua New Guinea	Birks & Edwards (2002)
5	AF 394654.1	*Megapodius freycinet*	Waigeu Island, Indonesia	Birks & Edwards (2002)
6	AF 394656.1	*Megapodius layardi*	Ambrym Islands, Vanuatu	Birks & Edwards (2002)
7	AF 394658.1	*Megapodius pritchardii*	Motu MoleMole, Tonga	Birks & Edwards (2002)
8	AF 394658.1	*Megapodius reinwardt*	Mission Beach, Australia	Birks & Edwards (2002)
9	AF 394659.1	*Megapodius tenimberensis*	Tanimbar Island, Indonesia	Birks & Edwards (2002)
10	AF 394651.1	*Eulipoa wallacei*	Haruku Island, Indonesia	Birks & Edwards (2002)
11	AF 394649.1	*Macrocephalon maleo*	Captive individual, Bronx Zoo, USA	Birks & Edwards (2002)
12	AF394644.1	*Alectura lathami*	Brisbane, Australia	Birks & Edwards (2002)
13	AF 394645.1	*Aepypodius arfakianus*	Tetebedi, Papua New Guinea	Birks & Edwards (2002)
14	AF 394647.1	*Leipoa ocellata*	New South Wales, Australia	Birks & Edwards (2002)
15	AF 394648.1	*Talagella fuscirostris*	Veimauri, Papua New Guinea	Birks & Edwards (2002)

RESULTS AND DISCUSSION

Nucleotide Diversity of the RDP1 Gene

The nucleotide diversity of the RDP1 gene in this study was characterized using nucleotide composition data (Table 2) and a nucleotide substitution matrix (Table 3). The RDP1 nucleotide composition of *Megapodius cumingii* from Kabetan showed a highly consistent pattern across 758 base pairs, with T = 25.46%, C = 25.59%, A = 19.26%, and G = 29.68% (Table 2). This pattern was essentially identical to that of the reference *Megapodius cumingii* sequence KF834037.1 and remained comparable to those of other *Megapodius* species, which generally fell within the ranges of T = 25–27%, C = 25–26%, A = 19–21%, and G = 28–30%. The GC content of *Megapodius cumingii* from Kabetan was approximately 55.27%, exceeding the AT content and indicating GC dominance, which was also observed in most other members of the family Megapodiidae. High GC content contributes to greater DNA thermal stability because of stronger hydrogen bonding and nucleotide stacking interactions (Ke et al., 2025).

The compared sequences were dominated by lengths of 760 base pairs within the genus *Megapodius*, whereas the *Megapodius cumingii* samples from Kabetan were slightly shorter at 758 base pairs. Several genera outside *Megapodius* possessed somewhat longer sequences, exceeding 800 base pairs. These differences likely resulted from the presence of indels among taxa; however, after multiple sequence alignment, informative sites were retained, and both the compositional pattern and the transition-dominated substitution pattern remained consistent.

Table 2. Nucleotide composition of the RDP1 gene in *Megapodius cumingii* from Kabetan and comparative Megapodiidae taxa

No	Accession Code	Species	Nucleotide Content (%)				Total Base Pairs
			T	C	A	G	
1	-	Megapodius cumingii Kabetan 1	25.46	25.59	19.26	29.68	758
2	-	Megapodius cumingii Kabetan 2	25.46	25.59	19.26	29.68	758
3	KF 834037.1	*Megapodius cumingii*	25.46	25.59	19.39	29.55	758
4	KF 834038.1	*Megapodius decollatus*	25.92	25.26	19.34	29.47	760
5	AF 394652.1	*Megapodius eremita*	26.18	25.00	19.34	29.47	760

No	Accession Code	Species	Nucleotide Content (%)				Total Base Pairs
			T	C	A	G	
6	KF 834048.1	*Megapodius laperouse*	26.05	25.13	19.34	29.47	760
7	AF 394654.1	*Megapodius freycinet*	26.05	25.13	19.34	29.47	760
8	AF 394656.1	*Megapodius layardi*	25.92	25.26	19.21	29.61	760
9	AF 394658.1	*Megapodius pritchardii*	25.92	25.26	19.21	29.61	760
9	AF 394658.1	*Megapodius reindwart*	26.05	25.00	19.08	29.87	760
10	AF 394659.1	*Megapodius tenimberensis*	25.79	25.26	19.34	29.61	760
11	AF 394651.1	*Eulipoa wallacei*	26.72	25.37	18.63	29.29	816
12	AF 394649.1	*Macrocephalon maleo*	24.91	26.89	18.22	29.99	807
13	AF 394644.1	*Alectura lathami*	25.68	25.68	18.43	30.22	814
14	AF 394645.1	*Aepypodius arfakianus*	25.68	25.80	18.55	29.98	814
15	AF 394647.1	*Leipoa ocellata*	26.11	25.87	18.94	29.08	808
16	AF 394648.1	*Talagella fuscirotris*	25.46	26.20	18.70	29.64	813

Substitution parameter analysis showed that molecular evolution within the sequence group including *Megapodius cumingii* from Kabetan had a maximum log likelihood value of -2569.049, based on a total of 672 nucleotide positions analyzed. The substitution matrix indicated a strong dominance of transitions over transversions, with the highest substitution rates occurring between purines (A↔G) and between pyrimidines (T↔C), with probability values reaching 19.04 and 15.39, respectively. In contrast, transversion rates, such as A↔T or G↔C, were substantially lower, ranging from 3.48 to 4.31. The overall higher frequencies of G and C nucleotides (27.66%) relative to A and T (22.34%) further supported the nucleotide composition analysis, confirming the high GC content of this gene.

Table 3. Nucleotide substitution matrix of the RDP1 gene in *Megapodius cumingii* from Kabetan and comparative Megapodiidae taxa using the maximum likelihood approach

	A	T	C	G
A	-	3,48	4,31	**19,04**
T	3,48	-	**19,04**	4,31
C	3,48	**15,39**	-	4,31
G	**15,39**	3,48	4,31	-

Phylogenetic Analysis

The accuracy of the phylogenetic tree topology was supported by the selection of the best-fit DNA model (Table 4). Based on the analysis of 19 nucleotide sequences comprising 672 positions, the Tamura 3-parameter model with Gamma distribution (T92+G) under the maximum likelihood framework was identified as the best-fitting model. This selection was based on the lowest Bayesian Information Criterion (BIC) value of 5497.567 and a Corrected Akaike Information Criterion (AICc) value of 5214.522. Determining the most appropriate substitution model is a crucial step in phylogenetic analysis because it helps minimize errors in tree topology reconstruction (Garg & Hochberg, 2025; Sharma & Kumar, 2022). The selection of the T92+G model indicates that the analyzed sequence data were characterized by GC dominance and variation in substitution rates among nucleotide sites. The Gamma parameter (+G) value of 1.03 further confirms that not all nucleotide positions evolved at the same rate and that some sites were more conserved than others.

Table 4. Maximum likelihood approach for assessing the goodness of fit of various substitution models, including parameter estimation

Model	Parameters	BIC	AICc	*lnL*	(+*I*)	(+*G*)	*R*	*f*(A)	*f*(T)	*f*(C)	*f*(G)	*r*(AT)	*r*(AC)	*r*(AG)	*r*(TA)	*r*(TC)	*r*(TG)	*r*(CA)
T92+G	38	5497.567	5214.522	-2569.144	n/a	1.03	2.19	0.223	0.223	0.277	0.277	0.035	0.043	0.190	0.035	0.190	0.043	0.035
T92+I	38	5499.188	5216.142	-2569.955	0.34	n/a	2.15	0.223	0.223	0.277	0.277	0.035	0.044	0.189	0.035	0.189	0.044	0.035
T92+G+I	39	5507.022	5216.534	-2569.144	0.00	1.03	2.19	0.223	0.223	0.277	0.277	0.035	0.043	0.190	0.035	0.190	0.043	0.035
K2+G	37	5509.207	5233.604	-2579.692	n/a	0.97	2.20	0.250	0.250	0.250	0.250	0.039	0.039	0.172	0.039	0.172	0.039	0.039
HKY+G	40	5509.787	5211.857	-2565.799	n/a	1.06	2.18	0.195	0.251	0.260	0.293	0.039	0.040	0.202	0.030	0.179	0.045	0.030
K2+I	37	5510.766	5235.163	-2580.471	0.35	n/a	2.17	0.250	0.250	0.250	0.250	0.039	0.039	0.171	0.039	0.171	0.039	0.039
HKY+I	40	5511.112	5213.182	-2566.462	0.33	n/a	2.15	0.195	0.251	0.260	0.293	0.039	0.041	0.201	0.031	0.178	0.046	0.031
T92	37	5513.325	5237.723	-2581.751	n/a	n/a	1.93	0.223	0.223	0.277	0.277	0.038	0.047	0.183	0.038	0.183	0.047	0.038
K2+G+I	38	5518.662	5235.616	-2579.692	0.00	0.97	2.20	0.250	0.250	0.250	0.250	0.039	0.039	0.172	0.039	0.172	0.039	0.039
HKY+G+I	41	5519.053	5213.681	-2565.705	0.00	1.06	2.18	0.195	0.251	0.260	0.293	0.039	0.040	0.202	0.030	0.179	0.045	0.030
TN93+G	41	5519.148	5213.776	-2565.753	n/a	1.06	2.18	0.195	0.251	0.260	0.293	0.039	0.040	0.208	0.030	0.175	0.045	0.030
TN93+I	41	5520.686	5215.314	-2566.522	0.33	n/a	2.15	0.195	0.251	0.260	0.293	0.039	0.041	0.206	0.031	0.175	0.046	0.031
HKY	39	5524.486	5233.998	-2577.877	n/a	n/a	1.93	0.195	0.251	0.260	0.293	0.042	0.044	0.194	0.033	0.172	0.049	0.033
K2	36	5526.951	5258.791	-2593.291	n/a	n/a	1.92	0.250	0.250	0.250	0.250	0.043	0.043	0.164	0.043	0.164	0.043	0.043
TN93+G+I	42	5528.414	5215.601	-2565.659	0.00	1.06	2.18	0.195	0.251	0.260	0.293	0.039	0.040	0.208	0.030	0.175	0.045	0.030
TN93	40	5533.754	5235.824	-2577.783	n/a	n/a	1.93	0.195	0.251	0.260	0.293	0.042	0.044	0.200	0.033	0.167	0.049	0.033
GTR+G	44	5533.959	5206.263	-2558.976	n/a	1.02	2.17	0.195	0.251	0.260	0.293	0.028	0.052	0.207	0.022	0.175	0.022	0.039
GTR+I	44	5535.233	5207.537	-2559.613	0.34	n/a	2.14	0.195	0.251	0.260	0.293	0.028	0.053	0.205	0.022	0.175	0.023	0.040
GTR+G+I	45	5543.601	5208.465	-2559.070	0.00	1.02	2.17	0.195	0.251	0.260	0.293	0.028	0.052	0.207	0.022	0.175	0.022	0.039
GTR	43	5550.024	5229.769	-2571.736	n/a	n/a	1.93	0.195	0.251	0.260	0.293	0.033	0.056	0.200	0.026	0.168	0.028	0.042
JC+G	36	5649.093	5380.933	-2654.362	n/a	1.27	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+I	36	5650.948	5382.788	-2655.289	0.30	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+G+I	37	5658.547	5382.945	-2654.362	0.00	1.27	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC	35	5658.590	5397.873	-2663.838	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083

Phylogenetic tree reconstruction included RDP1 sequences from other Galliformes, namely *Ortalis vetula* (family Cracidae) and *Numida meleagris* (family Numididae), obtained from NCBI as outgroups. The resulting phylogenetic topology revealed three major clades, with the principal nodes of clades 1 and 2 supported by very high bootstrap values (100) (Figure 1). Clade 1, one of the ingroups, comprised all members of the genus *Megapodius* together with the genus *Eulipoa*, with low to high bootstrap support (61–86) across several internal branches. Within this clade, *Megapodius cumingii* from Kabetan formed a monophyletic group with the reference sequence *Megapodius cumingii* KF834037.1, supported by bootstrap values ranging from low to high (61–86), and exhibited a very short branch length (scale = 0.05 substitutions/site), indicating low intraspecific genetic distance. Clade 2, which was also part of the ingroup, included non-*Megapodius* and non-*Eulipoa* megapode genera, namely *Macrocephalon*, *Talegalla*, *Leipoa*, *Alectura*, and *Aepypodius*, with low to high bootstrap support (67–99). Clade 3, the outgroup, consisted of *Numida meleagris* and *Ortalis vetula*, thereby stabilizing the root topology.

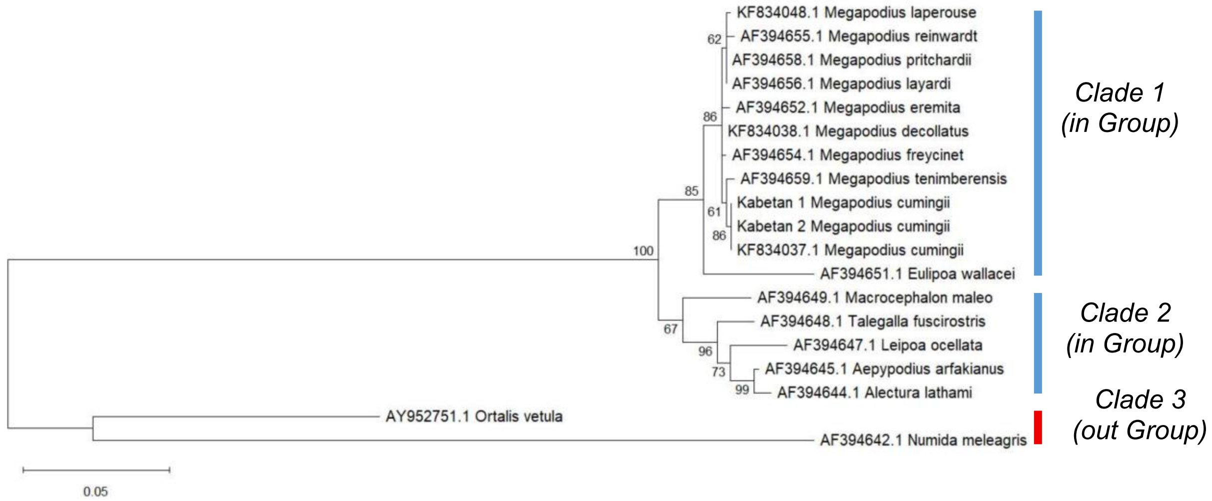

Figure 1. Phylogenetic tree reconstructed using the maximum likelihood approach under the Tamura 3-parameter model

The clustering of *Megapodius cumingii* from Kabetan with *Megapodius cumingii* KF834037.1, supported by a bootstrap value of 86, confirms that the sample is taxonomically consistent with *Megapodius cumingii* and derives from a very closely related population unit, as indicated by the low divergence observed on the 0.05 substitutions/site scale. This subclade was embedded within clade 1 together with other *Megapodius* species, with *Megapodius tenimberensis* representing the closest sister taxon. This finding is supported by Novitasari et al. (2025), who reported a megapode phylogenetic tree topology based on the EEF2 gene that is similar to that obtained in the present study. The low to high branch support within clade 1 indicates sufficient phylogenetic signal to discriminate among species, whereas the short branches within the *Megapodius cumingii* group suggest limited nucleotide diversity in the analyzed gene. This pattern is consistent with the nucleotide composition results and substitution profile, both of which indicate a tendency toward conservation in this gene region. The topology separating *Megapodius* and *Eulipoa* in clade 1 from the other megapode genera in clade 2, as well as from the outgroup in clade 3, was supported by a very high bootstrap value (100), thereby supporting the monophyly of Megapodiidae and strengthening the validity of the identification of *Megapodius cumingii* from Kabetan based on the RDP1 gene.

CONCLUSION

The maximum likelihood phylogenetic topology based on the RDP1 gene showed that, at the intrageneric level within *Megapodius*, *Megapodius cumingii* from Kabetan formed a subclade with the reference *Megapodius cumingii* sequence KF834037.1 (bootstrap = 86), with a very short branch length indicating low intraspecific divergence, and its closest sister taxon was *Megapodius tenimberensis*. At the intergeneric level, the clade containing *Megapodius* and *Eulipoa* was clearly separated from the clade comprising *Macrocephalon*, *Talegalla*, *Leipoa*, *Alectura*, and *Aepypodius* (bootstrap = 67–99), while the outgroups *Numida meleagris* and *Ortalis vetula* stabilized the root and confirmed the monophyly of Megapodiidae (bootstrap at the principal node = 100). The nucleotide composition data, substitution pattern, and selection of the T92+G model consistently supported the taxonomic identification of *Megapodius cumingii* from Kabetan within the family Megapodiidae.

RECOMMENDATION

Future studies should increase the sample size at each location and expand the population coverage of *Megapodius cumingii* across islands. This is essential for generating more accurate maps of nucleotide diversity and phylogenetic relationships, producing more precise estimates, and enabling stronger statistical analyses.

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