

Electronic Supplementary Material for:

Diversification of Neoaves: integration of molecular sequence data and fossils

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Samples and sequence data

DNA was obtained from blood or tissue samples of 87 species in Neoaves representing 73 non-passerine and two passerine families. Two paleognaths, one megapode and one screamer were used as outgroups. Sample information and GenBank accession numbers are given in table ESM-1. The same species and individuals were sequenced when possible, but in a few cases when amplification failed we had to use sequences from other individuals or species.

Five gene regions were sequenced: *c-myc* (exon 3), RAG-1, myoglobin (intron 2), β -fibrinogen (intron 7) and ornithine decarboxylase (ODC) (introns 6 and 7, along with the intercepting exon 7). Laboratory procedures for the extraction, PCR-amplification, and sequencing follow published protocols (Prychitko & Moore 1997; Ericson et al. 2000; Irestedt et al. 2001; Johansson et al. 2002; Irestedt et al. 2002; Allen & Omland 2003). For myoglobin no sequences were obtained for *Rhinopomastus* and *Upupa*, and only the 3' end could be sequenced for *Fregata*. For β -fibrinogen we could not obtain sequences for *Upupa epops*. The β -fibrinogen sequences of the two parrot species were highly autapomorphic. *Indicator*, *Megalaima* and *Picumnus* could not be sequenced for ornithine decarboxylase. Especially for β -fibrinogen we used sequences published in GenBank (table ESM-1). Most often we could use sequences obtained from individuals of the same species or genus as our own samples, but in a few cases we instead used a representative of another genus of the same family. The effect of this on tree topology is probably negligible given the short genetic distances between extant members within a family compared to the distance to the closest taxon outside the family.

Alignment of the protein-coding genes (*c-myc* and RAG-1) was unproblematic with few indels observed. Also the myoglobin, β -fibrinogen and ODC introns could be aligned by eye although the number of indels was larger. The preferred alignments can be downloaded at <http://www.nrm.se/inenglish/researchandcollections/zoology/vertebratezoology/birds.4.4e32c81078a8d9249800014590.html>. The concatenated, aligned data set consist of 4408 bp (after the exclusion of ambiguously aligned regions) of which 2400 (54%) are potentially phylogenetically informative.

Phylogenetic analysis

The models for nucleotide substitutions were selected for each gene individually using the Akaike Information Criterion (Akaike 1973). The program MrModeltest 2.2 (Nylander 2002) in conjunction with PAUP* (Swofford 1998) was used to evaluate the fit of the data to different models for nucleotide substitutions. The same models and parameter settings chosen for the individual genes were also used for these partitions in the analysis of the combined data set. The posterior probabilities for tree, branch lengths and model parameters were approximated with a Metropolis-coupled Markov chain Monte Carlo (MCMCMC) Bayesian analysis as implemented in the computer program MrBayes 3.1.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). For each gene region, we ran duplicate analyses of 4 million generations each with trees sampled every 100 generation. The saved trees from each

analysis were pooled and the posterior probabilities were calculated after discarding the trees saved during the “burn-in phase” (as estimated graphically). The analysis of the combined data set was based on the 70,000 trees saved after “burn-in”. Substitution models, base frequencies, and parameter settings used in the analysis (after reaching stability) are listed in table ESM2.

In most analyses we used one galliform, one anseriform, and two palaeognathous birds as outgroup following the well-established understanding that Galloanseres and Neoaves are sistergroups within Neognathae, and that Palaeognathae is the sister of Neognathae (Groth & Barrowclough 1999). The five gene regions were analyzed individually (figures ESM-1 to ESM-5). The β -fibrinogen data yielded the most resolved tree and in order to investigate the influence of this gene region upon the results we also analyzed the data set with β -fibrinogen excluded (figure ESM-6). To investigate the influence by the choice of outgroup we also excluded the paleognaths and only used the two representatives of Galloanseres. All parts of the tree topology and the respective node supports remained essentially identical with one important exception; “Metaves” was not recovered as monophyletic when using only the galliform and anseriform birds as outgroups (figure ESM-7).

We also estimated the phylogenetic relationships within Neoaves by parsimony jackknifing (Farris et al. 1996) as implemented in *XAC: Parsimony Jackknifer* (Farris 1997). The analysis was performed with 1,000 replicates, each with 10 random additions of taxa and branch swapping. The overall topology and clade support values in the resulting tree (figure ESM-8) do not contradict those obtained in the Bayesian analysis.

We have searched preliminary neighbour-joining trees and likelihood phylograms for cases where long-branch attractions may be suspected to have caused artificial groupings of taxa, but we have found no obvious such case.

Divergence time estimations

Divergence times were estimated using two rate smoothing methods, PATHd8 (Britton et al. 2006) and penalized likelihood (PL) (Sanderson 2002), the latter as implemented in the software r8s 1.7 (Sanderson 2003). Both methods need a topology with branch lengths, plus an arbitrary number of calibrations for calculation of absolute ages. The same topology and constraints were used as input for both methods.

PATHd8 is a non-parametric method, which smoothes substitution rates sequentially by taking averages over paths lengths from an internode to all its descending terminals. The smoothing is thereby done between sister groups, as opposed to most other methods, where rate smoothing is done between mother and daughter lineages. This has the effect of preserving more of the pattern of heterogeneous branch lengths that we find in the phylogram. An other property of the method is that zero or near-zero branchlengths collapse, which seems reasonable, considering that these branch lengths probably represents short time or uncertainties in the phylogeny (or both).

PL is a semi-parametric method, which smoothes substitution rate variation simultaneously over the whole tree. A maximum likelihood model, that overfits the data, is combined with a smoothing factor, and a penalty for fast rate changes between mother and daughter lineages. The optimal smoothing value for the data set is chosen objectively by a statistical cross-validation procedure. In this data set, the smoothing value was set to 13, which is of the same magnitude as most non clock-like data sets.

Both PL and PATHd8 need one calibration point to be fixed in age. For this purpose we used a 47.5 MY old fossil assigned to the stem group of hummingbirds. The r8s program also requires the root node to be constrained either by a fixed age or a maximum age. We chose to set a maximum age of 95 MY on the divergence of the Neoaves, based on the age obtained from the PATHd8 analysis. An additional set of 21 stratigraphically and phylogenetically

well-studied fossils were used as minimum age constraints. All fossils are stem group representatives of extant higher-level taxa, and provide a minimum age for the divergence of the total group (stem and crown group).

All fossils used for calibrations are placed in table ESM-3. Chronograms from PATHd8 and r8s are compared in figure ESM-9. Age estimates for major bird groups, obtained from both dating methods, are found in table ESM-4.

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Figure and table legends

Table ESM-1. Samples used in the study. Taxonomy follows Morony et al. (1975). Museum acronyms: AM, Australian Museum; ANSP, Academy of Natural Sciences, Philadelphia; FMNH, Field Museum, Chicago; LSUMZ, Museum of Natural Science, Louisiana State University; MV, Museum Victoria; NMWN, National Museum of Natural History, Windhoek; NRM, Swedish Museum of Natural History; PFIAO, Percy Fitzpatrick Institute of African Ornithology; SMF, Senckenberg Museum; UWBM, Burke Museum of Natural History and Culture; ZMCU, Zoological Museum, University of Copenhagen. NS = not sequenced.

Table ESM-2. Mean Bayes estimates of parameters for the five studied genetic markers. Substitution rate parameters are calculated with the rate for G \leftrightarrow T set to 1. The models for nucleotide substitutions for the individual genes were used also in the analysis of the concatenated data set.

Table ESM-3. Table 3. Fossils used for the calibration of the molecular clock in the analyses. Note that “stem group” means that the taxon is outside the clade including the stem species of the modern taxa and its descendants, “crown group” means that it is within. All fossils provided minimum ages for the clades, except Q that was used as a fixed age for the split between hummingbirds and the swifts/treeswifts lineage in the PATHd8 analysis.

Table ESM-4. Comparison of age estimates of major groups, obtained from the PL and PATHd8 analyses. Note that the maximum age for Neoaves is set to 95 MYA based on the result in the initial PATHd8 analysis. Ages in MYA, rounded to nearest integer.

Figure ESM-1. Bayesian tree based on *c-myc* exon 3. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

Figure ESM-2. Bayesian tree based on RAG-1. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

Figure ESM-3. Bayesian tree based on myoglobin intron 2. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

Figure ESM-4. Bayesian tree based on β -fibrinogen intron 7. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

Figure ESM-5. Bayesian tree based on ornithine decarboxylase (ODC) introns 6 and 7, along with the intercepting exon 7. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

Figure ESM-6. Bayesian tree based on all genes except **S**fibrinogen in order to assess the influence of this gene upon the tree topology. Taxa included in Metaves are marked with red. All nodes recovered in 50% or more of the sampled trees are shown (only

nodes with 95% or larger posterior probabilities are labeled).

Figure ESM-7. Bayesian tree based on all genes, but with the paleognaths excluded and rooted with Galloanseres. All nodes recovered with 95% (or larger) posterior probabilities are shown.

Figure ESM-8. Parsimony jack-knifing tree (Farris et al. 1996) as implemented in XAC: *Parsimony Jackknifer* (Farris 1997). The analysis was performed with 1,000 replicates, each with 10 random additions of taxa and branch swapping.

Figure ESM-9. Comparison between the results obtained from the PATHd8 and the PL analyses. The red PL chronogram suggests an earlier divergence, than the black, dotted chronogram from the PATHd8 analysis. The PL tree is less consistent with the fossil record, in the sense that it adds a "ghost range" of in average 21 MY to the fossil constrained nodes. The boundary between the Cretaceous and Tertiary at 65 MYA is marked with a green line.

Table ESM-1. Samples used in the study. Taxonomy follows Morony et al. (1975). Museum acronyms: AM, Australian Museum; ANSP, Academy of Natural Sciences, Philadelphia; FMNH, Field Museum, Chicago; LSUMZ, Museum of Natural Science, Louisiana State University; MV, Museum Victoria; NMWN, National Museum of Natural History, Windhoek; NRM, Swedish Museum of Natural History; PFIPO, Percy Fitzpatrick Institute of African Ornithology; SMF, Senckenberg Museum; UWBM, Burke Museum of Natural History and Culture; ZMCU, Zoological Museum, University of Copenhagen. NS = not sequenced. References: 1) Lerner & Mindell 2005, 2) Fain & Houde 2004, 3) Ericson et al. 2001, 4) Groth & Barrowclough 1999, 5) Johansson & Ericson 2003, 6) Pereira et al. 2002, 7) Johansson et al. 2001, 8) Thomassen et al. 2005, 9) Moyle 2004, 10) Ericson et al. 2003b., 11) Johnson & Clayton 2000, 12) Ericson & Johansson 2003, 13) Ericson et al. 2003c, 14) Mayr et al. 2003, 15) Johansson & Ericson 2005, 16) Mayr & Ericson 2004, 17) Pritchko & Moore 2003, 18) Johansson et al. 2002, 19) Fjeldsá et al. 2003, 20) Chesser 2004.

Family	Species	Sample used for new sequences	c-myc	RAG-1	Myoglobin	Beta-fibrinogen	ODC
Accipitridae	<i>Accipiter gentilis</i>	NRM 966488	new	new	new	new	new
Accipitridae	<i>Heterospizias meridionalis</i>	NRM 947034	new	new	new	new	new
Accipitridae	<i>Leptodon cayanesis</i>	NRM 947125	new	new	new	AY987168 (1)	new
Aegothelidae	<i>Aegotheles albertsii</i>	MV E044	new	new	new		new
Aegothelidae	<i>Aegotheles cristatus</i>					AY695138 (2)	
Alcedinidae	<i>Alcedo atthis</i>	NRM 968171	AF295143 (7)	AF294671 (7)	AY165800 (5)		new
Alcedinidae	<i>Ceryle alcyon</i>					AY695158 (2)	
Anhimidae	<i>Chauna torquata</i>	NRM uncat.	AY034413 (3)	AF143728 (4)	AY165805 (5)	AY140702 (6)	new
Anhingidae	<i>Anhinga anhinga</i>	NRM 947129	new	new	new	AY695210 (2)	new
Apodidae	<i>Apus apus</i>	ZMCU P3	AF295136 (7)	AF294664 (7)	new	AY513096 (8)	new
Apterygidae	<i>Apteryx australis</i>	NRM uncat.	new	new	new	new	new
Aramidae	<i>Aramus guarauna</i>	NRM 947123	new	new	new	AY695250 (2)	new
Ardeidae	<i>Ardea alba</i>					AY695235	
Ardeidae	<i>Ardea cocoi</i>	NRM 947128	new	new	new		new
Ardeidae	<i>Tigrisoma lineatum</i>	NRM 937362		new	new	new	new
Ardeidae	<i>Tigrisoma lineatum</i>	LMS B 1212	AY034420 (3)				
Balaenicipitidae	<i>Balaeniceps rex</i>	LSUMZ B-13372	new	new	new	AY695218 (2)	new
Brachypteraciidae	<i>Brachypteracias leptosomus</i>	FMNH 345686	AF295149 (7)	AF294676 (7)	new	new	new
Bucconidae	<i>Nystalus maculatus</i>	NRM 947240	AF295153 (7)	AF294680 (7)	AY165817 (5)	new	new
Bucerotidae	<i>Tockus erythrorhynchus</i>	ZMCU P487	AF295152 (7)	AF294679 (7)	new		new
Bucerotidae	<i>Tockus flavirostris</i>					AY695160 (2)	
Cacatuidae	<i>Cacatua sulphurea</i>	NRM 20026154	new	new	NS	new	new
Capitonidae	<i>Megalaima virens</i>	LSUMZ B-20788	AY165829 (5)	AY165793 (5)	AY165814 (5)	AY279227 (9)	NS
Caprimulgidae	<i>Podager nacunda</i>	NRM 947016	AF295132 (7)	AF294660 (7)	new	new	new
Cariamidae	<i>Cariama cristata</i>	LSUMZ B-8656	new	new	new	AY695168 (2)	new
Cathartidae	<i>Cathartes aura</i>	NRM 956732	new	new	new	AY695169 (2)	new
Cathartidae	<i>Coragyps atratus</i>	NRM 947124	new	new	new	AY695170 (2)	new
Charadriidae	<i>Charadrius collaris</i>	NRM uncat.	new	AY339106 (10)	AY339079 (10)		new
Charadriidae	<i>Charadrius vociferus</i>					AY695205 (2)	
Ciconiidae	<i>Jabiru mycteria</i>	NRM 947193	new	new	new	new	new
Ciconiidae	<i>Mycteria americana</i>	NRM 956665	new	new	new	AY695227 (2)	new
Coliidae	<i>Colius striatus</i>	ZMCU P398	new	AF294669 (7)	new	AY695164 (2)	new
Columbidae	<i>Scardafella squammata</i>	NRM 956728	new	new	new	AF182651 (11)	new
Coraciidae	<i>Coracias caudata</i>	NMWN 750	AF295148 (7)	AF143737 (4)	AY165807 (5)	AY695155 (2)	new
Cuculidae	<i>Cuculus canorus</i>	NRM 996341	AF295127 (7)	AF294655 (7)	AY165808 (5)	new	new
Cuculidae	<i>Guira guira</i>	NRM 937391	AY165835 (5)	AY165799 (5)	AY165818 (5)	new	new
Diomedidae	<i>Diomedea nigripes</i>	LSUMZ B-13340	new	new	new	new	new
Diomedidae	<i>Phoebastria palpebrata</i>	MV 979	new	new	new	new	new
Eurypygidae	<i>Eurypyga helias</i>	LSUMZ B-20749	new	new	new	AY695141 (2)	new

Falconidae	<i>Falco ruficularis</i>	NRM uncat.		new				
Falconidae	<i>Falco subbuteo</i>	????	new		new	new	new	new
Falconidae	<i>Polyborus plancus</i>	NRM 947200	new	new		new		
Fregatidae	<i>Fregata magnificens</i>	NRM uncat.	new	new	new			
Fregatidae	<i>Fregata minor</i>						AY695216 (2)	
Galbulidae	<i>Galbula cyanescens</i>	NRM uncat.	AF295155 (7)	AF294682 (7)	AY165810 (5)		AY695154 (2)	new
Gaviidae	<i>Gavia arctica</i>	NRM 976202	new	AF143733 (4)	new			new
Gaviidae	<i>Gavia immer</i>						AY695223 (2)	
Gruidae	<i>Grus canadensis</i>	NRM uncat.	new	AY339110 (10)	AY339083 (10)			
Gruidae	<i>Grus grus</i>	????					AY695255 (2)	new
Heliornithidae	<i>Podica senegalensis</i>	NMWN 1827	new	new	new		AY695247 (2)	new
Hemiprocidae	<i>Hemiprocne longipennis</i>	ANSP 1273	AF295137 (7)	AF294665 (7)	new		new	new
Hydrobatidae	<i>Hydrobates pelagicus</i>	ZMCU P734	new	new	new		new	new
Indicatoridae	<i>Indicator minor</i>	S.A. 2000:66	AY165830 (5)	AY165794 (5)	AY165812 (5)		AY279222 (9)	NS
Jacaniidae	<i>Jacana jacana</i>	NRM 937364	new	AY339112 (10)	AY339085 (10)			new
Jacaniidae	<i>Jacana spinosa</i>						AY695179 (2)	
Laniidae	<i>Lanius collurio</i>	NRM 986403	AY228000 (12)	new	new		AY395617 (13)	new
Leptosomidae	<i>Leptosomus discolor</i>	FMNH uncat.	AY233355 (14)	AY233361 (14)	AY233370 (14)		new	new
Megapodiidae	<i>Alectura lathami</i>	LSUMZ B-20851	AF296417 (7)	AF294687 (7)	AY165801 (5)		new	new
Meropidae	<i>Merops viridis</i>	ZMCU P935	AF295147 (7)	AF294675 (7)	AY165815 (5)		AY600483 (15)	new
Mesitornithidae	<i>Mesitornis unicolor</i>	FMNH 345610	new	AY756082 (16)	AY756085 (16)		new	new
Mesitornithidae	<i>Monias benschi</i>	SMF 3734-AL	new	new	new		new	new
Momotidae	<i>Momotus momota</i>	NRM 947281	AF295170 (7)	AF295192 (7)	AY165816 (5)		AY695159 (2)	new
Musophagidae	<i>Corythaixoides leucogaster</i>	ZMCU P509	AF295126 (7)	new	new			new
Musophagidae	<i>Corythaixoides personata</i>						AY695208 (2)	
Nyctibiidae	<i>Nyctibius aethereus</i>	LSUMZ B-11236	AF295131 (7)	AF294659 (7)	new		new	new
Opisthocomidae	<i>Opisthocomus hoazin</i>	LSUMZ B-10753	AY 233351 (14)	AY233357 (14)	AY233363 (14)		AY695134 (2)	new
Otididae	<i>Afrotis atra</i>	LSUMZ B-8672	new	AY339100 (10)	AY339073 (10)		AY695149 (2)	new
Pandionidae	<i>Pandion haliaetus</i>	NRM 956317	new	new	new		AY695172 (2)	new
Pelecanidae	<i>Pelecanus erythrorhynchos</i>						AY695217 (2)	
Pelecanidae	<i>Pelecanus onocrotalus</i>	LSUMZ B-18956	new	new	new			new
Pelecanoididae	<i>Pelecanoides urinatrix</i>	MV 1870	new	new	new		new	new
Phaethontidae	<i>Phaethon rubricauda</i>	UWBM 68951	new	new	new		new	new
Phalacrocoracidae	<i>Phalacrocorax auritus</i>						AY695211 (2)	
Phalacrocoracidae	<i>Phalacrocorax carbo</i>	NRM 937348	new	new	new			new
Phoenicopteridae	<i>Phoenicopus chilensis</i>	NRM P5	AY034424 (3)	new	new			new
Phoenicopteridae	<i>Phoenicopus ruber</i>						AY695139 (2)	
Phoeniculidae	<i>Rhinopomastus cyanomelas</i>	ZMCU P916	AF295150 (7)	AF294677 (7)	NS		new	new
Picidae	<i>Picumnus cirratus</i>	NRM 976666	AF295174 (7)	AF295195 (7)	AY165819 (5)		new	NS
Podargidae	<i>Podargus strigoides</i>	S. Dunham uncat.	AF295134 (7)	AF294662 (7)	AY233372 (14)		AY082408 (17)	new
Podicepsitidae	<i>Podiceps cristatus</i>	ZMCU P726	new	new	new		new	new
Procellariidae	<i>Fulmarus glacialis</i>	ZMCU P760	new	new	new		new	new
Procellariidae	<i>Puffinus lherminieri</i>	NRM uncat.	new	new	new			new
Procellariidae	<i>Puffinus tenuirostris</i>						AY695220 (2)	
Psittacidae	<i>Pyrhura frontalis</i>	NRM 966989	new	new	new		new	new
Psophidae	<i>Psophia leucoptera</i>	LSUMZ B-10733	new	new	new		new	new
Psophidae	<i>Psophia viridis</i>						AY695249 (2)	
Pteroclididae	<i>Pterocles bicinctus</i>						AY695147 (2)	
Pteroclididae	<i>Pterocles gutturalis</i>	PFAO 37 YtS	new	AY339116 (10)	AY339089 (10)			new
Rallidae	<i>Aramides ypecaha</i>	NRM 937389	new	AY756084 (16)	AY756087 (16)		new	new
Rallidae	<i>Laterallus albigularis</i>	NRM uncat.	new	new	new			new

Rallidae	<i>Laterallus melanophaius</i>					AY695238 (2)	
Ramphastidae	<i>Pteroglossus castanotis</i>	NRM 937285	AF295159 (7)	AF294686 (7)	AY165821 (5)	new	new
Rheidae	<i>Rhea americana</i>	LSUMZ B-5799	new	new	new	new	new
Rhynchotidae	<i>Rhynchotus jubatus</i>	C. Lambert uncat.	new	new	new	AY695140 (2)	new
Rynchopidae	<i>Rynchops niger</i>	LSUMZ B-2457	new	AY339119 (10)	AY339092 (10)	AY695191 (2)	new
Sagittariidae	<i>Sagittarius serpentarius</i>	NMWN 2610F	new	new	new	AY695171 (2)	new
Scopidae	<i>Scopus umbretta</i>	LSUMZ B-16327	AY034419 (3)	new	new	AY695219 (2)	new
Spheniscidae	<i>Spheniscus humboldti</i>	NRM uncat.	new	AF143734 (4)	new	new	new
Steatornithidae	<i>Steatornis caripensis</i>	LSUMZ B-7474	AF295135 (7)	AF294663 (7)	AY233371 (14)	AY695135 (2)	new
Strigidae	<i>Asio flammeus</i>	S. Dunham uncat.	AF295129 (7)	AF294657 (7)	AY233366 (14)	new	new
Sulidae	<i>Sula bassana</i>	ZMCU P570	new	new	new	AY695213 (2)	new
Threskiornithidae	<i>Harpiprion caerulescens</i>	NRM 937350	AY034416 (3)	new	new	new	new
Threskiornithidae	<i>Theristicus caudatus</i>	NRM 966880	new	new	new	new	new
Todidae	<i>Todus mexicanus</i>	LSUMZ B-11311	AF295145 (7)	AF294673 (7)	AY165824 (5)	new	new
Trochilidae	<i>Hylocharis chrysura</i>	NRM 937161	AF295139 (7)	AF294667 (7)	AY233377 (14)	new	new
Trogonidae	<i>Trogon melanurus</i>	ZMCU P494	AF295142 (7)	AF294670 (7)	AY165828 (5)	AY600479 (15)	new
Turnicidae	<i>Turnix pyrrhothorax</i>	AM O.64938					new
Turnicidae	<i>Turnix varia</i>	AM LAB1062	new	AY756083 (16)	AY756086 (16)	AY695197 (2)	
Tyrannidae	<i>Todirostrum cinereum</i>	NRM 947036	AF453782 (18)	AF453796 (18)	AY338740 (19)	AY489421 (20)	new
Tytonidae	<i>Tyto alba</i>	????	new	new	new	AY082407 (17)	new
Upupidae	<i>Upupa epops</i>	ZMCU P502	AF295151 (7)	AF294678 (7)	NS	NS	new

Table ESM-2. Mean Bayes estimates of parameters for the five studied genetic markers. Substitution rate parameters are calculated with the rate for G \leftrightarrow T set to 1. The models for nucleotide substitutions for the individual genes were used also in the analysis of the concatenated data set.

	c-myc	RAG-1	myoglobin	fibrinogen	ODC
No. of sites (aligned sequence)	510	930	1069	1085	814
No. of variable sites (%)	180 (35%)	476 (51%)	663 (62%)	923 (85%)	584 (72%)
No. of informative sites (%)	131 (26%)	382 (41%)	502 (47%)	822 (76%)	513 (63%)
ML model selected	GTR+I+G	GTR+I+G	GTR+G	GTR+I+G	GTR+I+G
r(AC)	1.29	1.97	1.13	1.14	1.00
r(AG)	10.19	7.53	4.93	3.56	3.81
r(AT)	0.67	0.72	0.69	0.63	0.67
r(CG)	1.34	1.49	1.69	1.48	1.33
r(CT)	20.68	12.65	5.16	3.59	3.22
r(GT)	1.00	1.00	1.00	1.00	1.00
Shape	0.3259	0.9247	1.8110	5.8062	2.7865
Proportion of invariant sites	0.4269	0.3979	N/A	0.0054	0.1522

Table ESM-3. Fossils used for the calibration of the molecular clock in the analyses. Note that “stem group” means that the taxon is outside the clade including the stem species of the modern taxa and its descendants, “crown group” means that it is within. All fossils provided minimum ages for the clades, except Q that was used as a fixed age for the split between hummingbirds and the swifts/treeswifts lineage in the PATHd8 analysis.

Symbol in tree	Taxon	Geological dating	MYA	Reference
A	Crown group Pici (Ramphastidae, Indicatoridae, Picidae]	early Oligocene	30-34	Mayr 2005a
B	Stem group (Upupidae + Phoeniculidae)	early Eocene	47.5	Mayr 2000
C	Stem group (Coraciidae + Brachypteraciidae)	early Eocene	47.5	Mayr & Mourer-Chauviré 2000
D	Stem group Trogoniformes	early Eocene	53	Mayr 2005b
E	Stem group Coliiformes	latest Paleocene/earliest Eocene	55	Houde & Olson 1992
F	Stem group Strigiformes	Paleocene	55-65	Rich & Bohaska 1976
G	Crown group Pandionidae	late Eocene	37-38	Harrison & Walker 1976
H	Stem group Cariamidae	early Eocene	47.5	Peters 1995
I	Stem group Phalacrocoracidae	late Oligocene	25	Mayr 2001
J	Crown group Sulidae	early Oligocene	33-34	Mayr 2002
K	Stem group Fregatidae	early Eocene	53	Olson 1977
L	Stem group Sphenisciformes	Paleocene	55-65	Fordyce & Jones 1986
M	Crown group Balaenicipitidae	early Oligocene	30-34	Rasmussen et al. 1987
N	Crown group Heliornithidae	middle Miocene	14	Olson 2003
O	Stem group Jacanidae	early Oligocene	30-34	Rasmussen et al. 1987
P	Stem group Apodiformes (Apodidae, Hemiprocnidae, Trochilidae)	early Eocene	53	Mayr 2003
Q	Stem group Trochilidae	early Eocene	47.5	Mayr 2005c
R	Crown group Pteroclididae	early Oligocene	30-33	Mourer-Chauviré 1993
S	Stem group Phoenicopteriformes	early Oligocene	30-34	Mayr 2005d
T	Stem group Phaethontidae	late Paleocene	55-60	Bourdon et al. 2005
U	Stem group Galliformes	early Eocene	53	Mayr & Weidig 2004
V	Stem group (Gruidae + Aramidae)	early Oligocene	30-34	Mayr 2005e
X	Stem group Gaviiformes	early Oligocene	30-34	Mayr 2004

Table ESM-4. Comparison of age estimates of major groups, obtained from the PL and PATHd8 analyses. Note that the maximum age for Neoaves is set to 95 MYA based on the result in the initial PATHd8 analysis. Ages in MYA, rounded to nearest integer.

Clade name	Colour coding in Figure 1	PL - crown group ages	PATHd8 - crown group ages	Difference absolute age, PL - PATHd8
"Metaves"	purple + orange clades	81	67	14
"Caprimulgiforms", hummingbirds and swifts	purple clade	70	62	8
"Coronaves"	green + blue clades	87	74	13
Terrestrial and arboreal birds	green clades	86	68	18
Parrots, passerines, falcons, seriemas	light green clade	85	68	17
Aquatic and semi-aquatic birds (plus Cuculidae, Otididae and Musophagidae)	dark blue clade	83	60	23
Shorebirds	light blue clade	79	57	22

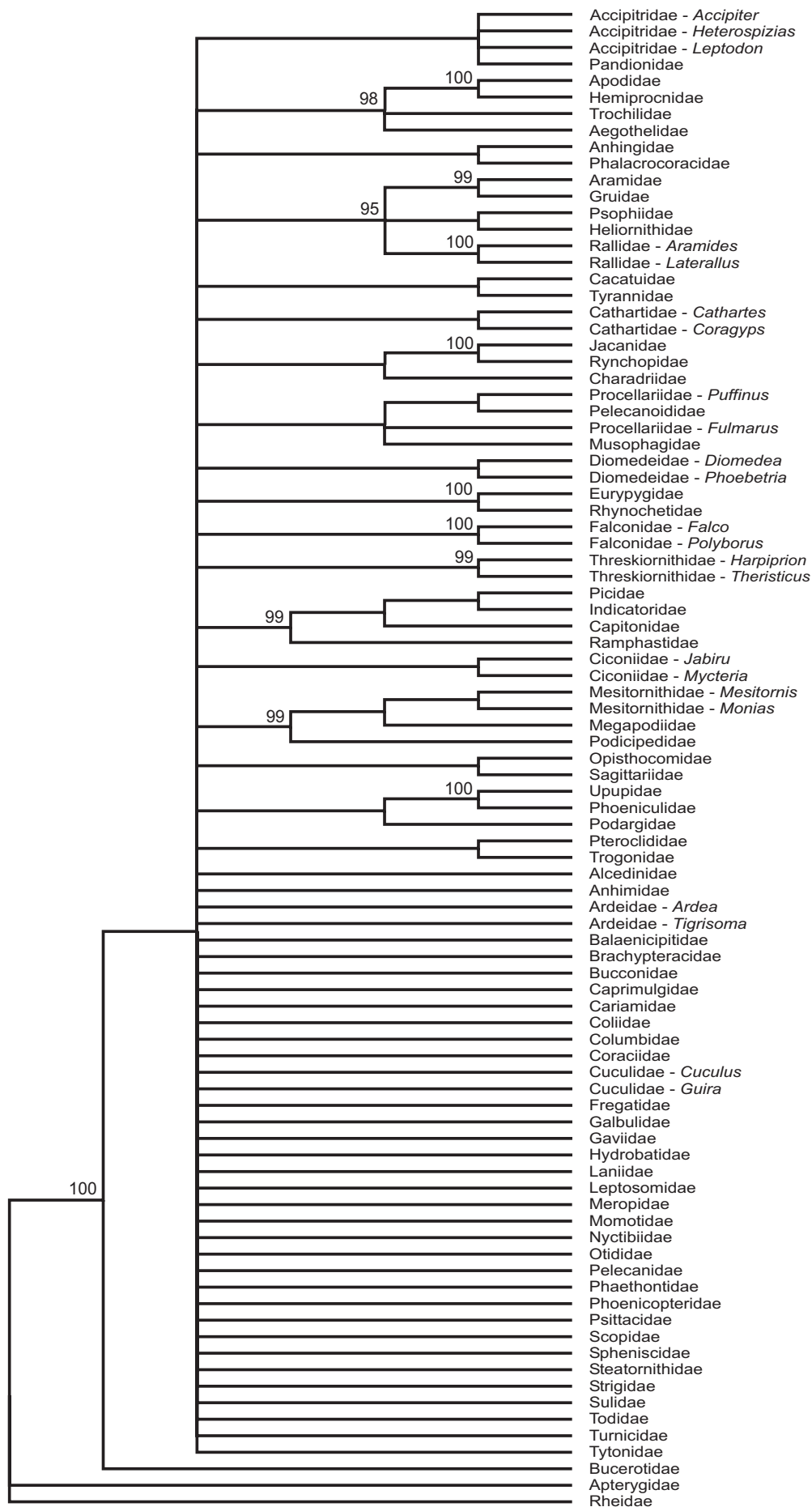


Figure ESM-1. Bayesian tree based on *c-myc* exon 3. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

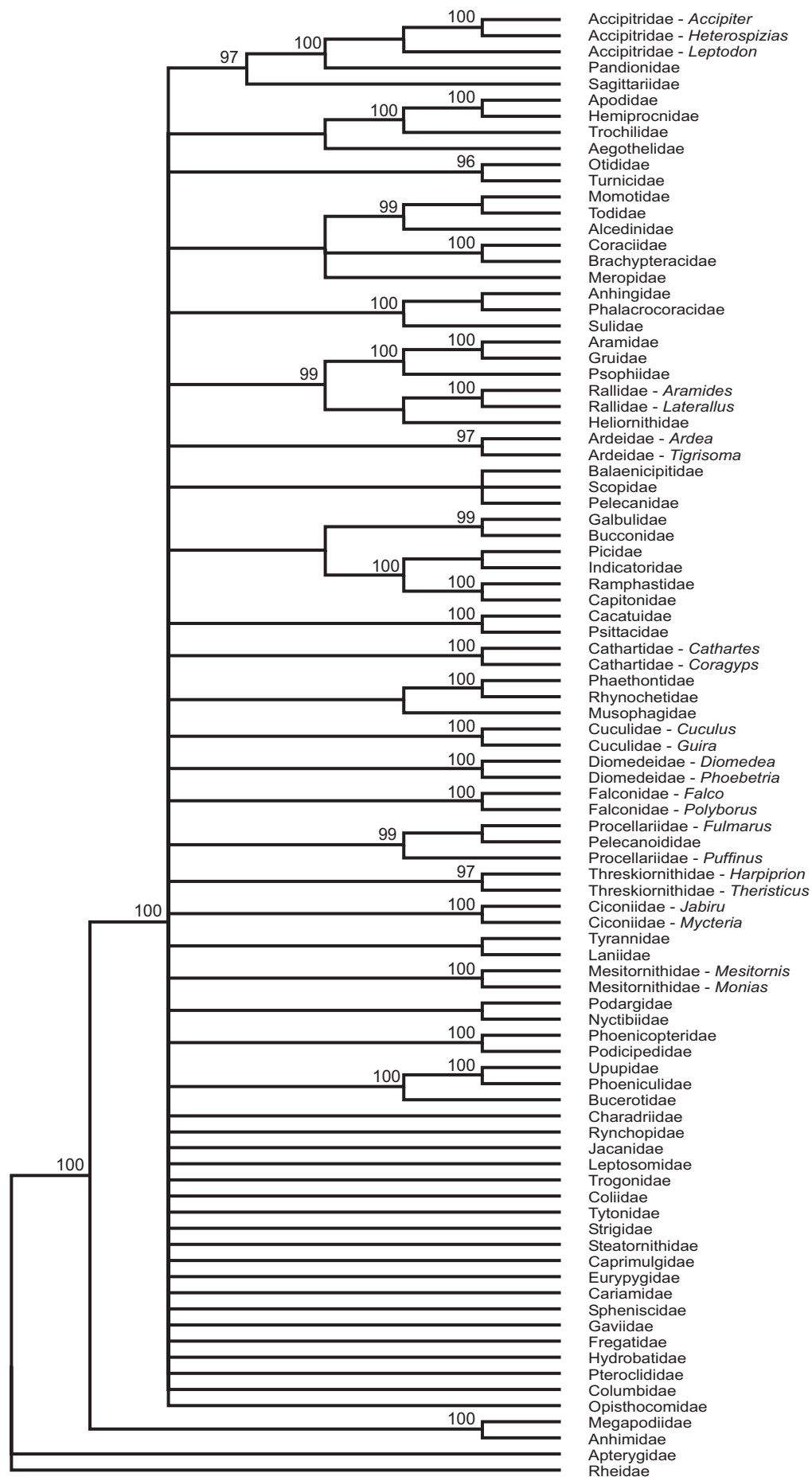


Figure ESM-2. Bayesian tree based on RAG-1. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

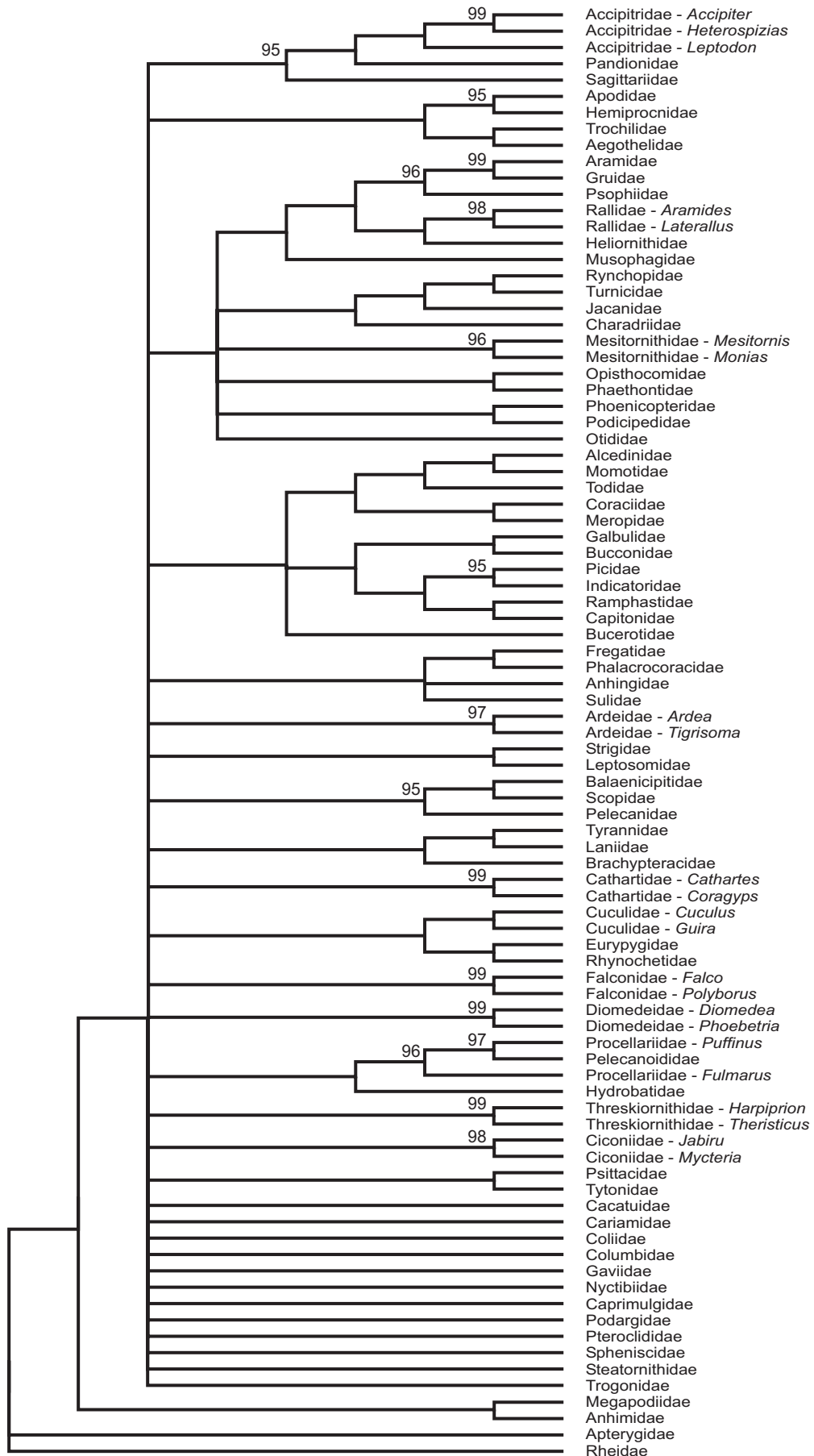


Figure ESM-3. Bayesian tree based on myoglobin intron 2. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

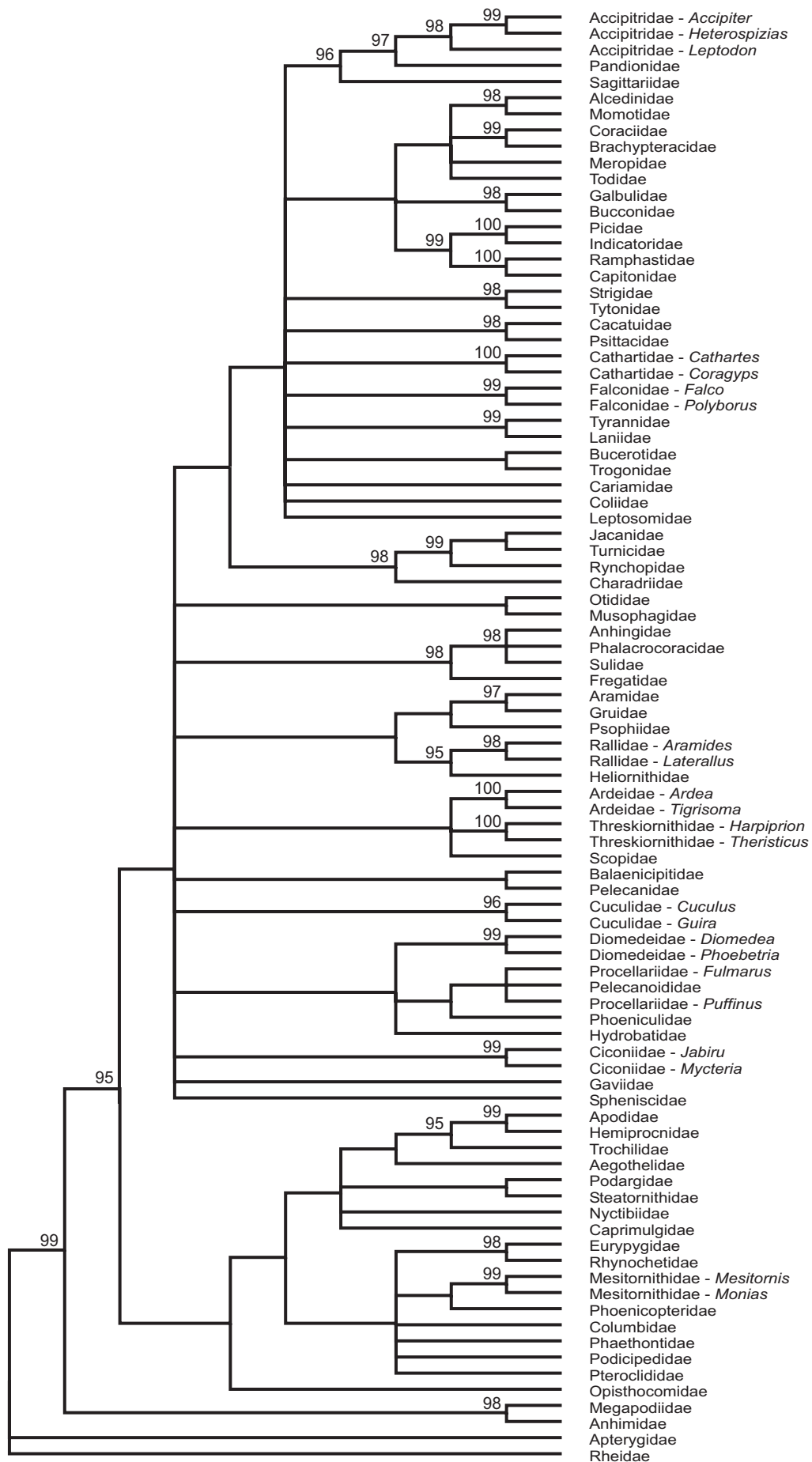


Figure ESM-4. Bayesian tree based on α -fibrinogen intron 7. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

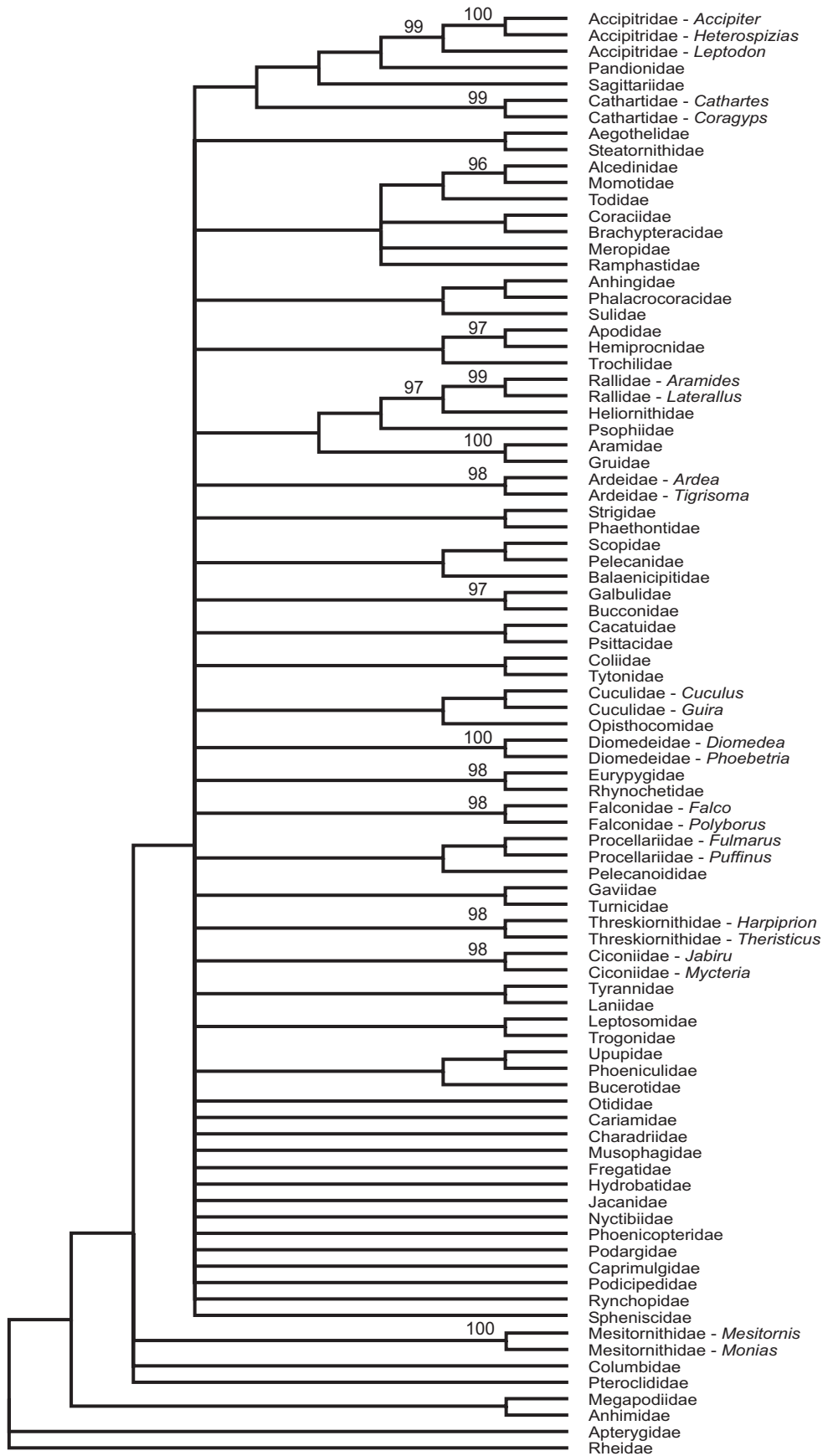


Figure ESM-5. Bayesian tree based on ornithine decarboxylase (ODC) introns 6 and 7, along with the intercepting exon 7. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

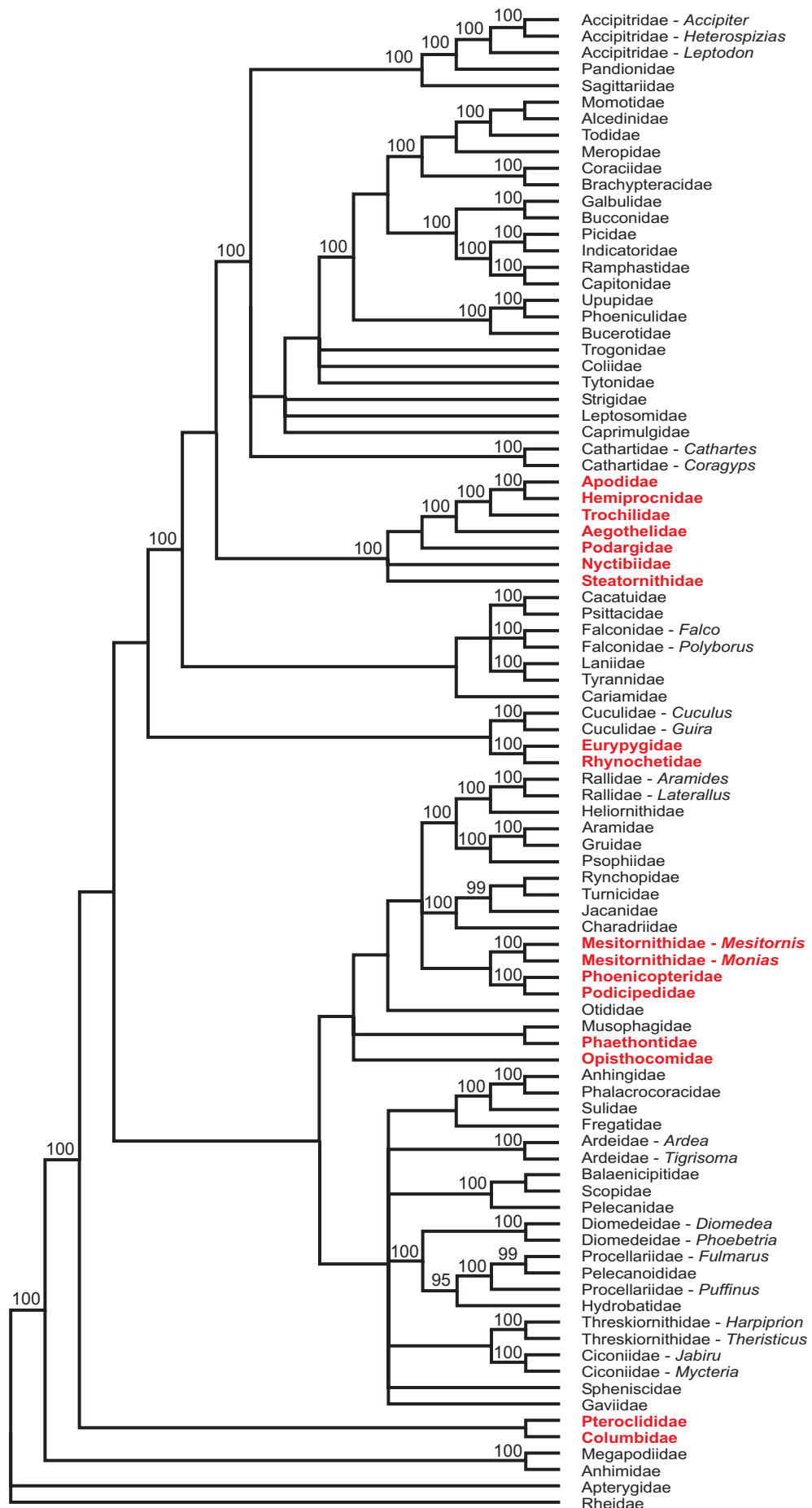


Figure ESM-6. Bayesian tree based on all genes except β -fibrinogen in order to assess the influence of this gene upon the tree topology. Taxa included in Metaves are marked with red. All nodes recovered in 50% or more of the sampled trees are shown (only nodes with 95% or larger posterior probabilities are labeled).

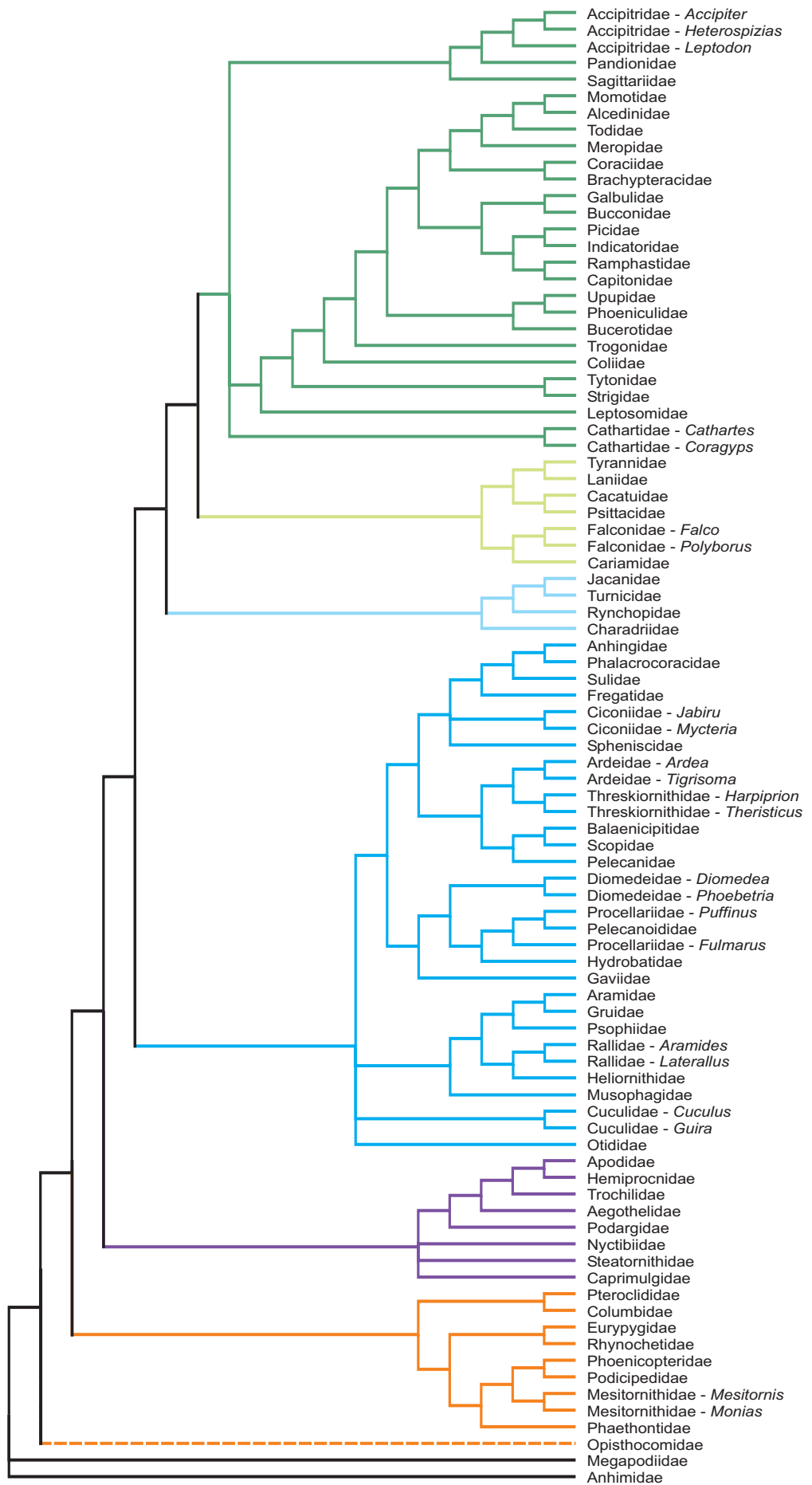


Figure ESM-7. Bayesian tree based on all genes, but with the paleognaths excluded and rooted with Galloanseres. All nodes recovered with 95% (or larger) posterior probabilities are shown.

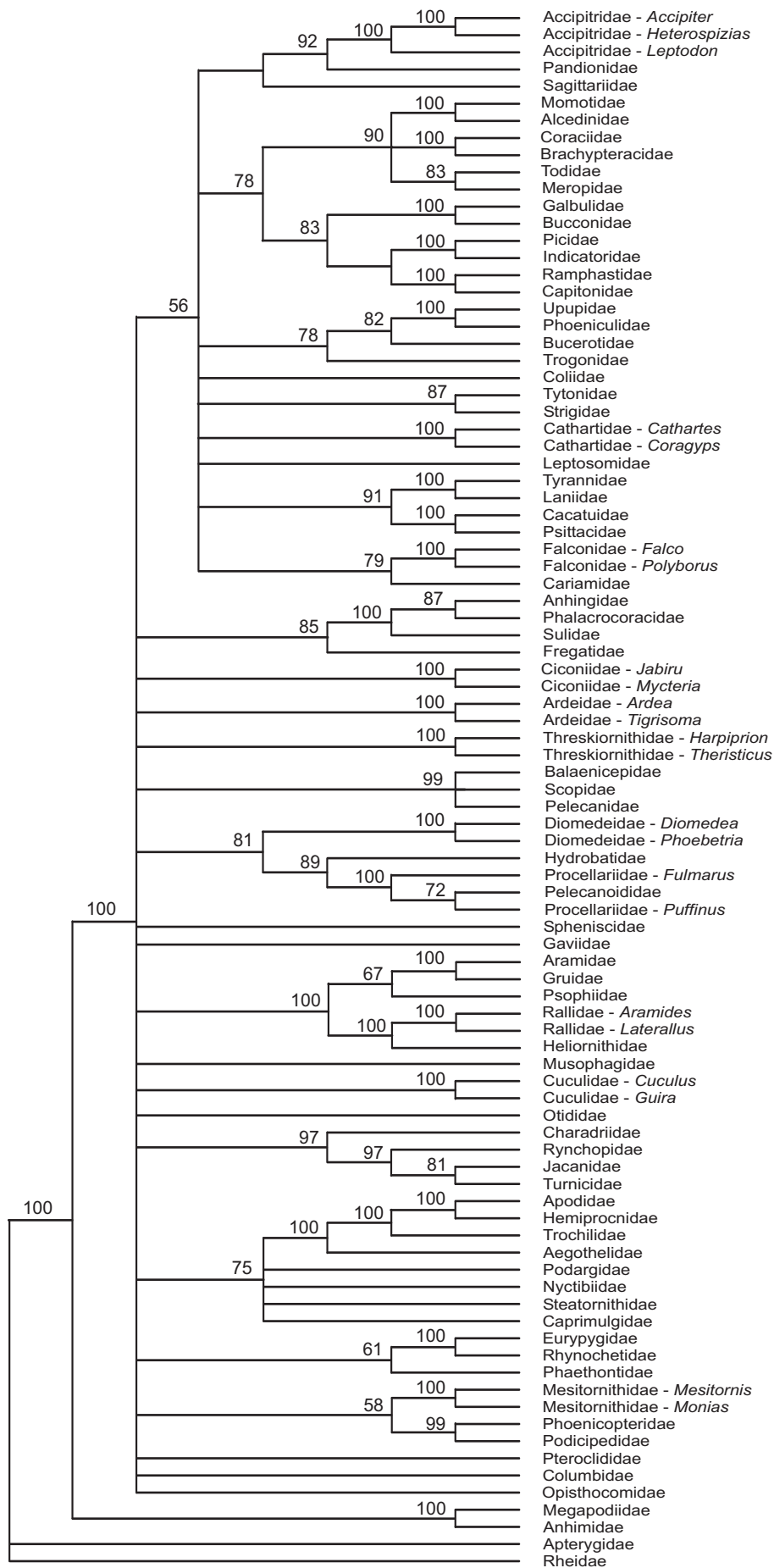


Figure ESM-8. Parsimony jack-knifing tree (Farris et al. 1996) as implemented in *XAC: Parsimony Jackknifer* (Farris 1997). The analysis was performed with 1,000 replicates, each with 10 random additions of taxa and branch swapping.

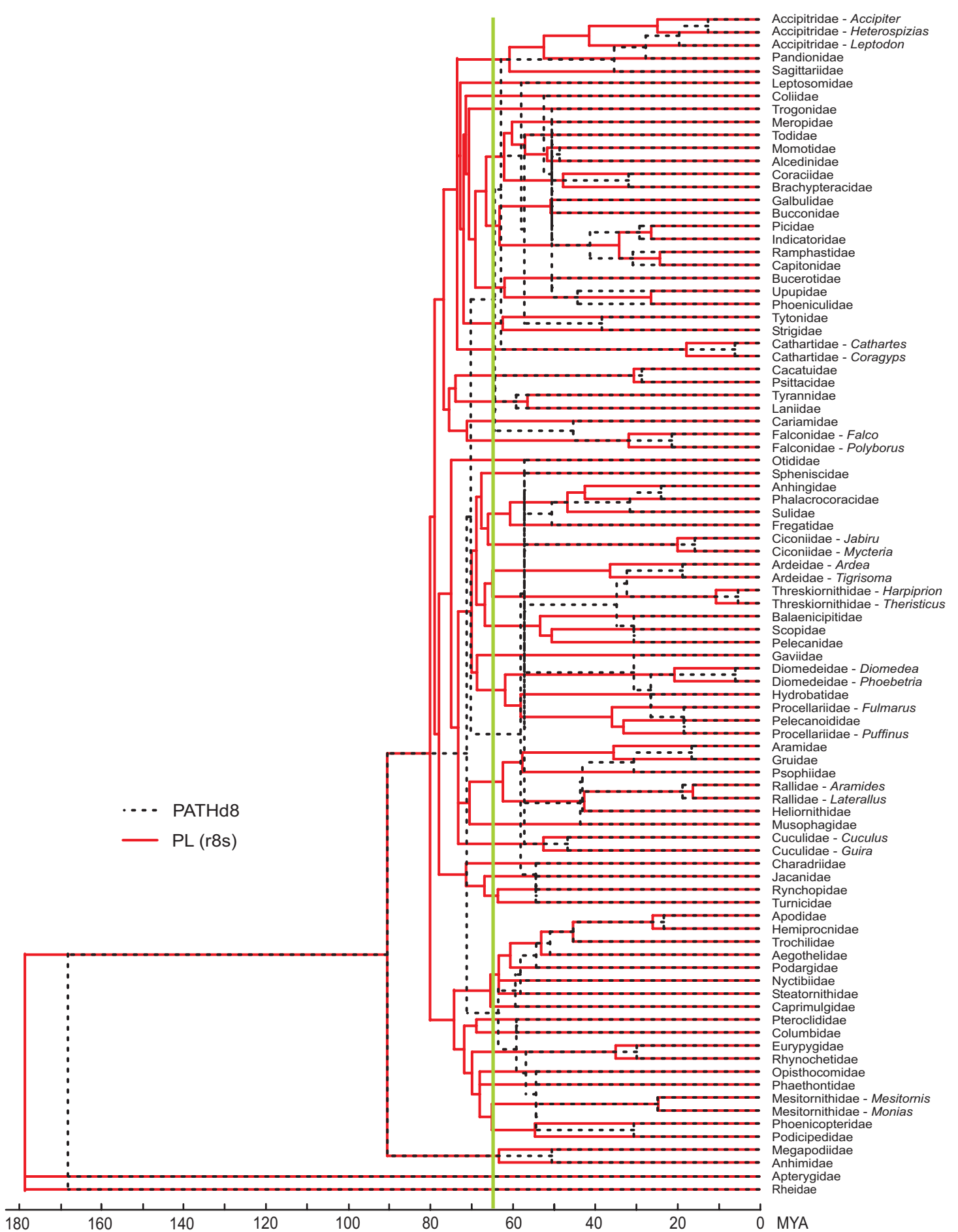


Figure ESM-9. Comparison between the results obtained from the PATHd8 and the PL analyses. The red PL chronogram suggests an earlier divergence, than the black, dotted chronogram from the PATHd8 analysis. The PL tree is less consistent with the fossil record, in the sense that it adds a "ghost range" of in average 21 MY to the fossil constrained nodes. The boundary between the Cretaceous and Tertiary at 65 MYA is marked with a green line.