Contents of Supplementary Information:

Table S1. Samples used in this study with information on the museum collection and accession number.

Scientific name	Common name[1]	Museum and Collection #[2]	Genbank Accession # CLTC	CLTCL1	Collection Locality[3]	Collector[3]
Anhinga anhinga	**Anhinga**	N-622528	EU302706	EU302749	Florida	M. E. Kennedy
Anser erythropus	Lesser White-fronted **Goose**	L-19457	EU302707	EU302750	Captive	—
Arenaria interpres	Ruddy **Turnstone**	N-607650	EU302708	EU302751	Panama	T. J. Parsons
Aythya americana	Redhead	L-2651	EU302709	EU302752	USA	D. L. Dittman
Buteo jamaicensis	Red-tailed **Hawk**	L-33264	EU302710	EU302753	Louisiana	—
Caprimulgus longirostris	Band-winged **Nightjar**	L-32361	EU302711	EU302754	Peru	D. G. Christian
Ciconia ciconia	White **Stork**	K-90088	EU302712	EU302755	—	—
Coccyzus americanus	Yellow-billed **Cuckoo**	N-626461	EU302713	EU302756	Florida	C. A. Gebhard
Colibri coruscans	Sparkling Violet-ear	L-5574	EU302714	EU302757	Peru	T. J. Davis
Colius colius	White-backed **Mousebird**	N-622447	EU302715	EU302758	Captive	—
Columba livia	Rock **Dove**	F-428785	EU302716	EU302759	Wisconsin	BBWS
Corvus corone	Carrion **Crow**	N-621224	EU302717	EU302760	Russia	B. K. Schmidt
Coturnix coturnix	Common **Quail**	N-621265	EU302718	EU302761	UK	T. West
Crax alector	Black **Curassow**	N-625104	EU302719	EU302761	Guyana	C. M. Milensky
Dromaius novaehollandiae	**Emu**	L-5895	EU302720	EU302763	Captive	S. M. Lanyon
Dryocopus pileatus	Pileated **Woodpecker**	L-20929	EU302721	EU302764	Louisiana	T. S. Sillett
Eudromia elegans	**Elegant Crested-tinamou**	L-5893	EU302722	EU302765	Captive	D. L. Dittmann
Eudyptula minor	Little Blue **Penguin**	V-I224	EU302723	EU302766	Australia	—
Eurypyga helias	**Sunbittern**	N-586303	EU302724	EU302767	Guyana	M. J. Braun
Falco mexicanus	Prairie **Falcon**	S-103400	EU302725	EU302768	New Mexico	—
Fregata magnificens	Magnificent **Frigatebird**	N-613348	EU302726	EU302769	Panama	S. L. Olson
Gallus gallus	Red Junglefowl	L-19436	EU302727	EU302770	Captive	D. L. Dittmann
Haematopus ostralegus	Eurasian **Oystercatcher**	L-163409	EU302728	EU302771	—	—

[1] The common names used in the text and figures are emphasized in bold, with the exception of the Redhead (**Duck**), Sparkling Violet-ear (**Hummingbird**) and Red Junglefowl (**Chicken**).

[2] Museums are indicated with a single letter: F=Field Museum of Natural History; K=University of Kansas Natural History Museum & Biodiversity Center; L=Louisiana State University Museum of Natural Science; S=Museum of Southwestern Biology, University of New Mexico; V=Museum Victoria, Australia; N=National Museum of Natural History (Smithsonian Institution). The tissue or voucher number follows the museum designation.

[3] Abbreviations for collection localities are: CAR=Central African Republic, UK=United Kingdom and USA=unspecified location within United States. Collecting organizations are: BBWS= Bay Beach Wildlife Sanctuary and ABS=Archibold Biological Station. Unspecified collecting locations or collectors are indicated with a dash.

Table S1, continued. Samples used in this study with information on the museum collection and accession number.

Scientific name	Common name[1]	Museum and Collection #[2]	Genbank Accession # CLTC	CLTCL1	Collection Locality[3]	Collector[3]
Mesitornis unicolor	Brown **Mesite**	F-345610	EU302729	EU302772	Madagascar	S. M. Goodman
Nothoprocta perdicaria	**Chilean Tinamou**	L-23841	EU302730	EU302773	Captive	D. L. Dittmann
Numida meleagris	Helmeted **Guineafowl**	F-384667	EU302731	EU302774	Madagascar	S. M. Goodman
Pandion haliaetus	**Osprey**	F-385893	EU302732	EU302775	Florida	ABS
Phalacrocorax carbo	Great **Cormorant**	L-45740	EU302733	EU302776	Kuwait	J. M. Bishop
Phoenicopterus chilensis	Chilean **Flamingo**	N-614545	EU302734	EU302777	Argentina	J. P. Angle
Podargus strigoides	Tawny **Frogmouth**	N-612702	EU302735	EU302778	Australia	F. Smith & T. Smith
Psophia crepitans	Common **Trumpeter**	N-621709	EU302736	EU302779	Guyana	M. J. Braun
Rallus limicola	Virginia **Rail**	F-380034	EU302737	EU302780	Illinois	D. E. Willard
Rhea americana	Common **Rhea**	N-541231	EU302738	EU302781	Captive	—
Rhynochetos jubatus	**Kagu**	Fl-42714	EU302739	EU302782	Captive	—
Smithornis rufolateralis	Rufous-sided **Broadbill**	F-429425	EU302740	EU302783	CAR	D. E. Willard
Speotyto cunicularia	Burrowing **Owl**	F-396871	EU302741	EU302784	Florida	—
Streptoprocne zonaris	White-collared **Swift**	N-626065	EU302742	EU302785	Guyana	C. M. Milensky
Struthio camelus	**Ostrich**	L-1526	EU302743	EU302786	Captive	D. L. Dittmann
Tauraco erythrolophus	Red-crested **Turaco**	L-5354	EU302744	EU302787	Captive	D. L. Dittmann
Tinamus guttatus	**White-throated Tinamou**	F-389673	EU302745	EU302788	Brazil	B. D. Patterson
Treron vernans	Pink-necked Green **Pigeon**	L-47229	EU302746	EU302789	Malaysia	F. H. Sheldon
Trogon personatus	Masked **Trogon**	L-7644	EU302747	EU302790	Peru	G. H. Rosenberg
Vidua chalybeata	Village **Indigobird**	L-39547	EU302748	EU302791	Ghana	B. D. Marks

[1] The common names used in the text and figures are emphasized in bold, with the exception of the Redhead (**Duck**), Sparkling Violet-ear (**Hummingbird**) and Red Junglefowl (**Chicken**).

[2] Museums are indicated with a single letter: F=Field Museum of Natural History; K=University of Kansas Natural History Museum & Biodiversity Center; L=Louisiana State University Museum of Natural Science; S=Museum of Southwestern Biology, University of New Mexico; V=Museum Victoria, Australia; N=National Museum of Natural History (Smithsonian Institution); A=Auckland Museum; Fl=Florida Museum of Natural History. The tissue or voucher number follows the museum designation.

[3] Abbreviations for collection localities are: CAR=Central African Republic, UK=United Kingdom and USA=unspecified location within United States. Collecting organizations are: BBWS= Bay Beach Wildlife Sanctuary and ABS=Archibold Biological Station. Unspecified collecting locations or collectors are indicated with a dash.

Table S2. Sequences of primers used to amplify *CLTC* and *CLTCL1*.

Locus	Primer name	Primer sequence	Notes[1]
CLTC	CLTC.e6Fnew	CTACATGAACAGAATCAGTGGAGAGAC	
CLTC	CLTC.e7Rnew	GCTGCCACTTTTGCTGCCTCTGAATA	
CLTC	CLTC.e7Falt	CAGAATCCTGATCTAGCTTTACGAATGGC	
CLTC	CLTC.e8Ralt	CATTTCTCCAGAAGTTGTTTGCGTCC	NP
CLTC	CLTC.e7Fpass2	CAGGTGCTCTCAGTGTGTGTGGAAGA	PO
CLTC	CLTC.e8Rpass	TGWGCTGGAACACTCTGGAACCG	PO
CLTCL1	CLTCL1.e7F	CACCAATGTTCTGCAGAATCCTGA	
CLTCL1	CLTCL1.e8Rnew	CCAGCTTATCTTCCTTNAGCCATTTCTC	

[1] NP and PO indicate primers appropriate for non-passerines and for passerines only, respectively. Primers CLTC.e7Falt and CLTC.e8Ralt tend to amplify *CLTCL1* rather than *CLTC* in passerines so a nested PCR using CLTC.e6Fnew and CLTC.e8Ralt was used in passerines.

Table S3. Information about fossils used for analyses.

Fossil	Age	Reference
Oligocorax spp.	26 MYA	Mayr (2001); also see Becker (1986)
Limnofregata spp.	49 MYA	Olsen and Matsuoka (2005)
Parvigrus pohli	30 MYA	Mayr (2005a)
Juncitarsus spp.	45 MYA	Mayr (2004)
Jiliniornis huadianensis	45 MYA	Hou and Ericson (2002)
Massiliraptor parvunguis	48.6 MYA	Mayr (2006)
Milvoides kempi	37 MYA	Harrison and Walker (1979)
Messelornis spp.	58 MYA	Livezey (1998); Mayr (2005b)
Ogygoptynx wetmorei	58 MYA	Rich and Bohaska (1976)
Laputavis robusta	55.4 MYA	Dyke (2001)
Jungornithidae	47 MYA	Mayr (2005b)
Massilapodargus	48 MYA	Mayr (2005b)
Murgon fossils	55 MYA	Boles (1997)
Weislochia weissi	33.9 MYA	Mayr and Manegold (2006a)
Luberon fossil	28.4 MYA	Mayr and Manegold (2006b)
Sandcoleidae	57 MYA	Dyke and Waterhouse (2001)
Septentrogon madseni	57 MYA	Kristoffersen (2001)
Waimanu manneringi	60.5 MYA	Slack et al. (2006)
Vegavis iaai	67 MYA	Clarke et al. (2005)
Paraortygoides	52 MYA	Dyke and Gulas (2002)
Amitabha	48.5 MYA	Gulas-Wroblewski and Wroblewski (2003)

Table S4. Estimates of divergence times for select nodes in the avian tree of life after rate smoothing.

Node[1]	NPRS Estimate for Calibration Set A (±SD)[2]	NPRS Estimate for Calibration Set B (±SD)[2]	Credible Interval for Calibration Set A[3]	Credible Interval for Calibration Set B[3]
Paleognathae	64 ± 22 MYA	77 ± 12 MYA	—[4]	—
Neognathae	87 ± 29 MYA	104 ± 16 MYA	96 (88 - 105) MYA	99 (89 - 111) MYA
Galloanserae	66 ± 22 MYA	79 ± 12 MYA	69 (63 - 73) MYA	74 (63 - 86) MYA
Neoaves	70 ± 24 MYA	84 ± 13 MYA	75 (69 - 81) MYA	77 (72 - 83) MYA
Clade 1	69 ± 23 MYA	78 ± 12 MYA	72 (66 - 78) MYA	75 (69 - 82) MYA
Clade 2	66 ± 22 MYA	82 ± 12 MYA	72 (67 - 77) MYA	71 (68 - 75) MYA
α	43 ± 15 MYA	52 ± 7.7 MYA	39 (32 - 47) MYA	44 (36 - 53) MYA
β	60 ± 20 MYA	71 ± 11 MYA	57 (50 - 64) MYA	63 (56 - 70) MYA
γ	53 ± 18 MYA	63 ± 9.5 MYA	29 (28 - 30) MYA	29 (28 - 30) MYA
δ	60 ± 21 MYA	73 ± 11 MYA	50 (44 - 57) MYA	62 (55 - 70) MYA
ε	61 ± 21 MYA	73 ± 11 MYA	54 (47 - 61) MYA	61 (54 - 68) MYA
ζ	27 ± 8.9 MYA	32 ± 4.8 MYA	25 (18 - 34) MYA	27 (20 - 36) MYA
η	59 ± 20 MYA	70 ± 11 MYA	64 (58 - 70) MYA	63 (59 - 68) MYA
θ	15 ± 4.9 MYA	17 ± 2.6 MYA	14 (11 - 18) MYA	18 (14 - 24) MYA
ι	35 ± 12 MYA	42 ± 6.3 MYA	33 (30 - 34) MYA	41 (35 - 48) MYA
κ	45 ± 15 MYA	53 ± 8 MYA	50 (47 - 55) MYA	61 (60 - 63) MYA
λ	58 ± 19 MYA	73 ± 11 MYA	63 (58 - 69) MYA	67 (65 - 69) MYA
μ	29 ± 9.6 MYA	34 ± 5 MYA	59 (58 - 60) MYA	31 (24 - 38) MYA
ν	41 ± 14 MYA	49 ± 7.4 MYA	46 (41 - 48) MYA	44 (38 - 50) MYA
ξ	61 ± 21 MYA	73 ± 11 MYA	69 (64 - 74) MYA	64 (62 - 65) MYA
ο	52 ± 18 MYA	62 ± 9.3 MYA	58 (51 - 65) MYA	57 (51 - 62) MYA
π	53 ± 18 MYA	63 ± 9.5 MYA	51 (49 - 52) MYA	60 (49 - 71) MYA
ρ	29 ± 9.8 MYA	35 ± 5.2 MYA	29 (24 - 34) MYA	34 (26 - 44) MYA
σ	59 ± 20 MYA	70 ± 11 MYA	—	—
τ	53 ± 18MYA	63 ± 9.4 MYA	—	—
υ	22 ± 7.4 MYA	26 ± 3.9 MYA	—	—

[1] The node labels are shown in Figure S3.

[2] Divergence time estimates based upon non-parametric rate smoothing (Sanderson, 1997). Standard deviations reflect the variance associated different calibrations. Calibration set A includes all of the fossils in Figure S1 and calibration set B included only owls, nightbirds, swifts and hummingbirds (using the dates from Bleiweiss, 1998).

[3] Maximum *a posteriori* estimates and 95% credible intervals (in parentheses) for avian divergence times obtained using the Bayesian method by Thorne and Kishino (2002). Fossil calibration sets are identical to those used with non-parametric rate smoothing.

[4] Multidivtime does not provide divergence time estimates for the outgroup taxa. Multidivtime runs used a 'bigtime' setting of 1000.0.

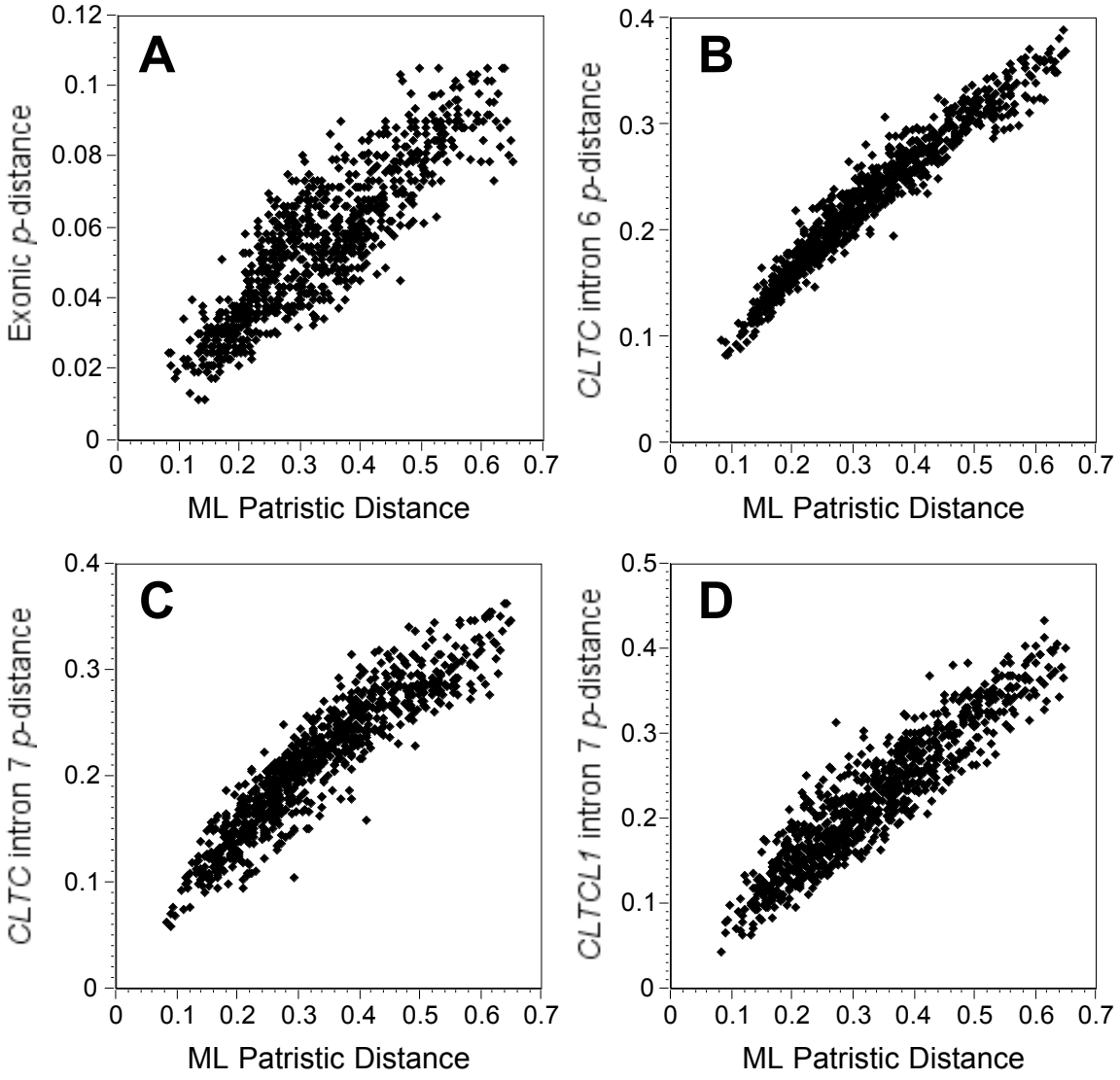

Fig. S1. Saturation plots for the clathrin heavy chain partitions. All of the graphs compare uncorrected distances (*p*-distances) for pairwise comparisons of taxa (*y* axis) to patristic distances using branch lengths from a multigene ML analysis in PAML (*x* axis). Note the differences in the scales used for the *y* axis of each graph. A. Combined dataset including all *CLTC* and *CLTCL1* exons. B. *CLTC* intron 6. C. *CLTC* intron 7. D. *CLTCL1* intron 7.

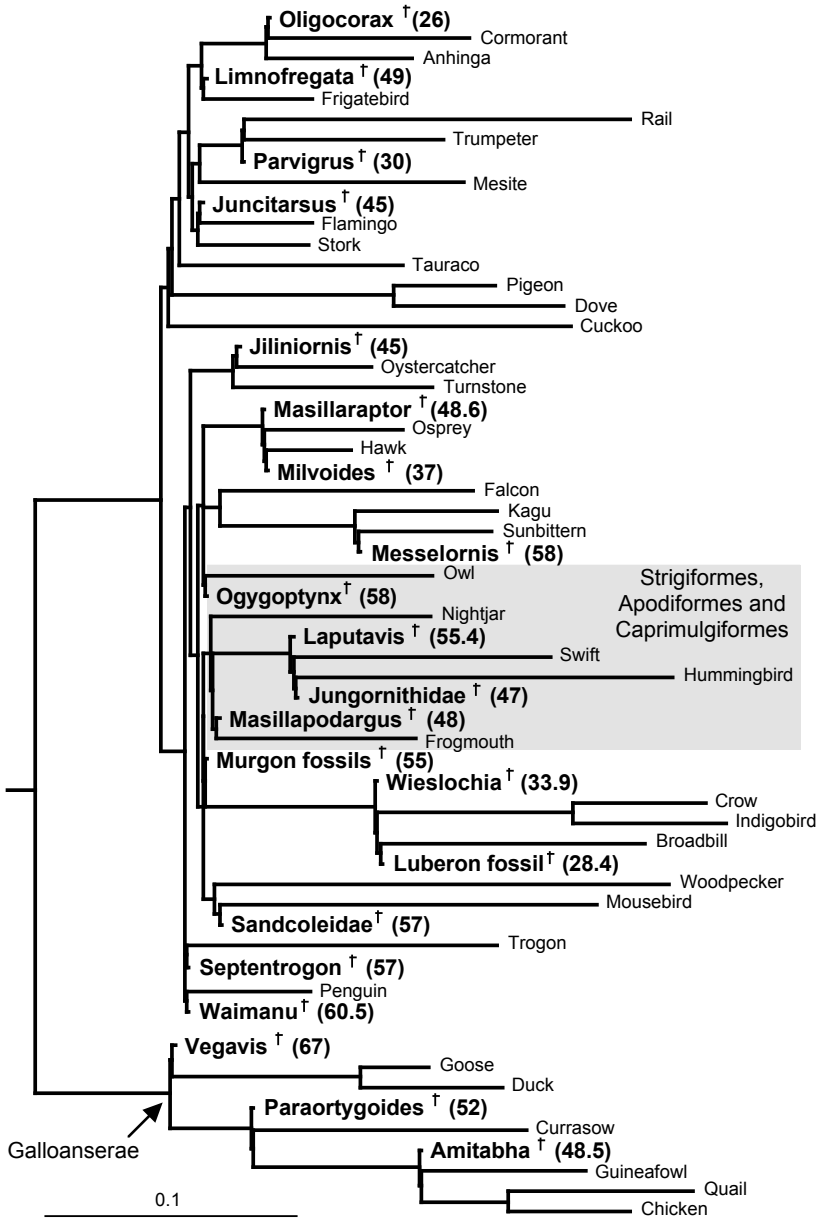

Figure S2. Fossil information used to calibrate relaxed molecular clock analyses. Fossil taxa, indicated using bold type and a dagger and followed by their age in parentheses, have been added to the ML tree in the appropriate positions (see Table S3 for details). Divergence times estimates were obtained using two distinct sets of calibrations: 1) the full set of divergence times based upon the earliest occurrence of all fossils; and 2) the oldest dates consistent with the 95% confidence interval for the origin of nightbirds (Caprimulgiformes; 67 MYA), owls (Strigiformes; 63 million years ago [MYA]), and the swift-hummingbird clade (Apodiformes; 62 MYA) (emphasized by shading). The second calibration set was used to reduce any bias due to the incompleteness of the fossil record, because Bleiweiss (1998) used fossil gap analysis (a statistical method that estimates the bottom of a fossil range accommodating the incompleteness of the fossil record) to establish earliest appearance of an order in the fossil record.

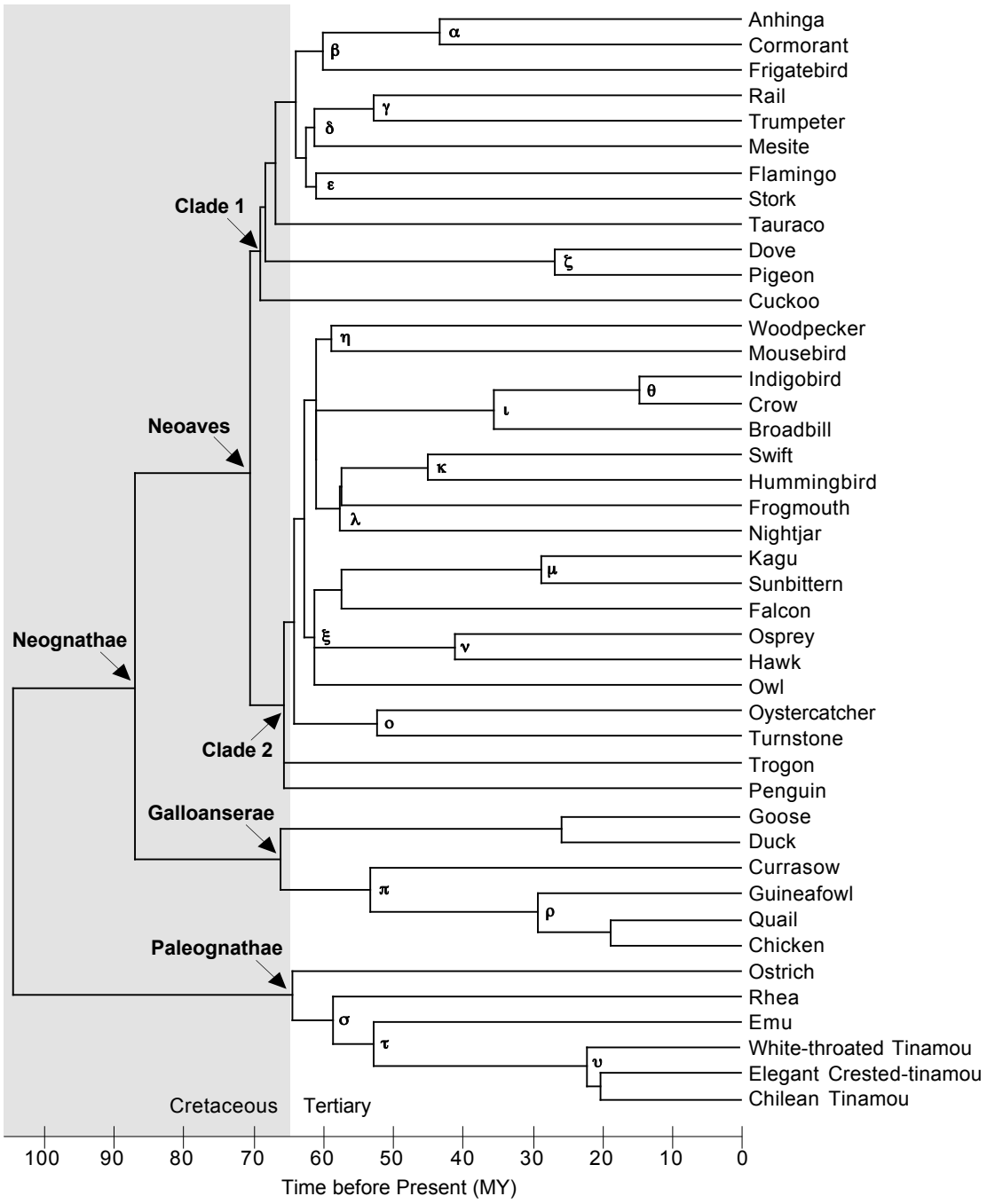

Figure S3. Chronogram showing estimates of avian divergence times based upon the combined dataset. This chronogram was generated using non-parametric rate smoothing (Sanderson, 1997) and branch lengths from a multigene analysis with linked branch lengths. The scale bar shows time in millions of years before present and the shading indicates the Cretaceous period. Credible intervals on divergence times and the sensitivity to fossil calibrations are provided in Table S4. The subset of clades that are included in Table S4 are indicated using names or Greek letters.

Supplementary References

Becker, J.J. 1986. Reidentification of "*Phalacrocorax*" *subvolans* Brodkorb as the earliest record of Anhingidae. *The Auk* 103, 804-8.

Bleiweiss, R. 1998. Fossil gap analysis supports early Tertiary origin of trophically diverse avian orders. *Geology* 26, 323-6.

Boles, W.E. 1997. Fossil Songbirds (Passeriformes) from the Early Eocene of Australia. *Emu* 97, 43-50.

Clarke, J.A., Tambussi, C.P., Noriega, J.I., Erickson, G.M. and Ketcham, R.A. 2005. Definitive fossil evidence for the extant avian radiation in the Cretaceous. *Nature* 433, 305-8.

Dyke, G.J. 2001. A primitive swift from the London Clay and the relationships of fossil apodiform birds. *Journal of Vertebrate Paleontology* 21, 195-200. [also see Dyke, G.J. 2001. *Laputavis*, a replacement name for *Laputa* Dyke 2001 (preoccupied name) *Journal of Vertebrate Paleontology* 21, 401.]

Dyke, G.J. and Gulas B.E. 2002. The fossil galliform bird *Paraortygoides* from the Lower Eocene of the United Kingdom. *American Museum Novitates* 3360, 1-14.

Dyke, G.J. and Waterhouse, D.M. 2001. A mousebird (Aves: Coliiformes) from the Eocene of England. *Journal für Omithologie* 142, 7-15.

Felsenstein, J. 1988. Phylogenies from molecular sequences: Inference and reliability. *Annual Review of Genetics* 22, 521-65.

Graur, D. and Martin, W. 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics* 20, 80-6.

Gulas-Wroblewski, B.E. and Wroblewski, A.F.-J. 2003. A crown group galliform bird from the Middle Eocene Bridger Formation of Wyoming. *Paleontology* 46, 1269-80.

Harrison, C.J.O. and Walker, C.A. 1979. Birds of the British Middle Eocene. *Tertiary Research* 5, 19-27.

Hou, L. and Ericson, P.G.P. 2002. A middle Eocene shorebird from China. *The Condor* 104, 896-9.

Kristoffersen, A.V. 2001. An early Paleogene Trogon (Aves:Trogoniformes) from the Fur Formation, Denmark. *Journal of Vertebrate Paleontology* 22, 661-6.

Livezey, B.C. 1998. 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* 353, 2077-151.

Mayr, G. 2001. A cormorant from the late Oligocene of Enspel, Germany (Aves, Pelecaniformes, Phalacrocoracidae). *Senckenbergiana Lethaea* 81, 329-33.

Mayr, G. 2004. Morphological evidence for sister group relationship between flamingos (Aves: Phoenicopteridae) and grebes (Podicipedidae). *Zoological Journal of the Linnean Society* 140, 157-69.

Mayr, G. 2005a. A chicken-sized crane precursor from the early Oligocene of France. *Naturwissenschaften* 92, 389-93.

Mayr, G. 2005b. The Paleogene fossil record of birds in Europe. *Biological Reviews* 80, 515-42

Mayr, G. 2006. A new raptorial bird from the Middle Eocene of Messel, Germany. *Historical Biology* 18, 95-102.

Mayr, G. and Manegold, A. 2006a. New specimens of the earliest European passeriform bird. *Acta Palaeontologica Polonica* 51, 315-23.

Mayr, G. and Manegold, A. 2006b. A small suboscine-like passeriform bird from the early Oligocene of France. *The Condor* 108, 717-20.

Olsen, S.L. and Matsuoka, H. 2005. New specimens of the early Eocene frigatebird *Limnofregata* (Pelecaniformes: Fregatidae), with the description of a new species *Zootaxa* 1046, 1-15.

Rabinowicz, P.D., Braun, E.L., Wolfe, A.D., Bowen, B. and Grotewold, E. 1999. Maize R2R3 Myb genes: Sequence analysis reveals amplification in the higher plants. *Genetics* 153, 427-44.

Rich, P.V. and Bohaska, D.J. 1976. The world's oldest owl: A new strigiform from the Paleocene of southwestern Colorado. *Smithsonian Contributions Paleobiology* 27, 87-93.

Sanderson, M.J., 1997. A nonparametric approach to estimating divergencetimes in the absence of rate constancy. *Molecular Biology and Evolution* 19, 1218-31.

Shaul, S. and Graur, D. 2002. Playing chicken (Gallus gallus): methodological inconsistencies of molecular divergence date estimates due to secondary calibration points. *Gene* 300, 59-61.

Slack, K.E., Jones, C.M., Ando, T., Harrison, G.L.(A.), Fordyce, R.E., Arnason, U. and Penny, D. 2006. Early Penguin fossils, plus mitochondrial genomes, calibrate avian evolution. *Molecular Biology and Evolution* 23, 1144-55.

Swofford, D.L., 2003. PAUP*: Phylogenetic analysis using parsimony (* and other methods). Sinauer, Sunderland, MA.

Swofford, D.L., Olsen, G.J., Waddell, P.J. and Hillis, D.M. 1996. Phylogenetic inference. pp. 407–514. In: Hillis, D.M., Moritz, C. and Mable B. (Eds.), Molecular Systematics. Sinauer, Sunderland, MA.

Thorne, J.L. and Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology* 51, 689-702.