Electronic Supplementary Material for:

Phylogenetic relationships and divergence times of Charadriformes genera: multigene evidence for the Cretaceous origin of at least 14 clades of shorebirds

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Taxon sampling

We obtained blood or tissue samples from 90 out of 96 genera of Charadriiformes, including two species of *Turnix* which is known to be embedded within Charadriiformes (Paton et al. 2003) and seven outgroup species (Supplementary Table 1) for DNA amplification and sequencing. These samples are deposited at the LSU Museum of Natural Science Collection of Genetic Resources, USA (*Hydrophasianus*, *Irediparra*, *Pluvianus* and *Rhinoptilus*), Zoological Museum of the University of Copenhagen, Denmark (*Anous*, *Creagrus*, *Gygys* and *Rhodostethia*) and the Royal Ontario Museum (all remaining species). DNA or tissue samples from six other genera (*Dromas*, *Prosobonia*, *Metopidius, Gabianus, Leucopheus* and *Procelsterna*) were not available. We also did not have DNA or tissue samples for *Pinguinus impennis*, but included the small ribosomal subunit (12S rDNA) and cytochrome b (cyt *b*) sequences for this species deposited in GenBank in our analyses.

DNA isolation, amplification, sequencing, and sequence alignments

DNA was extracted from blood or tissue samples using standard protocols (Sambrook et al. 1989). Chosen fragments for amplification and sequencing were the mitochondrial 12S rDNA, NADH dehydrogenase subunit 2 (ND2) and cyt *b* genes [primers designed by O. Haddrath and described in Pereira and Baker (2004)] and the nuclear recombination activating protein (RAG-1) gene [primers R13, R18, R17, R22, R21 and R2b described in Groth and Barrowclough (1999)]. Mitochondrial and nuclear amplifications were performed as previously described in Pereira and Baker (2005) and Groth and Barrowclough (1999), respectively. PCR products were recovered from 1% agarose gels, purified by centrifuging each through a filter tip, and were cycle-sequenced and run on a Li-Cor 4200 bidirectional automated DNA sequencer or an ABI 3100 automated DNA sequencer according to the manufacturer's suggested protocols. We used Sequencher 4.1.2 (GeneCodes Corp, Inc, Ann Arbor, Michigan) to check both L- and H-strands sequences for ambiguities and scored them following the standard IUB codes. Final consensus sequences for all gene fragments were exported and visually aligned in MacClade 4.0 (Maddison & Maddison 2000), and then concatenated into a matrix of 5,199 base pairs (bp), including alignment gaps. All sequences obtained in this study were deposited in GenBank (See Supplementary Table 1 for accession numbers).

Phylogenetic Bayesian inference

We inferred the phylogenetic relationships among Charadriiformes by applying a Metropolis-coupled Markov chain Monte Carlo (MCMC) Bayesian approach as implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). We ran two simultaneous independent runs, each starting with a different random tree. Each run was set to have one cold and five heated chains to allow better mixing of the MCMC chain and minimize the chance of being trapped in local optima. The addition of more heated chains allows for quicker convergence of large and complex data sets such as the one gathered in our study. We considered runs to have reached convergence when the average standard deviation of the split frequencies between both simultaneous runs was smaller than 0.01. We performed runs under the GTR + i + g model of DNA substitution (as selected by MrModeltest 2.0[Nylander, 2004]), assuming the same topology to be shared among all partitions but model parameters to be unlinked across partitions. We also assumed *a priori* among-partition rate variation. All trees were considered equally likely. The tree was rooted with sequences from the Ostrich (*Struthio camelus*). Other priors applied for all partitions were: unconstrained:exponential (10.0) for branch lengths, flat Dirichlet (1,1,1,1) for stationary base frequencies, flat Dirichlet (1,1,1,1,1,1) for the nucleotide substitution ratio, uniform distribution (0,200) for the shape parameter of the gamma distribution of rate variation and uniform distribution (0,1) for the proportion of invariable sites. MCMC samples were taken in every 1000th cycle. We plotted the log likelihood of sampled topologies to determine the burn-in period in which the MCMC chain had reached a stationary status. Post-burn-in samples from both simultaneous and independent runs were used to construct a 50% majority rule consensus tree. The proportion of trees in which nodes were recovered after the burn-in period is interpreted as the posterior probability (PP) of that node, or the probability that that node is true. Nodes receiving PP ≥ 0.95 were considered to be strongly supported.

Molecular dating

For each data partition used in the Bayesian inference of phylogeny, we obtained maximum likelihood estimates of the transition/transversion ratio, and nucleotide frequencies in PAML 3.14 (Yang 1997) under the F84 model of DNA substitution assuming rate variation across sites to follow a gamma distribution with five discrete rate categories (Hasegawa et al. 1985). These parameters were used to estimate branch lengths for each data partition and their approximate variance-covariance matrix to derived estimates of divergence times and 95% credibility intervals (95% CrI) based on all data partitions in a Bayesian framework (Supplementary Table 2) (Thorne & Kishino 2002; Thorne et al. 1998). These methods are implemented in the software ESTBRANCHES and MULTIDIVTIME from the MULTIDISTRIBUTE package, freely available from J. Thorne's website: http://statgen.ncsu.edu/thorne/multidivtime.html. The method requires an outgroup to root the tree and imposes the condition that the rate of change in the rate of DNA substitution at the root node is the same at the beginning and at the end of that branch (Kishino et al. 2001; Thorne et al. 1998).

Bayesian dating was run assuming a burnin period = 5,000, sample frequency = 200, number of samples = 10,000. We set the following gamma priors: expected time between tip and root (rttm) = 122.2 Mya (Pereira & Baker 2006) with standard deviation (SD) = 20 Mya, rate of the root node (rtrate) and its SD = 0.00556 substitutions per site per million years as estimated from the median of the tip-to-root branch lengths for all genes. However, it seems of little practical importance to specify these priors because they do not appear to have any appreciable effect on the Bayesian posterior distribution of node ages and rates of evolution, and because sequence data and time constraints should determine the overall rate and the age of the root (Yang & Yoder 2003). We also set the prior for the rate change between ancestral (brownmean) and descendant nodes = 0.00818 (SD = 0.00818) substitutions per site per million of years, so that rttm × brownmean = 1. This later prior follows the suggestion that this is a meaningful value for real and simulated data sets (Wiegmann et al. 2003). Because *a priori* information for rate change is unknown, a large SD value was chosen as suggested (Thorne & Kishino 2002), which allows a gene to have *a priori* a large variation in rate change over time. We assessed the convergence of the MCMC algorithm by running multiple analyses (each one starting with a different randomly selected initial state) and comparing the posterior distribution of divergence times, branch lengths and the proportion of successful changes of those parameters along the Markov chain.

Temporal constraints in molecular dating

The Bayesian method allows the use of prior information about the age of diversification to be incorporated in the analysis. Hence, we used multiple fossils to set a minimum age for 14 nodes spread throughout the tree (Supplementary Table 3). Additionally, we fixed the ages for the separation of Galloanserae and Neoaves at 122.2 Mya and between Galliformes and Anseriformes at 101.7 Mya based on a Bayesian molecular time estimates derived from complete mitochondrial genomes and assuming several independent time constraints from the fossil record (Pereira & Baker 2006).

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Supplementary Table 1. Taxon sampling and GenBank accession numbers.

Family	**Species**	**RAG-1**	**12S rDNA**	**ND2**	**cyt *b***
Alcidae	*Aethia cristatella*	EF373165	EF373064	EF373219	U37087
	Alca torda	AY228788	EF373065	EF373220	U37288
	Alle alle	EF373166	AJ242684	EF373221	U37287
	Brachyramphus brevirostris	EF373172	EF373070	EF373227	U63058
	Cepphus columba	EF373173	X76349	EF373229	U37293
	Cerorhinca monocerata	EF373174	EF373072	EF373230	U37295
	Cyclorrhynchus psittacula	EF373179	EF373077	EF373235	U37296
	Fratercula arctica	AY228787	DQ385279	DQ385092	DQ385228
	Pinguinus impennis	-	AJ242685	-	AJ242685
	Ptychoramphus aleuticus	EF373204	EF373103	EF373261	U37302
	Synthliboramphus antiqus	EF373212	EF373111	EF373269	U37303
	Uria lomvia	EF373216	AJ242687	EF373273	U37308
Burhinidae	*Burhinus vermiculatus*	AY228771	EF380264	EF380265	-
	Esacus magnirostris	AY228769	DQ385270	DQ385083	DQ385219
Charadriidae	*Anarhynchus frontalis*	EF373167	EF380263	EF373222	EF373118
	Charadrius vociferus	AF143736	DQ385269	DQ385082	DQ385218
	Elseyornis melanops	EF373180	EF373078	EF373236	EF373128
	Erythrogonys cinctus	EF373181	EF373079	EF373237	EF373129
	Eudromias morinellus	EF373182	EF373080	EF373238	EF373130
	Oreopholus ruficollis	EF373197	EF373096	EF373254	EF373146
	Peltohyas australis	EF373199	EF373098	EF373256	EF373148
	Phegornis mitchelli	AY228781	EF373099	EF373257	EF373149
	Pluvialis squatarola	EF373202	EF373101	EF373259	EF373151
	Thinornis novaeseelandiae	EF373214	EF373113	EF373271	EF373161
	Vanellus chilensis	AY228772	EF373115	EF373274	EF373163
Chionidae	*Chionis minor*	AY228782	DQ385272	DQ385085	DQ385221
Glareolidae	*Cursorius temminckii*	AY228780	DQ385277	DQ385090	DQ385226
	Glareola maldivarus	-	EF373083	EF373241	EF373133
	Glareola nuchalis	AY228798	-	-	-
	Pluvianus aegyptius	EF373203	EF373102	EF373260	EF373152
	Rhinoptilus chalcopterus	EF373205	EF373105	EF373263	EF373154
	Stiltia isabella	EF373211	EF373110	EF373268	EF373159
Haematopodidae	*Haematopus ater*	AY228794	NC_003713	NC_003713	NC_003713
Ibidorhynchidae	*Ibidoryncha struthersii*	EF373188	EF373086	EF373244	EF373136
Jacanidae	*Actophilornis africana*	AY228791	EF373063	EF373218	EF373117
	Hydrophasianus chirurgis	EF373186	EF373085	EF373243	EF373135
	Irediparra gallinacea	EF373189	EF373087	EF373245	EF373137
	Jacana jacana	AY228776	DQ385273	DQ385086	DQ385222
	Microparra capensis	EF373194	EF373094	EF373252	EF373144
Laridae	*Creagrus furcatus*	EF373178	EF373076	EF373234	EF373127
	Larus marinus	AY228799	EF373088	EF373246	EF373138
	Pagophila eburnea	EF373198	EF373097	EF373255	EF373147
	Rhodostethia rosea	EF373206	EF373106	EF373264	EF373155
	Rissa tridactyla	AY228785	DQ385280	DQ385093	DQ385229

	Xema sabini	EF373217	EF373116	EF373275	EF373164
Pedionomidae	*Pedionomus torquatus*	AY228789	DQ385276	DQ385089	DQ385225
Pluvianellidae	*Pluvianellus socialis*	AY228793	DQ385271	DQ385084	DQ385220
Recurvirostridae	*Cladorhynchus leucocephalus*	EF373176	EF373074	EF373232	EF373125
	Himantopus mexicanus	AY228795	DQ385268	DQ385081	DQ385217
	Recurvirostra americana	AY228800	EF373104	EF373262	EF373153
Rostratulidae	*Nycticryphes semicollaris*	AY228790	DQ385274	DQ385087	DQ385223
	Rostratula benghalensis	AY228801	EF373107	EF373265	EF373156
Rynchopidae	*Rynchops niger*	AY228784	DQ385281	DQ385094	DQ385230
Scolopacidae	*Actitis hypoleucos*	AY894212	AY894144	AY894178	AY894229
	Aphriza virgata	EF373169	EF373067	EF373224	EF373120
	Arenaria interpres	AY228792	NC_003712	NC_003712	NC_003712
	Bartramia longicauda	EF373171	EF373069	EF373226	EF373122
	Calidris canutus	AY228777	EF373071	EF373228	EF373123
	Catoptrophorus semipalmatus	AY894207	AY894139	AY894173	AY894224
	Coenocorypha aucklandica	EF373177	EF373075	EF373233	EF373126
	Eurynorhynchus pygmeus	EF373183	EF373081	EF373239	EF373131
	Gallinago gallinago	AY228775	EF373082	EF373240	EF373132
	Heteroscelus incanus	AY894213	AY894145	AY894179	AY894230
	Limicola falcinellus	EF373191	EF373089	EF373247	EF373139
	Limnodromus scolopaceus	AY228774	EF373090	EF373248	EF373140
	Limosa haemastica	AY228773	EF373091	EF373249	EF373141
	Lymnocryptes minimus	EF373192	EF373092	EF373250	EF373142
	Micropalama himantopus	EF373193	EF373093	EF373251	EF373143
	Numenius minutus	EF373195	EF373095	EF373253	EF373145
	Phalaropus tricolor	AY228778	AY894155	AY894189	AY894240
	Philomachus pugnax	EF373201	EF373100	EF373258	EF373150
	Scolopax minor	EF373207	EF373108	EF373266	EF373157
	Tringa melanoleuca	AY228779	AY894147	AY894181	AY894232
	Tryngites subruficollis	EF373215	EF373114	EF373272	EF373162
	Xenus cinereus	AY894221	AY894153	AY894187	AY894238
Stercoriidae	*Catharacta skua*	AY228783	DQ385278	DQ385091	DQ385227
	Stercorarius longicaudus	EF373208	EF373109	EF373267	EF373158
Sternidae	*Anous tenuirostris*	EF373168	EF373066	EF373223	EF373119
	Chlidonias leucoptera	EF373175	EF373073	EF373231	EF373124
	Gelochelidon nilotica	EF373184	AY631347	AY631383	AY631311
	Gygis alba	EF373185	EF373084	EF373242	EF373134
	Hydroprogne caspia	EF373187	AY631336	AY631372	AY631300
	Larosterna inca	EF373190	AY631328	AY631364	AY631292
	Onychoprion anaethetus	-	AY631332	AY631368	AY631296
	Onychoprion fuscata	EF373196	-	-	-
	Phaetusa simplex	EF373200	AY631329	AY631293	AY631293
	Sterna hirundo	EF373209	AY631342	AY631378	AY631306
	Sternula superciliaris	EF373210	AY631352	AY631388	AY631316
	Thalasseus eurygnatha	AY228786	AY631339	AY631375	AY631303
Thinocoridae	*Attagis gayu*	EF373170	EF373068	EF373225	EF373121
	Thinocorus rumicivorus	EF373213	EF373112	EF373270	EF373160
Turnicidae	*Turnix sylvatica*	EF380262	DQ385283	DQ385096	DQ385232
	Turnix hottentotta	AF143735	-	-	-

Outgroup	*Pterocles orientalis*	AY228767	-	-	-
	Pterocles namaqua	-	DQ385267	DQ385080	DQ385216
	Columba livia	EF373500	EF373295	AF353433	AF182694
	Anhima cornuta	AY140765	AY140699	AY140737	AY140735
	Chauna torquata	AY140766	AY140700	AY140738	AY140736
	Crax blumenbachii	AY140775	AF165444	AY140747	AF165468

Supplementary Table 2. Bayesian posterior distribution of divergence times. Mean, SD and 95% CrI are the mean estimate, standard deviation and 95% Credible Interval. Nodes are labeled as in Figure 2 in the main text.

Node	Mean ± SD	95% CrI	Node	Mean ± SD	95% CrI
A1	122.2 ± 0.0	122.2, 122.2	L20	22.9 ± 2.9	17.8, 28.6
A2	102.9 ± 4.3	94.5, 111.6	L21	62.9 ± 4.5	54.8, 72.0
A3	93.1 ± 4.5	84.7, 102.1	L22	52.0 ± 4.5	44.1, 60.8
A4	88.5 ± 4.6	79.8, 97.8	L23	45.2 ± 4.5	37.2, 54.3
A5	101.6 ± 0.0	101.6, 101.6	L24	42.8 ± 4.4	35.1, 51.7
A6	93.3 ± 3.3	86.1, 99.2	L25	40.2 ± 4.5	32.1, 49.4
A7	29.7 ± 4.7	21.3, 39.5	L26	32.6 ± 4.2	25.5, 40.8
A8	84.1 ± 4.5	75.5, 92.8	L27	30.0 ± 4.1	23.0, 38.2
A9	77.0 ± 5.2	66.7, 87.0	L28	22.5 ± 4.4	14.9, 31.3
C1	88.6 ± 4.6	79.8, 97.9	L29	46.9 ± 4.6	38.8, 56.4
C2	85.1 ± 4.7	75.9, 94.7	L30	21.0 ± 3.5	15.0, 28.2
C3	69.0 ± 4.9	60.0, 78.8	L31	14.7 ± 3.0	9.7, 20.9
C4	61.7 ± 4.8	52.9, 71.5	L32	17.3 ± 3.4	12.2, 24.2
C5	59.9 ± 4.8	51.1, 69.6	L33	24.0 ± 3.9	17.4, 31.6
C6	55.8 ± 4.9	46.9, 65.8	L34	72.4 ± 4.8	63.4, 82.2
C7	51.3 ± 5.1	42.1, 61.5	L35	62.3 ± 5.2	52.6, 72.6
C8	39.6 ± 4.7	31.2, 49.3	L36	29.6 ± 4.5	21.6, 38.9
C9	27.7 ± 4.3	20.1, 36.6	L37	0.1 ± 0.1	0.0, 0.2
C10	57.1 ± 5.1	47.6, 67.3	S1	73.4 ± 4.9	64.2, 83.3
C11	50.6 ± 5.2	41.0, 61.2	S2	63.7 ± 4.6	55.3, 72.9
C12	39.0 ± 4.9	30.2, 49.0	S3	57.1 ± 4.5	49.0, 66.1
C13	66.1 ± 4.9	57.1, 76.1	S4	50.9 ± 4.3	43.0, 59.3
C14	49.2 ± 5.1	40.0, 59.4	S5	47.3 ± 4.3	39.6, 55.7
C15	36.9 ± 5.2	27.6, 47.5	S6	41.0 ± 4.3	33.4, 49.4
C16	23.2 ± 4.2	15.9, 31.8	S7	29.0 ± 3.6	22.8, 36.1
C17	45.3 ± 5.2	35.6, 56.0	S8	27.1 ± 3.5	21.0, 33.9
C18	79.9 ± 5.3	69.8, 90.4	S9	16.1 ± 3.0	10.9, 22.3
C19	36.2 ± 6.4	24.6, 49.4	S10	23.6 ± 3.4	17.9, 30.3
C20	28.3 ± 4.3	20.7, 37.4	S11	26.9 ± 3.5	20.7, 34.0
L1	84.7 ± 4.8	75.6, 94.3	S12	42.8 ± 4.4	34.9, 51.4
L2	77.6 ± 4.5	69.2, 86.5	S13	39.2 ± 4.3	31.6, 47.7
L3	68.0 ± 4.4	60.1, 76.8	S14	36.8 ± 4.3	29.2, 45.3
L4	63.7 ± 4.5	55.5, 72.7	S15	20.6 ± 3.3	15.3, 26.9
L5	61.0 ± 4.4	53.0, 69.8	S16	18.8 ± 3.2	13.5, 25.0
L6	59.3 ± 4.4	51.3, 68.1	S17	46.4 ± 4.5	38.3, 55.2
L7	36.3 ± 4.1	29.0, 44.4	S18	41.6 ± 4.7	33.1, 50.8
L8	28.9 ± 3.7	22.6, 36.3	S19	27.4 ± 4.2	19.9, 35.9
L9	22.1 ± 3.5	16.4, 28.9	S20	42.7 ± 4.5	34.6, 51.7
L10	13.8 ± 3.1	9.0, 19.5	S21	47.7 ± 4.8	39.0, 57.3
L11	0.4 ± 1.6	0.0, 0.8	S22	65.2 ± 5.0	56.1, 75.3

L12	25.7 ± 3.7	19.5, 33.0	S23	60.1 ± 4.9	51.0, 70.2
L13	22.1 ± 3.5	16.1, 29.3	S24	48.5 ± 4.9	39.6, 58.5
L14	33.0 ± 4.2	25.5, 41.4	S25	37.6 ± 4.8	28.9, 47.4
L15	55.2 ± 4.5	46.9, 64.2	S26	26.0 ± 4.2	18.6, 34.9
L16	25.7 ± 2.8	21.0, 31.3	S27	28.1 ± 4.1	21.0, 36.4
L17	19.3 ± 1.8	16.8, 22.8	S28	44.7 ± 5.1	35.5, 55.3
L18	17.8 ± 1.4	16.2, 20.7	S29	55.3 ± 5.2	45.6, 65.8
L19	16.8 ± 1.0	15.9, 19.0	S30	39.6 ± 5.0	30.2, 49.8

Supplementary Table 3. Time constraints based on the fossil record or molecular dates. Node labels as in Fig. 1 in the main text. Fossil constraints are labeled A to N and are taken from Brodkorb (1964), except *Vegavis iaii* (Clarke et al. 2005) and were set as mininum ages. Molecular time constraints O and P are from Pereira and Baker (2006) and were fixed as shown.

Node	Age (Myr)	Fossil	Split
A	15.9	Four spp of *Larus*	*Larus* x *Rissa*
B	11.6	*Uria antiquua*	*Uria* x *Alca*, *Alle*
C	2.6	*Brachyramphus pliocenus*	*Brachyramphus* x *Cepphus*, *Synthliboramphus*, *Uria*, *Alca*, *Alle*
D	2.6	*Ptychoramphus tenuis*	*Ptychoramphus* x *Aethia*, *Cyclorhynca*
E	11.6	*Cerorhincha dubia*	*Cerorhincha* x *Fratercula*
F	3.6	*Calidris pacis*	*Calidris* x *Aphriza*
G	1.8	*Micropalama hesternus*	*Micropalama* x *Tryngites*
H	33.9	*Limosa gypsorum*	*Limosa* x *Prosobonia*
I	11.6	*Numenius antiquus*	*Numenius* x *Bartramia*
J	28.4	*Charadrius sheppardianus*	*Charadrius* x *Elseyornis*, *Thinornis*
K	28.4	*Vanellus selysii*	*Vanellus* x *Anarhynchus*, *Peltohyas*, *Erythrogonys*
L	65.5	Three spp of *Paleotringa*	Scolopaci x Lari
M	65.5	Three spp of *Cimolopteryx, Ceramornis major,* Three spp of *Paleotringa*	Charadriiformes x outgroups
N	65.5	*Vegavis iaii*	*Anas* x *Anhima*, *Chauna*
O	101.6	Molecular date	Anseriformes x Galliformes
P	122.2	Molecular date	Galloanserae x Neoaves