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Convergence and divergence in the evolution of aquatic birds

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Aquatic birds exceed other terrestrial vertebrates in the diversity of their adaptations to aquatic niches. For many species this has created difficulty in understanding their evolutionary origin and, in particular, for the flamingos, hamerkop, shoebill and pelecaniforms. Here, new evidence from nuclear and mitochondrial DNA sequences and DNA–DNA hybridization data indicates extensive morphological convergence and divergence in aquatic birds. Among the unexpected findings is a grouping of flamingos and grebes, species which otherwise show no resemblance. These results suggest that the traditional characters used to unite certain aquatic groups, such as totipalmate feet, foot-propelled diving and long legs, evolved more than once and that organismal change in aquatic birds has proceeded at a faster pace than previously recognized.

Keywords: phylogeny; flamingo; grebe; avian; DNA sequence; DNA–DNA hybridization

1. INTRODUCTION

Many adaptations in birds to life around water are related to feeding style (Storer 1971). Examples include the traditional pelecaniforms with their webbing around all four toes (totipalmate feet), loons and grebes with their posteriorly positioned legs for diving and storks and allies with their long legs adapted for wading. Traditionally, such morphologically distinct groups have been given taxonomic and evolutionary status (Cracraft 1981, 1988; Feduccia 1996) (figure 1). Although flamingos have most often been placed with other long-legged waders (Cracraft 1981, 1988; Sibley & Ahlquist 1990), some characters have suggested an affinity with ducks (Sibley & Ahlquist 1990; Feduccia 1996) or with shorebirds (Olson & Feduccia 1980). Furthermore, the five living species of flamingos show many unique characters related to their unusual filter-feeding lifestyle in tropical saline waters (Olson & Feduccia 1980; Zweers *et al*. 1994). The shoebill and hamerkop are two additional enigmatic species, which show a blend of morphological characters shared with either waders or non-waders (Sibley & Ahlquist 1990; Feduccia 1996). For these reasons, close relationships of these enigmatic birds to other long-legged wading birds have remained tenuous at best.

Among molecular phylogenetic investigations of aquatic birds, one DNA–DNA hybridization study, which was performed by Sibley & Ahlquist (1990), is unique in both the number of their species comparisons and the nature of their findings. Except for ducks, cranes and rails, the aquatic birds formed a single evolutionary group (Ciconiiformes). The relationships within this large aquatic group were non-traditional and the 'Pelecaniformes' appeared as polyphyletic. In particular, some birds with divergent morphologies formed sister groups (e.g. storks with condors and pelicans with shoebill), while other birds with similar morphologies seemed more distantly related (e.g. loons with grebes and pelicans with cormorants). In order to account for such relationships, rapid rates of morphological evolution were implied. Such scenarios have generally received little support (Feduccia 1996). However, both morphological convergence and divergence have been described before in aquatic organisms (Storer 1971; Nikaido *et al*. 1999). It is therefore surprising that we still lack subsequent molecular investigations with complete familial representations of these aquatic birds. In order to address this issue, we examined the phylogenetic position of the enigmatic flamingos, hamerkop and shoebill among the other major aquatic bird families by obtaining new DNA sequences and DNA–DNA hybridization data.

2. METHODS

(a) *DNA sequence analyses*

Mitochondrial gene sequences were obtained from the 12S rRNA, $tRNA^{Val}$ and 16S rRNA genes, yielding *ca.* 3 kb per sequence. Twenty-six representatives of the major families of Ciconiiformes and Gruiformes (Sibley & Ahlquist 1990) were included, with the domestic duck and fowl serving as outgroups. With Ciconiiformes as part of a neoavian clade, galloanserine birds have been shown to provide appropriate outgroup taxa to this clade (Sibley & Ahlquist 1990; Van Tuinen *et al*. 2000). Most mitochondrial sequences have been obtained previously (Van Tuinen *et al*. 2000): *Balaeniceps rex* (AF173569), *Charadrius semipalmatus* (AF173565), *Ciconia nigra* (AF173571), *Fregata magnificens* (AF173576), *Gavia immer* (AF173577), *Gavia stellata* (AF173578), *Grus canadensis* (AF173564), *Gymnogyps californianus* (AF173574), *Larus glaucoides* (AF173566), *Neophron percnopterus* (AF173581), *Pelecanus occidentalis* (AF173570), *Phaethon aethereus* (AF173592), *Phalacrocorax brasilianus* (AF173580), *Phoenicopterus ruber* (AF173568), *Podiceps auritus* (AF173567), *Puffinus gravis* (AF173572), *Pygoscelis adeliae* (AF173573), *Sula nebouxii* (AF173579) and *Vultur gryphus* (AF173575). A heron (*Nycticorax nycticorax*), spoonbill (*Platalea alba*), hamerkop (*Scopus umbretta*) and a second grebe (*Aechmophorus occidentalis*) were

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added to this data set using standard primers and a sequencing protocol (Hedges & Sibley 1994; Van Tuinen *et al*. 2000). Other available representative sequences were obtained from GenBank (*Ciconia ciconia* (AB026818), *Ciconia boyciana* (AB026193), *Falco peregrinus* (AF090338), *Gallus gallus* (X52392) and *Anas platyrhynchos* (L16770)). A 750-base pair (bp) fragment of a grebe (*Podiceps auritus*) was sequenced, aligned and analysed for the mitochondrial cytochrome *b* gene, with more than 180 other aquatic bird sequences available for this gene. Accession numbers for those sequences and alignments and other supplementary data are available at http://www.evogenomics.org/publications/data/flamingo/index.htm.

Unless otherwise noted, sequences from two nuclear genes were obtained with representatives identical to the mitochondrial rRNA data set and using the same methods as for the mitochondrial sequences. A 600-bp exon fragment of the *c*-mos proto-oncogene was sequenced in 16 of the earlier mentioned aquatic species using previously described primers (Cooper & Penny 1997) and added to available sequences from a loon (*Gavia arctica* (U88423)), penguin (*Eudyptes pachyrhynchus* (U88420)), shearwater (*Puffinus griseus* (U88421)), tropic bird (*Phaethon rubricauda* (U88418)), gull (*Larus heermanni* (U88419)), guinea fowl (*Numida meleagris* (U88425)) and domestic fowl (*G. gallus* (M19412)). This exon fragment was only partially obtained (sequenced) in the domestic duck (391 bp). A 370-bp fragment of intron 11 (with respect to *G. gallus* (M11213)) of the glyceraldehyde-3-phosphodehydrogenase (G3PDH) gene was obtained for 18 birds (with one loon and grebe representative and *Porphyrio porphyrio* as a gruiform representative but excluding frigate bird and vulture representatives due to amplification problems) using the primer pairs G3P13/G3P14 and G3P13A/G3p14 (G3P13 = TCCAYCTTTGATGCGGRTGCTGGMAT, G3P13A = GGCATTGCACTGARYGAYCATTT and G3P14 = ARRTCCACAACACGGTTGCTGTA). The combined nuclear and mitochondrial data set included a shoebill, hamerkop and one flamingo as well as one heron, stork, spoonbill, pelican, cormorant, booby, tropic bird, penguin, shearwater, plover, gull, gruiform, grebe, loon, domestic duck and fowl. New sequences have been deposited in GenBank under accession numbers AF339322–AF339361.

Sequences were aligned by individual gene using the multiple alignment option in CLUSTAL W (Thompson *et al*. 1994). Phylogenetic analyses and estimation of most data parameters were performed in PAUP* (Swofford 1998) and MEGA (Kumar *et al*. 1993). The shape parameter of the gamma distribution for variable evolutionary rates among sites was estimated for each gene and the combined data set using PAML (Yang 1997). The domestic duck and fowl were used as outgroup species in all analyses and a pairwise deletion option (Nei & Kumar 2000) was used whenever nuclear genes were involved due to the nature of these data sets (randomly distributed indels in the G3PDH intron and a shorter *c*-mos sequence size of the domestic duck). The stability of the topologies based on the combined data set ($n = 19$) was tested using different tree-building methods (maximum likelihood, maximum parsimony and neighbour joining). Combined and gene-specific analyses (using individual genes) in conjunction with assessment of the effect of distance correction (*P*, gamma, Jukes–Cantor and Kimura two-parameter methods), differing substitution rates (transversion weighted analyses), biased base composition (Tamura–Nei distances), possible heterogeneous base composition among sequences (logarithmic determined-transformed distances), differing frequency of invariant sites (Swofford 1998) and differing order of sequence addition into initial alignment were performed with the neighbour-joining method. Unless stated otherwise, the Tamura–Nei distance was used because it takes into account the biased base composition of the respective data sets. The signal for alternative topologies was explored with Kishino–Hasegawa tests in conjunction with maximum likelihood (Swofford 1998) and through spectral analyses (Hendy & Penny 1993) on maximally 20×20 distance matrices from the separate and combined gene data. Spectral analyses were performed with SPECTRUM (Charleston & Page 1997). In order to assess the significance of resulting nodes, the bootstrap method was applied with 2000 (neighbour joining), 500 (maximum parsimony) and 100 (maximum likelihood) iterations using MEGA (Kumar *et al*. 1993) and PAUP* (Swofford 1998) and values of 95% were considered to be significant. Standard error tests were employed with neighbour joining only. Relative apparent synapomorphy analysis (RASA) was employed for assessing the overall signal of the separate and combined genes and the topological effect of subsequent noise reduction (Lyons-Weiler *et al*. 1996), performing optimal outgroup analyses (Lyons-Weiler *et al*. 1998) and identifying significant topology-altering long branches using taxon-variance plots. These analyses identified the *F. peregrinus* RNA sequence as a significant long branch and we excluded this sequence from the final analyses.

(b) *DNA–DNA hybridization analyses*

DNA hybridization experiments were performed using a protocol modified from that of Sibley & Ahlquist (1990) and described previously (Kirsch *et al*. 1990; Bleiweiss *et al*. 1994) and included 21 mostly aquatic species. Representative birds were as in the DNA sequence study except for *Accipiter melanoleucus*, *Amaurornis phoenicurus*, *Anhinga rufa*, *Bubo virginianus*, *Diomedea bulleria*, *Egretta novaehollandiae*, *Mycteria americana*, *Phaethon rubricauda*, *Phalacrocorax carunculatus*, *Plegadis falcinellus*, *Pygoscelis papua* and *Sula dactylatra*. Every species was labelled and the final matrix was complete except for 14 heterologous comparisons, with an average of 3.2 replicates per cell. Missing measurements were estimated by reflection from known reciprocals after symmetrization of the matrix (Sarich & Cronin 1976). The G, S and P = 0 options were employed in FITCH tree calculation, the input order of birds was randomly varied 100 times and the tree was validated by bootstrapping and jackknifing procedures (Krajewski & Dickerman 1990; Felsenstein 1993; Lapointe *et al*. 1994). See http://www.evogenomics.org/publications/data/flamingo/index.htm for the hybridization matrices and a nearly congeneric Sibley & Ahlquist (1990) data set ($n = 18$) used for comparison.

3. RESULTS AND DISCUSSION

We obtained sequences from four mitochondrial and two nuclear genes from representative aquatic birds as well as other possible flamingo relatives (the crane, rail and domestic duck) and outgroup species. The RASA analyses (Lyons-Weiler *et al*. 1996) showed that each gene contained a significant ($p < 0.01$), non-random phylogenetic signal and that, for all of these genes, the domestic duck and fowl sequences were not long branches and, thus, provided valid outgroup species ($t\mathrm{RASA}_{\mathrm{rooted}} > t\mathrm{RASA}_{\mathrm{unrooted}}$) (Lyons-Weiler *et al*. 1998). Because the combined data set, which comprised 19 birds and 4062 sites, displayed the largest signal ($t\mathrm{RASA} = 7.2$) among sets with equal numbers of birds, we performed phylogenetic analyses on

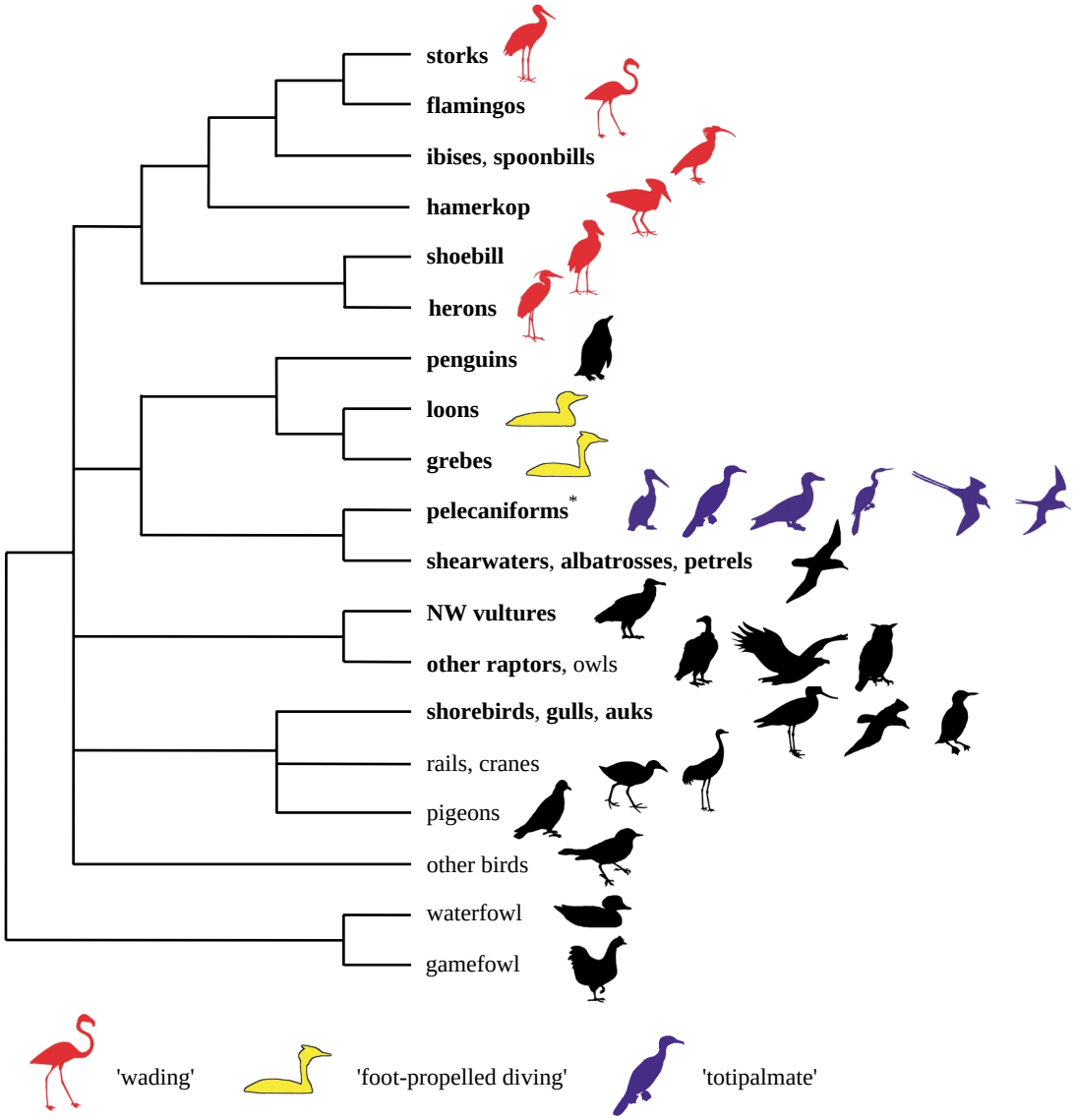

Figure 1. Traditional relationships of aquatic birds based on morphological studies (Cracraft 1981). More recently, herons were instead placed near the shorebirds, cranes and pigeons and the New World vultures were placed closest to the storks (Cracraft 1988). Bold-faced birds form a single order Ciconiiformes based on DNA–DNA hybridization data (Sibley & Ahlquist 1990). Pelecaniforms include the pelicans, frigate birds, tropic birds, darters, boobies and cormorants.

this larger data set. Although this data set was unable to resolve the entire aquatic bird phylogeny, significant resolution was found for the three enigmatic waders in question. Together with other widely accepted clusters (booby–cormorant, gull–plover and penguin–shearwater), the bootstrap consensus topology showed significance for an assemblage of the hamerkop with the shoebill and pelican and an unexpected cluster formed by the flamingo and grebe (figure 2). These five clusters appeared regardless of tree building method, distance correction, frequency of invariant sites, use of noise reduction (excluding 1070 sites) or when varying the order of sequence input during the aligning process.

Because the latter two clusters (hamerkop plus shoebill plus pelican and flamingo plus grebe) conflict with other available molecular data (Sibley & Ahlquist 1990), we subsequently investigated phylogenetic consistency between genes regarding these birds. The separate gene trees all supported the flamingo–grebe cluster (72, 84 and 96%, respectively, for *c*-mos ($n=25$), G3PDH ($n=19$) and combined mitochondrial RNA ($n=27$ excluding *Falco*) genes using a Tamura–Nei distance on transversions only). In the species-dense ($n=181$ sequences) conditions of the mitochondrial cytochrome *b* data set, where few groupings were supported by significant bootstrap values, the flamingo and grebe clustered together either with (56%) or without (40%) transitions. The phylogenetic placement of the shoebill with the pelican was also supported by all (individual and combined) genes, as well as an alliance of this group with the hamerkop in the four individual non-protein coding genes. Although not in conflict with such a placement, the protein-coding *c*-mos fragment alone did not resolve the position of the hamerkop.

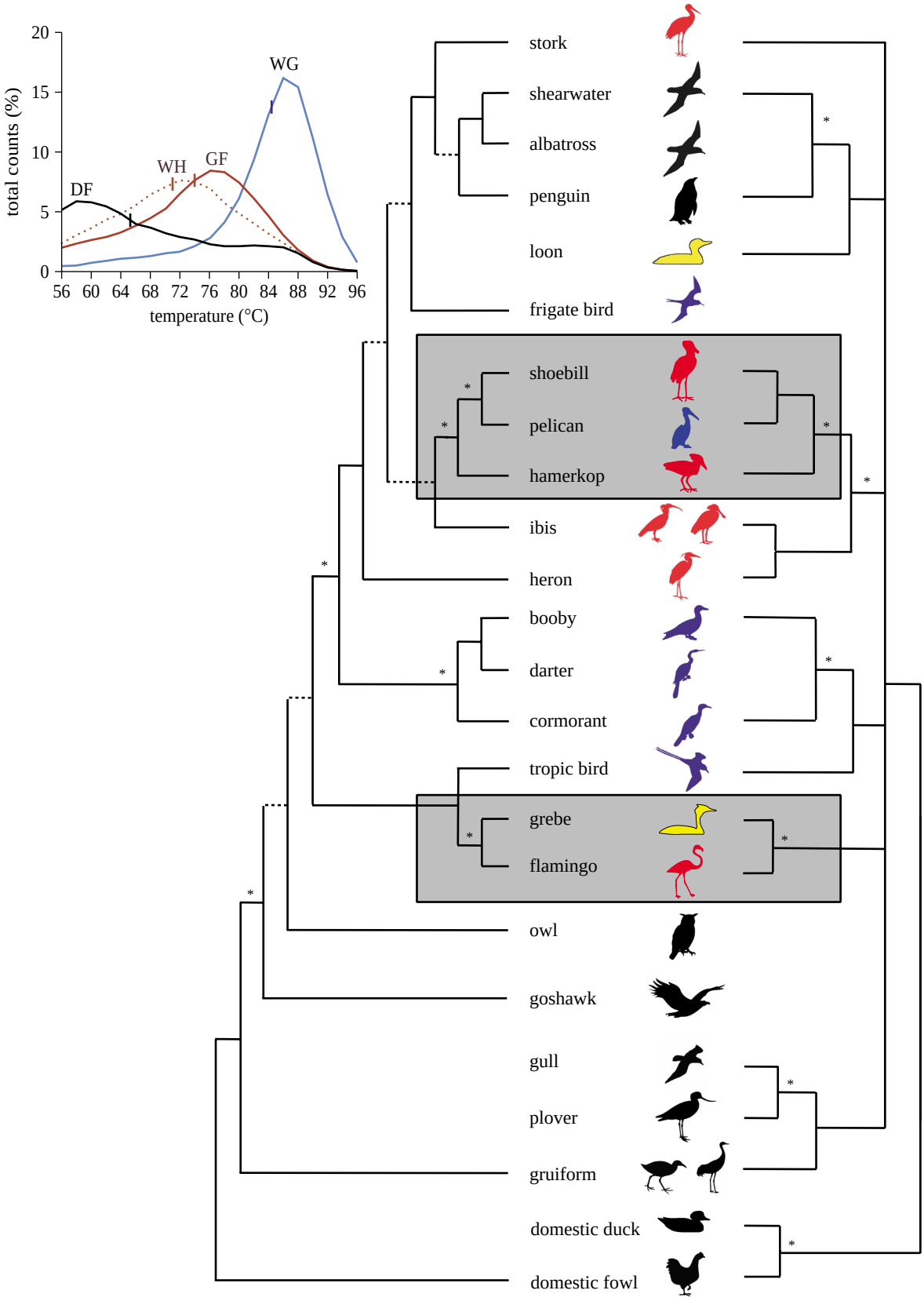

Figure 2. Molecular evidence bearing on the positions of flamingos, hamerkop and shoebill. Colour coding as in figure 1. Left: DNA–DNA hybridization phylogeny based on a FITCH (Felsenstein 1993) computation on ΔT_ms (median melting point differences) using the G, S and P = 0 options and varying the input order of taxa 100 times. The unexplained tree sum of squares was 0.9% of the total matrix sum of squares ($n = 1431$) (average repeated measurements per cell = 3.2, average standard deviation (s.d.) = 0.39 and correlation of s.d.s with distance = −0.01). The tree was tested by the bootstrap for distances (Krajewski & Dickerman 1990) and jackknife for weighted trees (Lapointe *et al.* 1994). Asterisks indicate nodes supported by 100% of the 1000 bootstrap replicates, except for that uniting all taxa including and above the booby–darter–cormorant clade, which received 98% support. All other nodes were supported by > 50% of the replicates. The shoebill–pelican–hamerkop and

In addition, we tested for the presence of an alternative signal in the combined sequence data set ($n = 19$) by comparing the maximum-likelihood values for trees based on the same observed data parameters (invariable proportion = 0.502, alpha = 0.27 and transition = 1.6) but under different topological constraints. These Kishino–Hasegawa tests (Swofford 1998; but see Goldman *et al.* 2000) significantly ($p < 0.0001$) supported the tree that yielded a grebe–flamingo cluster ($\ln L = -30001.37$) (shown in condensed form in figure 2), as opposed to a duck–flamingo relationship ($\ln L = -30120.882$) (Sibley & Ahlquist 1990), a plover–flamingo relationship ($\ln L = -30084.66$) (Olson & Feduccia 1980; Feduccia 1996), a topology based on previous hybridization data ($\ln L = -30151.45$) (Sibley & Ahlquist 1990) and the traditional phylogeny ($\ln L = -30320.34$) (Cracraft 1988). Spectral analyses also supported the five groupings with high support:low conflict while showing low or no support for other alternative associations. Specific to the position of the enigmatic aquatic birds in this study, the support:conflict ratio for a grouping of the flamingo with the grebe was 45.5, which was higher than those ratios for a grouping of the flamingo with either the duck (1.4×10^{-3}) or shorebird (3.1×10^{-3}). The support for the hamerkop as sister to a shoebill–pelican grouping yielded a support:conflict ratio of 114.9, as opposed to 4.1×10^{-4} for a monophyletic Pelecaniformes.

In order to investigate the apparent discrepancy between the sequence data and the available hybridization data further (Sibley & Ahlquist 1990), we constructed a new, nearly complete hybridization distance matrix comprising 21 representative birds. Although agreeing with earlier hybridization data (Sibley & Ahlquist 1990) in showing the polyphyletic associations of various 'pelecaniforms', these data also showed a significant flamingo–grebe relationship as well as a hamerkop–shoebill–pelican cluster (figure 2). Because of the latter difference from earlier hybridization results, we re-examined Sibley & Ahlquist's (1990) original data for birds, which approximately corresponded to those in figure 2. Among those data were two distances from the western grebe (which was radioactively labelled with iodine-125) to the flamingo that were on average shorter than those to other birds in the data set. We calculated bootstrapped trees with a 70% complete Sibley & Ahlquist (1990) data set for 18 species using an additive-estimation program in order to complete each pseudo-replicate matrix (Landry *et al.* 1996). The results, like those for our own matrix, also united the western grebe and flamingo, with 86% (T_{50}Hs-expressed: median melting points corrected for per cent hybridization) or 69% (T_{mode}s-expressed: temperatures at which the largest number of sequences melt) support in 1000 replicates on the Sibley & Ahlquist (1990) data. Other associations of 'pelecaniforms' such as the booby–darter–cormorant grouping and that of the pelican with the shoebill and hamerkop received 100% bootstrap support. The 'pelecaniform' frigate bird and tropic bird were separated from these trios and from each other as suggested previously (Sibley & Ahlquist 1990). Presumably, Sibley & Ahlquist (1990) did not report the flamingo–grebe and hamerkop–shoebill pairing because their comparisons were so few or because both members of a pair were not included in any tree calculated by them.

Thus, the hybridization and sequence data consistently united the flamingos with grebes and the hamerkop with the pelican and shoebill, thereby further disassociating the 'pelecaniforms'. Some of these findings have support from other data as well, including a smaller molecular data set (Hedges & Sibley 1994), fossil evidence (Olson 1985), middle ear morphology (Saiff 1978) and jaw articulation (Cottam 1957). However, to our knowledge, the flamingo–grebe grouping is novel. We further eliminated the possibility of contamination of either flamingos or grebes by cross-checking our sequences with other available sequences in public databases as well as using independent DNA sources in the sequence and hybridization experiments in this study. We propose that this unusual alliance of birds has been overlooked because the exceptional adaptations to their respective aquatic niches have obscured evolutionary history.

The distant relationship of grebes and loons within aquatic birds implies convergent evolution of morphology imposed by the aquatic niche. Their hind leg musculature, bill shape and streamlined body are clear adaptations for catching fish by means of foot-propelled diving (Storer 1971) and fossils have provided evidence for the antiquity of this lifestyle (Chiappe 1995; Feduccia 1996). Flamingos are divergent from both this body plan and lifestyle. Our data also indicated morphological convergence among the totipalmate birds ('pelecaniforms') and among the wading birds (figures 1 and 2). Specifically, the characters employed in uniting Pelecaniformes, e.g. the totipalmate condition and presence of a gular pouch in wading birds (*sensu* Cracraft 1981), e.g. related to long leg size, have probably converged in different aquatic bird lineages. In addition, these data suggest that the shoebill is not an aberrant pelecaniform, as proposed before (Cottam 1957; Saiff 1978), but instead that the pelicans are aberrant long-legged waders in which leg size has been secondarily reduced. Denser species sampling is needed in order to determine the closest relative of the shoebill–hamerkop–pelican assemblage. These new findings add to previous evidence for convergence among

Figure 2. (*Contd*) grebe–flamingo groups were also fully supported by the jackknife, which was based on all single and 5000 random multiple deletions of taxa. Nodes that were not supported by jackknifing are shown with dotted lines. Inset: representative stepwise thermal-elution curves for four taxa, corrected for percentage hybridization (from right to left, the homologous western grebe (WG), greater flamingo (GF), white-faced heron (WH) and domestic fowl (DF)). Vertical marks indicate T_{m}s. Note that the flamingo curve is some 4° closer to the homologue than are the other two heterologues. Right: sequence-based phylogeny, as shown by a 50% condensed bootstrap consensus tree (Nei & Kumar 2000) for the combined genes (*c*-mos proto-oncogene exon, G3PDH intron 11 and complete 12S rRNA, $\mathrm{tRNA}^{\mathrm{Val}}$ and 16S rRNA genes). The combined data parameters are as follows: 4062 total, 1855 variable and 1196 parsimony informative sites, alpha = 0.268 and transition/transversion = 1.6 and T = 21.2%, C = 26.1%, A = 30.0% and G = 22.7%. Asterisks indicate nodes that were significantly (> 95%) supported based on bootstrapping using maximum likelihood or a standard error test using neighbour joining.

auks and penguins and gulls and albatrosses (Storer 1971; Feduccia 1996).

Some insight into the early evolution of flamingos and grebes can be gleaned from their fossil record. Although some fossil flamingos (*Palaelodus*) might be interpreted as being grebe-like in appearance and behaviour (Feduccia 1996), the earliest flamingo fossils resemble a more typical shorebird (Olson & Feduccia 1980). The earliest extant shorebirds and gruiforms consist of small-bodied rail-like water birds (Sibley & Ahlquist 1990). The flamingo style of filter feeding may have evolved through accidental water intake during pecking at food in water (Zweers *et al*. 1994). Likewise, the grebe style of diving would appear to be equally derived. Thus, the flamingos and grebes probably each represent morphological divergence from a typical shorebird habitus and lifestyle.

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REFERENCES

Bleiweiss, R. E., Kirsch, J. A. W. & Matheus, J. C. 1994 DNA hybridization evidence for subfamily structure among hummingbirds. *Auk* **111**, 8–19.

Charleston, M. A. & Page, R. D. M. 1997 *SPECTRUM, version 2.0.* Glasgow, UK: University of Glasgow.

Chiappe, L. M. 1995 The first 85 million years of avian evolution. *Nature* **378**, 349–355.

Cooper, A. & Penny, D. 1997 Mass survival of birds across the K–T boundary: molecular evidence. *Science* **275**, 1109–1113.

Cottam, P. A. 1957 The pelecaniform characters of the skeleton of the shoebill stork *Balaeniceps rex*. *Bull. Br. Mus. Nat. Hist.* **5**, 51–72.

Cracraft, J. 1981 Toward a phylogenetic classification of the recent birds of the world (class Aves). *Auk* **98**, 681–714.

Cracraft, J. 1988 The major clades of birds. In *The phylogeny and classification of the tetrapods* (ed. M. J. Benton), pp. 339–361. Oxford, UK: Clarendon Press.

Feduccia, A. 1996 *The origin and evolution of birds*. New Haven, CT: Yale University Press.

Felsenstein, J. 1993 *PHYLIP, phylogenetic inference package, program and documentation*, v. 3.5c. Seattle, WA: University of Washington.

Goldman, N., Anderson, J. P. & Rodrigo, A. G. 2000 Likelihood-based tests of topologies in phylogenetics. *Syst. Biol.* **49**, 652–670.

Hedges, S. B. & Sibley, C. G. 1994 Molecules vs. morphology in avian evolution: the case of the 'pelecaniform' birds. *Proc. Natl Acad. Sci. USA* **91**, 9861–9865.

Hendy, M. D. & Penny, D. 1993 Spectral analysis of phylogenetic data. *J. Class.* **10**, 5–24.

Kirsch, J. A. W., Springer, M. S., Krajewski, C., Archer, M., Aplin, K. & Dickerman, A. W. 1990 DNA–DNA hybridization studies of carnivorous marsupials. I. The intergeneric relationships of bandicoots (Marsupialia: Perameloidea). *J. Mol. Evol.* **30**, 434–448.

Krajewski, C. & Dickerman, A. W. 1990 Bootstrap analysis of phylogenetic trees derived from DNA hybridization distances. *Syst. Zool.* **39**, 383–390.

Kumar, S., Tamura, K. & Nei, M. 1993 *MEGA: molecular evolutionary genetic analysis, version 1.01.* University Park, PA: Institute of Molecular Evolutionary Genetics, The Pennsylvania State University.

Landry, P.-A., Lapointe, F.-J. & Kirsch, J. A. W. 1996 Estimating phylogenies from lacunose distance matrices: additive is superior to ultrametric estimation. *Mol. Biol. Evol.* **13**, 818–823.

Lapointe, F.-J., Kirsch, J. A. W. & Bleiweiss, R. E. 1994 Jackknifing of weighted trees: validation of phylogenies reconstructed from distance matrices. *Mol. Phylogenet. Evol.* **3**, 256–267.

Lyons-Weiler, J., Hoelzer, G. A. & Tausch, R. J. 1996 Relative apparent synapomorphy analysis (RASA) I. The statistical measurement of phylogenetic signal. *Mol. Biol. Evol.* **13**, 749–757.

Lyons-Weiler, J., Hoelzer, G. A. & Tausch, R. J. 1998 Optimal outgroup analysis. *Biol. J. Linn. Soc. Lond.* **64**, 493–511.

Nei, M. & Kumar, S. 2000 *Molecular evolution and phylogenetics*. Oxford University Press.

Nikaido, M., Rooney, A. P. & Okada, N. 1999 Phylogenetic relationships among cetartiodactyls based on insertions of short and long interpersed elements: hippopotamuses are the closest extant relatives of whales. *Proc. Natl Acad. Sci. USA* **96**, 10 261–10 266.

Olson, S. L. 1985 The fossil record of birds. In *Avian biology* (ed. D. S. Farner, J. R. King & K. C. Parkes), pp. 79–252. New York: Academic Press.

Olson, S. L. & Feduccia, A. 1980 Relationships and evolution of flamingos (Aves: Phoenicopteridae). *Smithson. Contr. Zool.* **316**, 1–73.

Saiff, E. I. 1978 The middle ear of the skull of birds: the Pelecaniformes and Ciconiiformes. *Zool. J. Linn. Soc. Lond.* **63**, 315–370.

Sarich, V. M. & Cronin, J. E. 1976 Molecular systematics of the primates. In *Molecular anthropology, genes, and proteins in the evolutionary ascent of primates* (ed. M. Goodman & R. E. Tashian), pp. 141–170. New York: Plenum Press.

Sibley, C. G. & Ahlquist, J. E. 1990 *Phylogeny and classification of birds: a study in molecular evolution*. New Haven, CT: Yale University Press.

Storer, R. W. 1971 Adaptive radiation in birds. In *Avian biology* (ed. D. S. Farner & J. R. King), pp. 149–188. New York: Academic Press.

Swofford, D. L. 1998 *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, v. 4.0. Sunderland, MA: Sinauer Associates.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994 Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* **22**, 3488–3494.

Van Tuinen, M., Sibley, C. G. & Hedges, S. B. 2000 The early history of modern birds inferred from DNA sequence of mitochondrial and nuclear ribosomal genes. *Mol. Biol. Evol.* **17**, 451–457.

Yang, Z. 1997 PAML: a program package for phylogenetic analysis by maximum likelihood. *CABIOS* **13**, 555–556.

Zweers, G. A., De Jong, F., Berkhoudt, H. & Vandenberge, J. C. 1994 Filter-feeding in flamingos (*Phoenicopterus ruber ruber*). *Condor* **97**, 297–324.