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The phylogenetic relationships of the extant pelicans inferred from DNA sequence data

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ABSTRACT

The pelicans are a charismatic group of large water birds, whose evolutionary relationships have been long debated. Here we use DNA sequence data from both mitochondrial and nuclear genes to derive a robust phylogeny of all the extant species. Our data rejects the widespread notion that pelicans can be divided into white- and brown-plumaged groups. Instead, we find that, in contrast to all previous evolutionary hypotheses, the species fall into three well-supported clades: an Old World clade of the Dalmatian, Spot-billed, Pink-backed and Australian Pelicans, a New World clade of the American White, Brown and Peruvian Pelicans, and monospecific clade consisting solely of the Great White Pelican, weakly grouped with the Old World clade. We discuss possible evolutionary scenarios giving rise to this diversity.

1. Introduction

The pelicans are an almost cosmopolitan group of large water birds (see Fig. 1), with distinctive pouched bills and webbed (“totipalmate”) feet. This latter feature was long used to group pelicans with cormorants, gannets, boobies, frigatebirds and tropicbirds in the Pelecaniformes, although most recent work (e.g., Hackett et al., 2008) has not recovered this traditional order as monophyletic. Nevertheless, the pelicans themselves are uncontroversially monophyletic, with all species currently classified in the Linnaean genus *Pelecanus* within the monogeneric Pelecanidae. The pelicans have a long independent evolutionary history (as do their distinctive pouched bills), with the oldest fossil attributed to *Pelecanus* being ca. 30 million years old (Louchart et al., 2011).

Most authorities today recognize eight extant species, as listed in Table 1. The most recent taxonomic change was the elevation to species status of the Peruvian Pelican (*P. thagus*), previously treated as a subspecies of the American Brown Pelican (*P. occidentalis*), on the basis of its much greater size (approximately double the weight of the Brown), differences in plumage and bill colors, and a lack of hybridization in spite of a sizable area of range overlap (Jaramillo, 2007). Most of the pelican species today exist in much smaller numbers than a few decades ago and several are of conservation concern (Nelson, 2005), although the Brown Pelican has recovered considerably since the banning of DDT in North America (Anderson et al., 1975; USFWS, 2008).

Recognition of the evolutionary relationships among these species, however, has not been straightforward. Peters's (1931) taxonomy separated each of the American White Pelican and the Brown Pelican (including the Peruvian) into monospecific subgenera, a view endorsed by Dorst and Mougin (1979). These latter authors also followed the assertion of Delacour and Mayr (1945) that the Dalmatian and Spot-billed Pelicans were closely related, subspecifically distinct.

More recently, however, Elliott (1992) and Nelson (2005) considered the four largest species (Great White, American White, Australian and Dalmatian), which share a number of ecological and behavioral features, most notably nesting on the ground, to be closely related allospecies. Of these species, the Dalmatian was thought by Nelson (2005) to be the most divergent. The Spot-billed and Pink-backed, which are smaller and tree nesters, were considered by both Elliott (1992) and Nelson (2005) to be sister species and the plunge-diving, marine Brown (again including the Peruvian) was held to be the most divergent of all. Johnsgard (1993) hypothesized that the pelicans derived from an African or southern Asian ancestor and spread out through northern Asia and Australia and finally to North America, which would seem to imply that the Brown group and the American White would be sister taxa (unless they resulted from multiple invasions of North America). In contrast, in his species accounts, Johnsgard (1993) endorsed the widespread view of the Brown (+Peruvian) being the most divergent and suggested that the American White is closest to the Great White.

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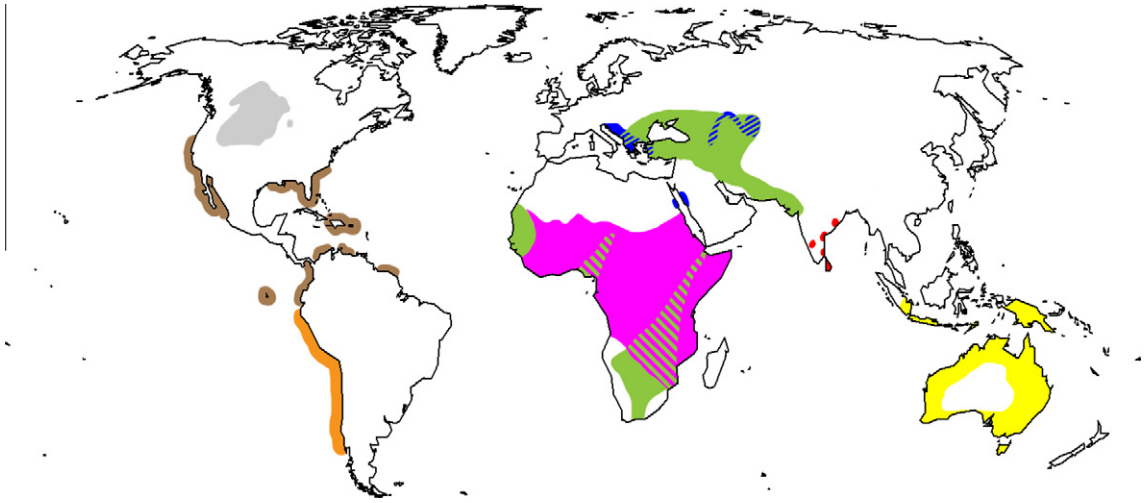

Fig. 1. Approximate distributions of the world's pelican species (after Nelson, 2005). Gray – American White Pelican, *Pelecanus erythrorhynchos*. Brown – Brown Pelican, *Pelecanus occidentalis*. Orange – Peruvian Pelican, *Pelecanus thagus*. Green – Great White Pelican, *Pelecanus onocrotalus*. Pink – Pink-backed Pelican, *Pelecanus rufescens*. Blue – Dalmatian Pelican, *Pelecanus crispus*. Red – Spot-billed Pelican, *Pelecanus philippensis*. Yellow – Australian Pelican, *Pelecanus conspicillatus*. The overlapping regions between the Great White/Pink-backed and Great White/Dalmatian are shown by hatched colors. The distributions for the Great White and Brown pelicans show the breeding ranges (and not those described as "non-breeding" and "occurs") from Nelson's (2005) Fig. 5.3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Trees derived from genetic data, however, do not agree. Sibley and Ahlquist's (1990) UPGMA tree based on DNA–DNA hybridization data omitted the Peruvian, Dalmatian and Spot-billed, but implied rather different relationships: unexpectedly, the American White and Pink-backed were sister species, this pair being sister to the Great White and, in turn, this triplet was sister to the Australian, with the Brown the most divergent.

In this paper we investigate the phylogeny of the pelicans, sampling all recognized species and deriving robust, well-supported trees based on both mitochondrial and nuclear DNA sequence data. Our phylogeny contradicts all the views summarized above, although it has elements of them all, and allows for an interpretation of pelican evolution that makes sense in light of biogeography.

2. Materials and methods

Tissue or blood from all living species of pelican was obtained from a number of sources (see Table 1). Given the relationships found in Hackett et al. (2008) samples from a Shoebill, gannet, cormorant and frigatebird were selected for use as outgroups. Total genomic DNA was extracted from each of the samples using a phenol/chloroform extraction, a 5% Chelex 100 solution or the DNeasy Tissue Kit (Qiagen) (e.g., see Kennedy and Spencer, 2004; Spencer et al., 2006). Negative controls were included with each extraction. Following extraction, the DNA was amplified for five mitochondrial genes (the 12S ribosomal RNA gene, the overlapping ATPase-8 and -6 genes, the NADH dehydrogenase subunit 2 gene [ND2] and the cytochrome oxidase subunit I gene [COI]) and the nuclear β-fibrinogen intron 7 (FIB7). The polymerase chain reaction (PCR) was used to amplify these regions with 45 °C annealing for ATPase-8 and -6 and the barcoding region of COI, 50 °C annealing for the other COI fragment, ND2 and FIB7, and 55 °C annealing for 12S. For 12S, ATPase and part of COI we used the primers and followed the procedures described in Kennedy and Spencer (2004), e.g., using the primers COIf and COIa (see Palumbi, 1996) for COI. For the other COI fragment (the barcoding region) we used either the primer pair BirdF1 and BirdR1 or (more commonly) FalcoFA and VertR1 from Kerr et al. (2007). This barcoding fragment overlapped with the fragment produced by the COIf and COIa primer pair, and they could subsequently be combined (or were sometimes amplified as a single piece using the FalcoFA and COIa primer combination). For ND2 the primer pair Av5199tMetF (5′-GGTCAGCTAAATAAGCTATCGGG-3′) (know as L-Met3841 in Lloyd, 2003) and Av6314tTrpR (5′-CTCTTRTTTAAGGCTTTGAAGG-3′) (a modified version of H6313 from Sorenson et al., 1999) were used (Gillian Gibb, pers. comm.). For FIB7 the primers FIB-BI7U and FIB-BI7L (Prychitko and Moore, 1997) were used.

The PCR conditions were an initial denaturation step of 94 °C (3 min), followed by 40 cycles of 94 °C (30 s), variable annealing temperatures (as described above) for 30 s to 1 min, and 72 °C (1 min) and a final extension phase at 72 °C for 4 min. Negative controls were included with each PCR reaction. The PCR products were either left unpurified or were purified using the PureLink PCR purification kit (Invitrogen), the High Pure PCR purification kit (Roche), or the Ultra-Sep Gel extraction kit (Omega), and then sequenced on an automated sequencer using the PCR primers for the majority of the genes and internal primers for 12S (see Kennedy and Spencer, 2004).

The mitochondrial sequences were aligned by eye following the procedure outlined in Kennedy et al. (2000). For 12S all gaps of more than one base were removed to avoid mistaken homology. The FIB7 sequences were initially aligned using ClustalX 2.0 (Larkin et al., 2007) using the default settings before being finally aligned by eye. The sequences have been submitted to, or downloaded from, GenBank (Accession Nos. JX683910–JX683987, AY009321, AY009333, AY009345, AY009357, AY369042, AY369048, AY369052, AY369058, AY369059, AY369066, AY369072, AY941805, AY941809, DQ881957, EF101668, EF101673, and EF101684) and the data matrix and resultant phylogenetic tree to TreeBASE (www.treebase.org). Phylogenetic analyses were performed with MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) for Markov-chain Monte-Carlo Bayesian analysis and posterior probabilities, PAUP* version 4b10 (Swofford, 2002) for maximum parsimony (MP) and Neighbor-joining (NJ) bootstrap searches (Felsenstein, 1985) and PhyML 3.0 (Guindon et al., 2010) for maximum likelihood (ML) bootstrap searches. We used the partition-homogeneity test (Swofford, 2002) to investigate whether the different gene sequences contain

Table 1
Species used in the analysis.

Species	Voucher information/source of sample	Collection location
Magnificent Frigatebird *Fregata magnificens*	Sample 5782, Museum of Natural Science at Louisiana State University	Mexico
Australasian Gannet *Morus serrator*	Unvouchered sample (CD3), Martyn Kennedy	Hawke's Bay, New Zealand
Little Pied Cormorant *Phalacrocorax melanoleucos*	Sample 958, Museum of Victoria	Australia
Shoebill *Balaeniceps rex*	Unvouchered sample, Blair Hedges	Unknown
Australian Pelican *Pelecanus conspicillatus*	Sample 1883, Museum of Victoria	Australia
Dalmatian Pelican *Pelecanus crispus*	Unvouchered sample (303005), Petr Nádvorník	Zoo Dvůr Králové (wild origin Romania)
Dalmatian Pelican *Pelecanus crispus*	Unvouchered sample (303018), Petr Nádvorník	Zoo Dvůr Králové (wild origin Unknown)
American White Pelican *Pelecanus erythrorhynchos*	Samples 388009 and 395693, Field Museum	USA
Brown Pelican *Pelecanus occidentalis carolinensis*	Samples 5797 and 10336, Museum of Natural Science at Louisiana State University (presumed *carolinensis* from sampling location)	Louisiana, USA
Brown Pelican *Pelecanus occidentalis californicus*	Sample ORN 90541, California Academy of Sciences	California, USA
Brown Pelican *Pelecanus occidentalis californicus*	Sample ORN 91010, California Academy of Sciences	California, USA
Great White Pelican *Pelecanus onocrotalus*	Unvouchered sample (302006), Petr Nádvorník	Zoo Dvůr Králové (wild origin Romania)
Great White Pelican *Pelecanus onocrotalus*	Unvouchered sample (302009), Petr Nádvorník	Zoo Dvůr Králové (wild origin Romania)
Spot-billed Pelican *Pelecanus philippensis*	Unvouchered sample (594001), Petr Nádvorník	Zoo Dvůr Králové (wild origin Sri Lanka)
Spot-billed Pelican *Pelecanus philippensis*	Unvouchered sample (594006), Petr Nádvorník	Zoo Dvůr Králové (wild origin Sri Lanka)
Pink-backed Pelican *Pelecanus rufescens*	Unvouchered sample (418009), Petr Nádvorník	Zoo Dvůr Králové (wild origin Unknown)
Pink-backed Pelican *Pelecanus rufescens*	Unvouchered sample (418010), Petr Nádvorník	Zoo Dvůr Králové (wild origin Unknown)
Peruvian Pelican *Pelecanus thagus*	Unvouchered sample (Chile31), Scott Taylor	Chile
Peruvian Pelican *Pelecanus thagus*	Unvouchered sample (Peru44), Scott Taylor	Peru

Note: The samples collected from the Zoo Dvůr Králové birds are identified by the birds Zoo breeding book number.

similar signals and could thus be analyzed as a single data set. For visualization purposes the gannet, cormorant and frigatebird were defined as outgroup taxa (see Hackett et al., 2008).

The models of nucleotide substitution for the Bayesian analysis were selected using the Akaike Information Criterion of Modeltest 3.7 (Posada and Crandall, 1998). The models selected for each gene region were sub-models of GTR+I+G with more than two substitution types (GTR+I+G for 12S, TIM+I+G for ATPase, GTR+I+G for ND2, TrN+G for COI, and TVM for FIB7); thus, it is more appropriate to use six, rather than two, substitution types with each partition (gene).

Bayesian analysis was performed using MrBayes v3.1.2 with the maximum likelihood model employing 6 substitution types ("nst = 6") for each partition. For 12S, ATPase and ND2 rate variation across sites was modelled using a gamma distribution, with a proportion of the sites being invariant ("rates = invgamma"). For COI rate variation across sites was modelled using a gamma distribution, with none the sites being invariant ("rates = gamma"). For FIB7 the model selected had no rate variation across sites, and none of the sites were invariant ("rates = equal"). Trees were estimated for each partition individually (see Supplementary material), and for the combined mitochondrial data (see Supplementary material) and all of the partitions combined. For the combined datasets the different model parameters (e.g., shape, proportion of invariable sites, state frequency and substitution rate) were estimated for each partition separately. For all of the analyses the branch length priors were set to "Unconstrained:Exponential(100)" to account for potential branch length estimation problems (see Brown et al., 2010). The Markov-chain Monte-Carlo searches were run twice with 4 chains for 5,000,000 generations, with trees being sampled every 100 generations. Convergence of the duplicate runs was assessed both in Tracer v1.4 (Rambaut and Drummond, 2007), and via the average standard deviation of split frequencies. Following this assessment, the first 10,000 trees, i.e., 1,000,000 generations, were discarded as "burnin" in each of the analyses.

Congruence with other measures of support was evaluated using NJ, MP and ML bootstrap analyses (for all datasets) and, on the combined dataset, spectral analysis. The NJ bootstrap analyses consisted of 10,000 replicates with GTR distances. The equally weighted MP bootstrap analyses consisted of 1000 replicates using a heuristic search (with 10 random addition sequence replicates and TBR branch-swapping). The ML bootstrap analyses consisted of 1,000 replicates with SPR and NNI branch-swapping and the appropriate model parameters estimated and optimized (for the combined datasets a GTR+I+G model was used). The program Spectrum 2.3 (Charleston, 1998) was used to perform spectral analysis (Hendy and Penny, 1993). In spectral analysis support for a split (a split is any bipartition of the set of sequences and thus equates to a branch in a tree) depends on the number of character columns in the alignment whose patterns correspond to that split, whereas the conflict for a split is the sum of the support for the splits that are incompatible with it. As a split may be incompatible with many other splits, its conflict may be much larger than its support. To make the level

Table 2
Dataset size and variability.

Fragment	Total length	# Of constant sites	# Of variable sites	# Of parsimony informative sites
12S	383	277	106	60
ATPase-8 and -6	819	499	320	222
ND2	1041	599	442	293
COI	1262	881	381	280
Combined mitochondrial DNA	3505	2256	1249	855
FIB7	934	736	198	75
All combined	4439	2992	1447	930

of conflict comparable to the level of support the conflict values are normalized (see Lento et al., 1995). Spectrum computes the support and conflict for all the terminal and possible internal branches, a threshold (in this case 0.0005) is used to avoid calculating extremely low, biologically irrelevant, support values. The resulting spectrum is plotted as a bar chart (see Lento et al., 1995), which allows the level of support and conflict for the internal (possibly mutually exclusive) branches of interest to be visually compared.

An approximation of the timing of the splits was made using a rate used previously for the Pelecaniformes, an average rate of 2% per million years (for the mitochondrial genes only), by Kennedy and Spencer (2004). Given the inherent uncertainly in mean rates like this we also evaluated other rates of sequence divergence, e.g., 0.2% per million years for transversions alone (see Kennedy et al., 2000). We evaluated hypotheses about the evolution of nest type in the pelicans (see Nelson, 2005) by mapping nest type onto our phylogeny using MacClade 4.08 (Maddison and Maddison, 2005).

3. Results

Our alignments resulted in a 383 bp fragment of 12S, a 819 bp fragment of ATPase-8/ATPase-6, 1041 bp of ND2, a 1262 bp fragment of COI, and a 934 bp fragment of FIB7. As an indication of the level of variation in each marker (and the combined data), the numbers of constant, variable and parsimony informative sites per fragment are shown in Table 2. As expected, the level of variation differed between the fragments with, for example, the nuclear fragment, FIB7, having a lower proportion of variable sites than the mitochondrial fragments.

A partition-homogeneity (PH) test showed that there was no significant difference in the phylogenetic signals among the mitochondrial sequences (1000 replicates, $P = 0.208$) and hence they were analyzed as a single 3505 bp dataset (see Supplementary material). A PH test showed that there was no significant difference in the phylogenetic signals among all the different sequence partitions (i.e., the mitochondrial genes and FIB7; 1000 replicates, $P = 0.089$) and hence they were subsequently analyzed as a single 4439 bp dataset. Interestingly, excluding the gannet, cormorant and frigatebird outgroups from the PH test for the mitochondrial genes made little difference to the results (1000 replicates, $P = 0.233$), whereas it made more of a difference for all the different sequence partitions (i.e., the mitochondrial genes and FIB7; 1000 replicates, $P = 0.338$). This finding suggests that there really in no incongruence between the phylogenetic signals among all the different sequence partitions, particularly within the ingroup.

Our results (see Fig. 2) show an Old World grouping of the Dalmatian, Spot-billed and Pink-backed Pelicans is sister to the Australian Pelican and this clade is in turn sister to the Great White Pelican. The New World species also form a monophyletic group, separate from the Old World taxa (Fig. 2). Each of the species is monophyletic in the combined tree (Fig. 2), with strong posterior probabilities and bootstrap support (spectral analysis shows support, with no conflict, for the monophyly of each species, Fig. 3). All but three of the branches in the combined tree are very strongly supported by posterior probabilities and bootstrapping (as shown by the asterisks). Of the less well-supported branches, branch O, the grouping of the Dalmatian and the Spot-billed Pelicans is nevertheless strongly supported (0.99 posterior probability and bootstraps of 77–97%) and, although it has relatively low spectral support (Fig. 3), it has no conflicting splits (above the relatively low threshold used). Branch P, the grouping of the California Brown Pelican subspecies individuals, has relatively weak posterior probability and bootstrap support and the lowest spectral support (so low at 0.000264953, that it was below our threshold and had to be calculated using the "Evaluate Bipartition" option in Spectrum) of the branches in our tree, but no conflict. Branch N, which groups the Great White Pelican with the Dalmatian, Spot-billed, Pink-backed and Australian Pelicans also has relatively weak posterior probability and bootstrap support (see Fig. 2), with relatively low spectral support and some, though much less, conflict from 2 splits (see Fig. 3).

4. Discussion

The tree for the combined dataset (Fig. 2) shows three species groups within the pelicans. The tree unequivocally recovers the three New World species as a monophyletic group (branch G) and finds a strongly supported Old World clade of the Dalmatian, Spot-billed and Pink-backed Pelicans (branch D), with the Australian Pelican as their sister (branch E), also with strong support. The third group, consisting solely of the Great White Pelican (branch B), is then sister to this group of four (branch N), but its placement has relatively weak statistical support (spectral analysis shows relatively low support and some, though much less, conflict from 2 splits for split N, Fig. 3). These relationships are also found in most of the single-gene trees (see Supplementary material), with the most frequent exceptions being those for the two least-informative genes, FIB7 and 12S, where the issue is usually one of poor resolution. The slight ambiguity about the position of the Great White Pelican in our combined analysis (i.e., branch N) appears to be caused by weak conflicting signals in some of the genes. The Great White Pelican is weakly placed by 12S and ATPase as sister to all the other extant pelicans, whereas COI very weakly places it as sister to the three New World species. Nevertheless, in spite of these weakly conflicting signals the combined mitochondrial data, like the combined dataset, places the Great White Pelican as sister to the Dalmatian, Spot-billed, Pink-backed and Australian Pelicans (see Supplementary material).

The combined phylogeny thus splits the pelicans into a New World (branch G) and two Old World groups (branches B and E). Most emphatically, our data reject the recent notion (e.g., Sibley and Ahlquist, 1990; Johnsgard, 1993; Nelson, 2005) that pelicans can be divided into white- and brown-plumaged groups. Using the "Evaluate Bipartition" option in Spectrum we calculated the support for a split grouping all the white-plumaged pelicans together, this grouping received no support and very high conflict (−0.0177777) from seven conflicting splits. The clustering of the three American species, however, fits with Johnsgard's (1993) evolutionary scenario (which implied a single colonization of North

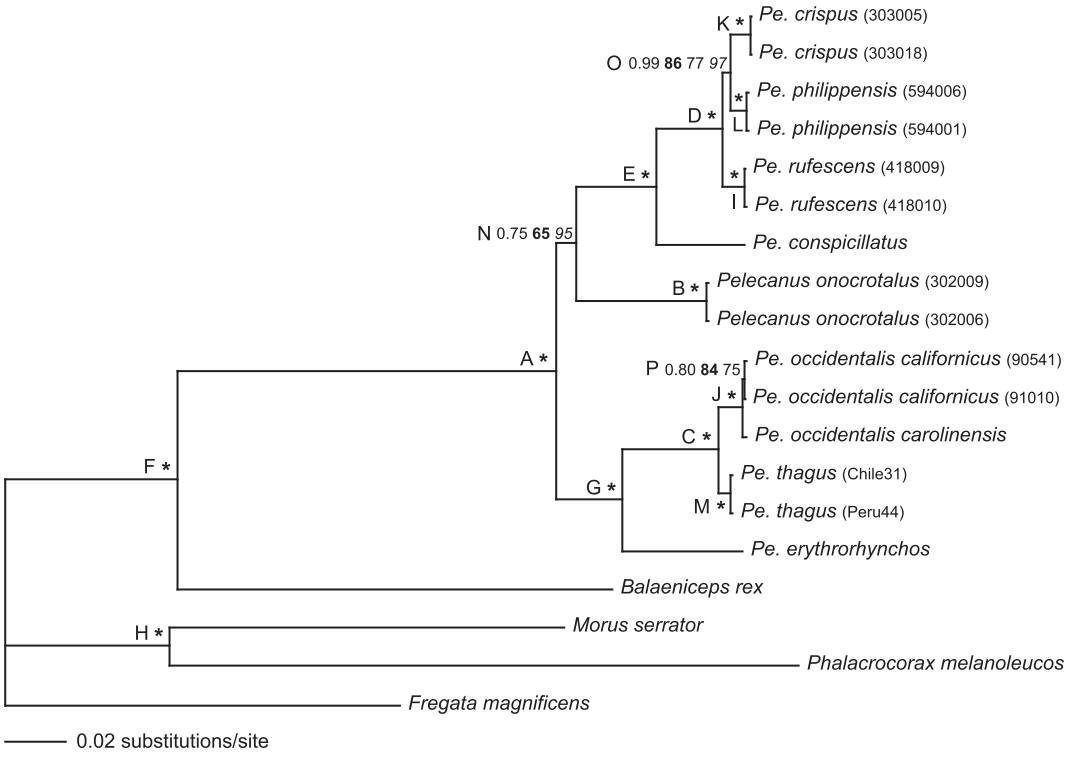

Fig. 2. The Bayesian phylogram for the combined dataset. The numbers associated with the branches represent posterior probabilities from Bayesian MCMC searches, ML bootstrap values (bold), equally weighted MP bootstrap values, and NJ bootstrap values (italic). Bootstrap values ⩾ 50% are shown. The asterisks indicate strong support from all of these measures, i.e., a Bayesian posterior probability of 0.95 and above and bootstrap values of 90% and above. The letters associated with the branches are labels for the splits from the spectral analysis (see Fig. 3). MP bootstrapping (like the other methods) favoured branch N, but with a value below 50% (44%).

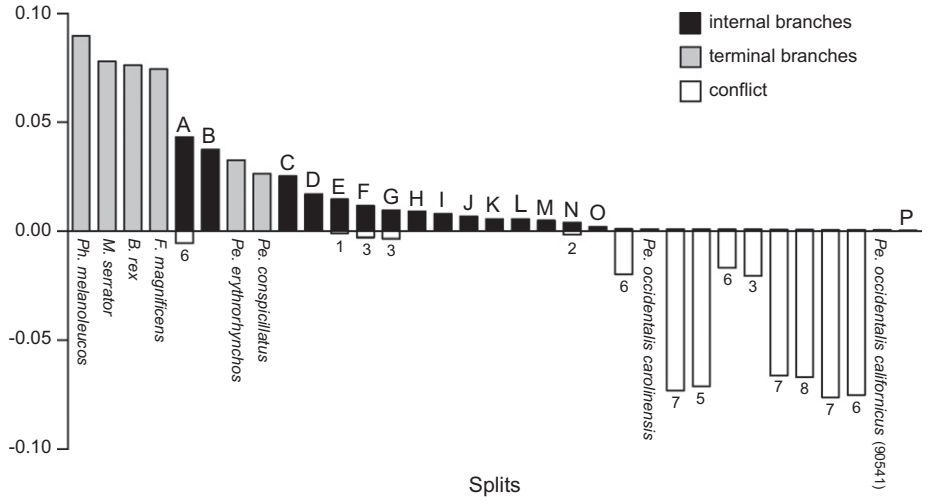

Fig. 3. The support/conflict spectrum with the Tamura–Nei distance option. The letters above the bars are the labels for the internal branches (splits) discussed and shown (e.g., see Fig. 2). The splits are ordered left to right by their (positive) support values (i.e., expected number of substitutions per site), with the (negative) conflict values normalized following Lento et al. (1995). The support values are differentiated into internal and terminal branches. The support value for split P fell below the threshold and was calculated using the "Evaluate Bipartition" option in Spectrum. The labels below the bars show the taxon of each terminal split and the number of splits (with support about the threshold level) that conflict with each split (>0).

American) that the pelicans derived from an African or southern Asian ancestor and spread out through northern Asia and into North America (as well as into Australia). Moreover, our tree is not incompatible with Peters's (1931) taxonomy (assuming the Great White Pelican does indeed cluster with the other Old World species). All the same, neither of these authors (nor anyone else, so far as we are aware) has suggested a sister relationship between the American White Pelican and the Brown + Peruvian clade; unanimously, opinion has been that the Brown and Peruvian Pelicans are the most different from all other pelicans.

The genetic distances between the American White and the Brown + Peruvian (6.27–6.57%, Table 3) suggest that the separation of these two clades is evolutionarily long-standing. A split between freshwater and coastal marine habitats may have resulted in the Brown and Peruvians's brown plumage, which could possibly be an adaptation to the wear and tear of salt water on feathers. In

Table 3
General time-reversible distance matrix (percentage divergence).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*Fregata magnificens*	–																		
2	*Morus serrator*	16.15	–																	
3	*Phalacrocorax melanoleucos*	17.31	16.87	–																
4	*Balaeniceps rex*	16.14	17.68	18.79	–															
5	*Pe. conspicillatus*	17.65	18.91	20.02	16.11	–														
6	*Pe. crispus* (303005)	17.08	18.57	19.37	16.07	5.12	–													
7	*Pe. crispus* (303018)	17.17	18.54	19.62	16.06	5.18	0.02	–												
8	*Pe. erythrorhynchos*	17.44	17.93	19.65	15.95	8.49	8.79	8.82	–											
9	*Pe. occidentalis carolinensis*	17.12	17.96	19.34	16.58	8.68	8.24	8.27	6.57	–										
10	*Pe. occidentalis californicus* (90541)	17.07	17.87	19.23	16.51	8.64	8.12	8.18	6.52	0.16	–									
11	*Pe. occidentalis californicus* (91010)	17.04	17.88	19.23	16.48	8.54	8.01	8.08	6.47	0.11	0.05	–								
12	*Pe. onocrotalus* (302009)	16.88	18.09	19.60	16.10	7.92	7.53	7.56	8.40	8.37	8.37	8.26	–							
13	*Pe. onocrotalus* (302006)	16.91	18.11	19.64	16.18	7.94	7.56	7.58	8.44	8.42	8.43	8.32	0.00	–						
14	*Pe. philippensis* (594006)	17.52	18.80	19.59	16.30	5.11	1.13	1.19	8.82	8.42	8.31	8.20	7.56	7.57	–					
15	*Pe. philippensis* (594001)	17.53	18.71	19.63	16.27	5.14	1.10	1.16	8.85	8.45	8.34	8.23	7.59	7.60	0.07	–				
16	*Pe. rufescens* (418009)	17.51	18.77	19.65	16.32	5.07	1.55	1.57	9.02	8.50	8.39	8.29	7.62	7.65	1.54	1.56	–			
17	*Pe. rufescens* (418010)	17.48	18.74	19.62	16.30	5.07	1.55	1.57	9.02	8.50	8.40	8.29	7.62	7.65	1.54	1.56	0.00	–		
18	*Pe. thagus* (Chile31)	17.17	17.84	19.22	16.15	8.33	7.92	8.03	6.27	1.23	1.27	1.18	8.02	8.05	8.18	8.21	8.21	8.21	–	
19	*Pe. thagus* (Peru44)	17.13	17.84	19.16	16.17	8.34	7.91	8.04	6.27	1.23	1.27	1.18	8.03	8.05	8.18	8.21	8.21	8.22	0.00	–

contrast, genetic distances between the Brown and Peruvian Pelicans are relatively small (1.18–1.27%, Table 3) and, unsurprisingly, all our trees position them as sister to one another (with very high support). Spectral analysis also shows that the branch grouping the Brown and Peruvian Pelicans (C) is one of the best-supported branches in the tree, with no conflict. The Brown and Peruvian Pelicans could be argued to have speciated recently as the result of allopatry and, assuming so, we can approximate the timing of this split. If we follow Kennedy and Spencer (2004) and use an average rate of 2% per million years (for the mitochondrial genes only) we get an estimate of 0.77 million years ago (mya). (Following Kennedy et al. (2000) and using a rate of 0.2% per million years for transversions alone gives a similar estimate, 0.64 mya.) Even if this rate of 2% per million years is too high (see Pereira and Baker, 2006), its halving (which may be more appropriate for mitochondrial genes, at least for deep avian phylogeny; see Pacheco et al., 2011) would only increase our estimate to 1.54 mya. Each of these estimates is close to the dates found previously by Patterson et al. (2011) for the geographically parallel Blue-footed and Peruvian Booby split (see Taylor et al., 2012). Patterson et al. (2011) estimated this split at 0.8 and 1.1 mya, and, following from the findings of Taylor et al. (2012), suggested that the environmental gradient created by non-terrestrial barriers such as the Humboldt Current meeting the Equatorial Counter Current in northern Peru may have facilitated this divergence. Given the concordance between Patterson et al. (2011) and our estimates of divergence time and the birds' distributions, it is possible that similar pressures (e.g., partial barriers created by oceanography combined with different selective regimes in the different environments) facilitated the divergence of the Brown and Peruvian Pelicans.

Like the American White and the Brown + Peruvian group, the trio of the Dalmatian, Spot-billed and Pink-backed Pelicans is also not one previously recognized. Previous workers have, however, argued that different pairs of these are each other's closest relatives: Delacour and Mayr (1945) and Dorst and Mougin (1979) paired the Dalmatian and Spot-billed; Johnsgard (1993) the Pink-backed and Spot-billed. Our combined dataset favors the former with good statistical support; the genetic distances between our Dalmatian and Spot-billed samples are the smallest between-species comparisons in Table 3 at 1.10–1.19%. Interestingly, the breeding ranges of these three species do not appear to overlap (Johnsgard, 1993) and they seem likely to be the result of almost simultaneous allopatric speciation. None of the pairwise genetic distances within this trio exceed 1.57% (Table 3). Using the rate of 2% per million years (for the mitochondrial genes only) these splits would be dated at ~0.7 mya for the Dalmatian and Spot-billed and ~1 mya for the Pink-backed and Dalmatian + Spot-billed, suggesting that only ~300,000 years separated these divergences (using a mean rate of 1% per million years instead would simply double this estimate to ~600,000 years). Thus, the speciation events are not particularly distinct from one another and could be considered almost contemporaneous. The Australian Pelican's position as sister to these three species fits with Johnsgard's (1993) suggestion that it diverged from a south Asian ancestor, although this separation clearly preceded speciation within the trio. Genetic distances from the Australian to these latter species are all more than three times those within the trio (5.07–5.18%, Table 3).

The degree of separation of the Great White Pelican from all other species is another unexpected finding. We note, however, that the range of this species overlaps significantly with two others (Pink-backed and Dalmatian), the largest number of species overlapped with and largest area of overlap for any pelican (see Fig. 1). Maybe the larger evolutionary distance alleviates possible ecological competition. For example, although the Pink-backed and Great White Pelicans co-occur over large parts of Africa, they have evolved different sizes and foraging strategies: the smaller Pink-backed Pelican usually forages individually in the shallows, close to shore, whereas the weightier Great White Pelican most often forages socially in deeper water (Johnsgard, 1993).

Given that Elliott (1992) and Nelson (2005) considered the four largest species (Great White, American White, Australian and Dalmatian) to be closely related allospecies because they share features like nesting on the ground, it is worthwhile evaluating how size and nesting behavior may have evolved. We calculated the support and conflict for a split grouping all these four largest species together (as would be expected if size and ground nesting were synapomorphic), but it received no support and extremely high conflict (−0.0672115) from 12 conflicting splits.

Given the distribution of ground nesting in the pelicans and the topology of our tree (see Fig. 4), we suggest that tree nesting evolved independently in the Spot-billed and Pink-backed Pelicans (which may have evolved their smaller morphologies as they moved from ground nesting to tree nesting). We note that our hypothesis is consistent with the almost simultaneous allopatric speciation of this group. Alternatively, tree nesting may have evolved once in Pink-backed and Dalmatian + Spot-billed ancestor, and the Dalmatian subsequently moved back to ground nesting.

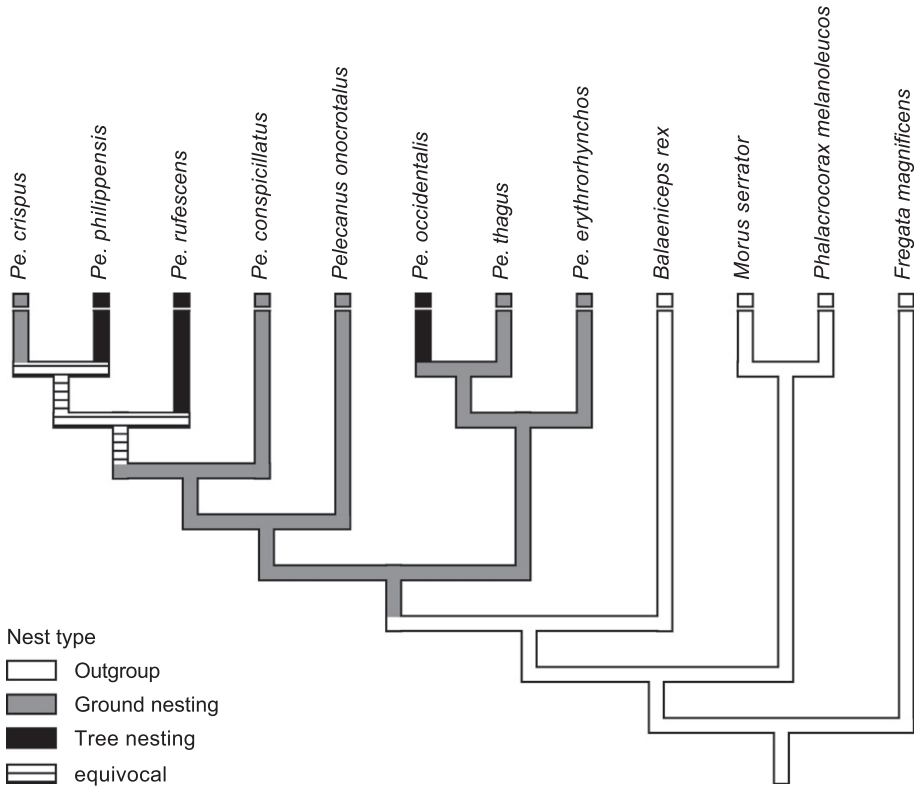

Fig. 4. Nest type mapped (for the pelicans only) onto our phylogeny (Fig. 2, summarized to include species only). While there is some variability within some species the states are characterized by their usual assignment (i.e., the tree nesters are wholly or at least partly arboreal, see Nelson, 2005).

Some Brown Pelicans are arboreal (they have variable nesting strategies, from ground nesting to using stick nests in trees) implying that they independently evolved this trait (see Fig. 4). It seems most reasonable to deduce that ground nesting is the ancestral trait in pelicans (given that it spans the root of the pelican part of our tree, see Fig. 4), a finding reinforced by the shoebill also being a ground nester, and thus it is not a useful character for grouping pelicans.

Finally, our trees allow us to make some taxonomic recommendations. The genetic distances among pelicans are all $\leqslant$9.02% and so we recommend that the use of a single genus be maintained. It would be possible to use a subgenus (or possibly two) for the American clade, but the uncertainty in the position of the Great White Pelican suggests that a further subgenus would then be needed for the remaining four Old World species. We doubt that the differences among pelicans warrant such fine taxonomic divisions. All currently recognized species, including the Peruvian, are well supported and should also be retained.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.09.034.

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