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The Phylogenetic Relationships of the Shags and Cormorants: Can Sequence Data Resolve a Disagreement between Behavior and Morphology?

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Taxonomic arrangements for the cormorants and shags (Phalacrocoracidae) had varied greatly until two quite similar arrangements, one based on behavior and the other on osteological characters, became the basis for current thought on the evolutionary relationships of these birds. The terms cormorant and shag, which had previously been haphazardly applied to members of the group, became the vernacular terms for the two major subdivisions within this family. The two taxonomies differ in places, however, with the behavioral taxonomy placing several species within the shags and the osteological taxonomy and phylogeny grouping those species (as the marine cormorants) and placing them within the cormorants. In an attempt to resolve the differences in the relationships hypothesized by behavior and morphology, we sequenced three mitochondrial genes (12S, ATPase 6, and ATPase 8). Initial equally weighted parsimony trees differed slightly from our two weighted parsimony trees, one of which was also our maximum-likelihood tree. Many of the branches within our trees were well supported, but some sections of the phylogeny proved difficult to resolve with confidence. Our sequence trees differ substantially from the morphological phylogeny and show that neither the shags nor the cormorants are monophyletic, but form an intermingled group. Some of the groups supported by both the behavioral and the morphological taxonomies (e.g., the cliff shags, *Stictocarbo*) appear to be polyphyletic. Conversely, the monophyly of the blue-eyed shags, a traditional group that the osteological analysis had found to be paraphyletic, was supported by the sequence data. Until more taxa are sampled and a fully robust phylogeny is obtained, a conservative approach accepting a single genus, *Phalacrocorax*, for the shags and cormorants is recommended.

INTRODUCTION

The monophyly of the traditional Order Pelecaniformes (pelicans, anhingas and darters, boobies, gannets, cormorants and shags, frigatebirds, and tropicbirds) is the subject of an ongoing debate (e.g., see Cracraft, 1985; Sibley and Ahlquist, 1990; Hedges and Sibley, 1994). This debate places two types of data in opposition to one another. Morphological evidence supports the monophyly of the pelecaniforms (Cracraft, 1985), whereas molecular evidence questions the monophyly of the group (Sibley and Ahlquist, 1990; Hedges and Sibley, 1994; Siegel-Causey, 1997). Similarly, two types of data support different taxonomic arrangements within the cormorants and shags (Phalacrocoracidae). The two most important recent classifications for the shags and cormorants (see Johnsgard, 1993) are the morphological taxonomy of Siegel-Causey (1988) and the behavioral taxonomy of van Tets (1976). Although these two taxonomies generally agree with one another, they also disagree on the placement of several taxa.

The cormorants and shags contain more than half the species within the pelecaniforms and are widely accepted as being most closely associated with the darters and anhingas (*Anhinga*), gannets (*Morus*), and boobies (*Sula*) (see Siegel-Causey, 1988; del Hoyo *et al.*, 1992; Johnsgard, 1993; Siegel-Causey, 1997). Although the morphological and behavioral taxonomies disagree (particularly in the placement of one group of species), they are also quite similar, differing mainly in rank (i.e., the use of genera and subgenera in one case or subfamilies and genera in the other, see Table 1). These taxonomies and the phylogeny of Siegel-Causey (1988) form the basis of current thought on the evolutionary relationships within the shags and cormorants (e.g., see Marchant and Higgins, 1990; del Hoyo *et al.*, 1992; Johnsgard, 1993).

van Tets (1976) used his knowledge of the behavior, ecology, and anatomy of the shags and cormorants to classify the Phalacrocoracidae into two genera and a

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1055-7903/00 $35.00

TABLE 1

Taxa Used in This Study

Common name	Taxon	van Tets' (1976) subgenera	Siegel-Causey's (1988) genera
Australasian Gannet	*Morus serrator*		
Red-footed Booby	*Sula sula*		
Japanese Cormorant	*Phalacrocorax capillatus*	*P.* (*Phalacrocorax*)	*Phalacrocorax*
Great (Black) Cormorant	*P. carbo*	*P.* (*Phalacrocorax*)	*Phalacrocorax*
Double-crested Cormorant	*P. auritus*	*P.* (*Hypoleucos*)	*Hypoleucos*
Neotropic Cormorant	*P. brasilianus*[a]	*P.* (*Hypoleucos*)	*Hypoleucos*
Little Black Cormorant	*P. sulcirostris*	*P.* (*Hypoleucos*)	*Hypoleucos*
Pied Cormorant	*P. varius*	*P.* (*Hypoleucos*)	*Hypoleucos*
Little Pied Cormorant	*P. melanoleucos*	*P.* (*Microcarbo*)	*Microcarbo*
Imperial Shag	*P. albiventer*[b]	*Leucocarbo* (*Leucocarbo*)	*Notocarbo*
Macquarie Island Shag	*P. purpurascens*	*L.* (*Leucocarbo*)	*Notocarbo*
Guanay Shag	*P. bougainvillii*	*L.* (*Leucocarbo*)	*Leucocarbo*
Cape Shag	*P. capensis*	*L.* (*Leucocarbo*)	*Leucocarbo*
Campbell Island Shag	*P. campbelli*	*L.* (*Leucocarbo*)	*Nesocarbo*
Stewart Island Shag	*P. chalconotus*	*L.* (*Leucocarbo*)	*Euleucocarbo*
Chatham Island Shag	*P. onslowi*	*L.* (*Leucocarbo*)	*Euleucocarbo*
Brandt's Cormorant	*P. penicillatus*	*L.* (*Leucocarbo*)	*Compsohalieus*[c]
European Shag	*P. aristotelis*	*L.* (*Stictocarbo*)	*Stictocarbo*
Pitt Island Shag	*P. featherstoni*	*L.* (*Stictocarbo*)	*Stictocarbo*
Red-legged Shag	*P. gaimardi*	*L.* (*Stictocarbo*)	*Stictocarbo*
Rock Shag	*P. magellanicus*	*L.* (*Stictocarbo*)	*Stictocarbo*
Pelagic Shag	*P. pelagicus*	*L.* (*Stictocarbo*)	*Stictocarbo*
Spotted Shag	*P. punctatus*	*L.* (*Stictocarbo*)	*Stictocarbo*
Red-faced Shag	*P. urile*	*L.* (*Stictocarbo*)	*Stictocarbo*

Note. We follow Marchant and Higgins (1990) use of a single genus for the Phalacrocoracidae and Siegel-Causey's (1988) use of Shag or Cormorant in the common name.

[a] As *P. olivaceus* (see Browning, 1989).

[b] Sometimes treated as a subspecies of *P. atriceps* (e.g., see del Hoyo *et al.*, 1992).

[c] Placed within the Phalacrocoracinae rather than the Leucocarboninae by Siegel-Causey (1988).

total of five subgenera (see Table 1). The two genera equate to what van Tets (1976) described as the shags and cormorants (*Leucocarbo* and *Phalacrocorax,* respectively), whereas previously the use of shag and cormorant in the coining of common names was generally haphazard (see del Hoyo *et al.*, 1992). Within the shags, van Tets (1976) subgenerically separated the king shags (*Leucocarbo sensu strictum*) from the cliff shags (*Stictocarbo*), whereas within the cormorants, he separated the macrocormorants (*Phalacrocorax s.str.*) from the mesocormorants (*Hypoleucos*) and the microcormorants (*Microcarbo*). Johnsgard (1993) described van Tets (1976) classification as being perhaps the first biologically meaningful taxonomy for this group.

Siegel-Causey (1988) used 137 osteological characters to produce a cladistic phylogeny for the Phalacrocoracidae (e.g., see Fig. 1). From this morphologically based phylogeny Siegel-Causey (1988) proposed a classification of the Phalacrocoracidae with two subfamilies (similar to the two genera of van Tets, i.e., the Leucocarboninae and Phalacrocoracinae; see Fig. 1) and nine genera (see Table 1). Siegel-Causey's genera equate to van Tets' subgenera (also in their vernacular terms), apart from splitting one of van Tets' subgenera, the king shags (*Leucocarbo s.str.*), into five separate genera: guano shags (*Leucocarbo*), blue-eyed shags (*Notocarbo*), Campbell Island Shag (*Nesocarbo*), New Zealand blue-eyed shags (*Euleucocarbo*), and the marine cormorants (*Compsohalieus*). Siegel-Causey (1988) placed four of these genera in the Leucocarboninae, but placed *Compsohalieus* in the Phalacrocoracinae (and hence he described them as the marine cormorants rather than the marine shags).

The grouping of the marine cormorants and their placement in the cormorants or shags is the main point of disagreement between Siegel-Causey's (1988) and van Tets (1976) taxonomies. In their parsimony analysis of many of van Tets' (1965) behavioral characters, Kennedy *et al.* (1996) found that Brandt's Cormorant (*P. penicillatus*), the only marine cormorant in the analysis, grouped with a shag rather than with any cormorants. Although the behaviorally based phylogeny is more congruent with Siegel-Causey's (1988) tree than would be expected by chance alone, the phylogenies disagree on the relative placement of several taxa (see Kennedy *et al.*, 1996). In an attempt to resolve the position of Brandt's Cormorant and the other differences between the behaviorally and the morphologically based taxonomies and phylogenies, we sequenced mitochondrial DNA from several species of shags and

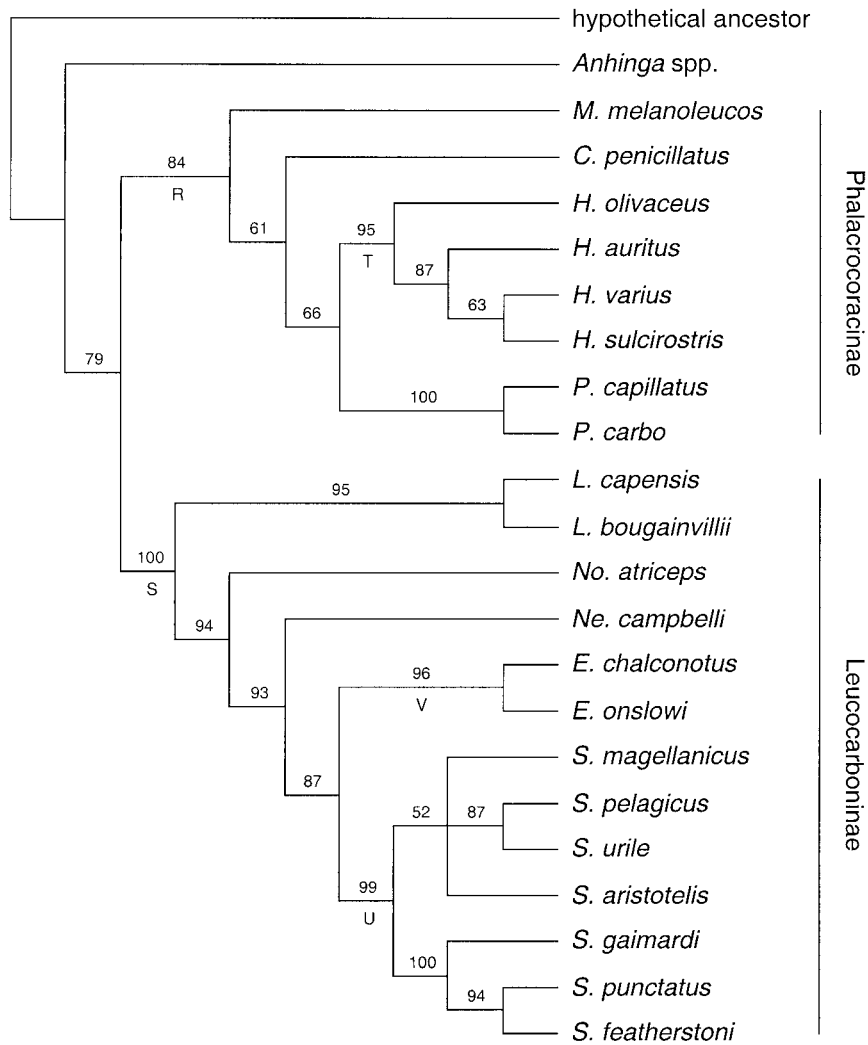

FIG. 1. The bootstrap maximum-parsimony tree (1000 replicates) for the shags and cormorants that are shared between Siegel-Causey's (1988) and our data sets (generated from Siegel-Causey's data set). In this tree *No. atriceps* is equivalent to our *P. albiventer* and *P. purpurascens.* Siegel-Causey (1988) used a hypothetical ancestor to root his tree. The data were treated as stated in Siegel-Causey (1988). The generic names used here follow Siegel-Causey (1988), who separated the shags and cormorants into two subfamilies (the Leucocarboninae and Phalacrocoracinae) and nine genera (see Table 1). Similar bootstrap values were found for an analysis with all of the taxa used in Siegel-Causey's (1988) study. (The most-parsimonious trees for Siegel-Causey's data set do not recover the branch with 52% bootstrap support uniting *magellanicus* + *urile* + *pelagicus* + *aristotelis.*) The letters associated with the branches refer to splits mentioned in the text.

cormorants. Sequence data can be used to estimate the shags' and cormorants' phylogenetic relationships and as a measure of the relative level of divergence among them. A phylogeny for the group would allow us to evaluate the existing evidence regarding their evolutionary relationships.

METHODS

Total genomic DNA was obtained for each of the samples using proteinase K followed by phenol/chloroform extraction (the taxa for which samples were obtained are listed in Table 1). Following extraction, the DNA was amplified for three mitochondrial DNA genes, the 12S ribosomal RNA gene and the overlapping ATPase 6 and 8 genes. The polymerase chain reaction (PCR) was used to amplify these regions using universal primers for 12S (Kocher *et al.*, 1989) and primers for ATPase 6 and 8 (Kennedy, 1999).

A typical 25-μl double-stranded PCR amplification contained 0.7–1 μl of extracted genomic DNA, 0.5 μM each primer, 1 unit of *Taq* polymerase (Promega), 2.5 μl of 10× *Taq* buffer (Promega), 1 mM $MgCl_2$ (Promega), and 200 μM each dNTP. Negative controls were included with each PCR and all the mixtures were covered with mineral oil. The reaction began with denaturation (94°C for 3 min) followed by 40 cycles of annealing (1 min) at 55–57°C (for 12S) or 42–45°C (for

ATPase), template extension at 72°C (1 min), and denaturation at 94°C (1 min). Final annealing (1 min) and extension (4 min) steps completed the reactions. The PCR product was purified using Gelase (Epicentre Tech.) and then sequenced by an automated sequencer using either the PCR primers or internal primers (following Kennedy, 1999). Wherever possible, two or more individuals of a species were sequenced for both strands of DNA to verify the accuracy of the sequencing and control for DNA contamination. Ambiguity codes were used when it was not possible to discriminate between alternative bases at a site and were analyzed as uncertainties.

Sequences were aligned by eye. The 12S sequence was aligned with reference to the seabird data of Paterson *et al.* (1995) and the waterfowl data of Kennedy and Spencer (2000) and used the secondary structure model and conserved motifs approach of Hickson *et al.*, (1996, 2000). All gaps of more than one base were removed to avoid mistaken homology. The sequences have been submitted to GenBank (Accession Nos. AY009321–AY009368) and the data matrix and resultant phylogenetic trees will be submitted to TreeBASE (http://herbaria.harvard.edu/treebase/). Analyses were conducted using test versions 4.0d64 and 4.0b4a of PAUP* (Swofford, 2000). Phylogenetic trees were constructed with both maximum-parsimony and maximum-likelihood. The Australasian Gannet (*Morus serrator*) and Red-footed Booby (*Sula sula*) were included as outgroup taxa. We used the partition-homogeneity test (Farris *et al.*, 1995; Swofford, 2000) to investigate whether the ATPase and 12S sequences can be analyzed as a single data set and the PTP test (Faith, 1991; Faith and Cranston, 1991) and g_1 statistic (Hillis and Huelsenbeck, 1992) to investigate whether the data contained significant phylogenetic signal. The parsimony trees were estimated using heuristic searches with 1000 random addition sequences and TBR branch-swapping. For the maximum-likelihood heuristic search, one of the equally weighted parsimony trees was used as the starting tree for branch-swapping (TBR). For the maximum-likelihood analysis, the TIM substitution model with invariable sites and among-site rate heterogeneity was selected using the Akaike information criterion of Modeltest (Posoda and Crandall, 1998). The nucleotide frequencies, gamma shape parameter for rate heterogeneity (with four rate categories), substitution rate matrix (with four substitution types: A-C and G-T; A-G; A-T and C-G; and C-T), and proportion of invariable sites were all estimated by maximum-likelihood.

To investigate the support for our trees and the phylogenetic signal in our sequence data we used bootstrap analysis (Felsenstein, 1985), decay indices (Donoghue *et al.*, 1992), and spectral analysis (Hendy and Penny, 1993). For the bootstrap analyses 1000 replicates were performed using a heuristic search with parsimony and a fast heuristic search with likelihood. The fast heuristic search has been shown to provide similar (though lower) values than the heuristic search with branch swapping, with the greatest discrepancy when the bootstrap values are relatively low (Mort *et al.*, 2000). We used the program Spectrum 2.2 (Charleston, 1998), which implements spectral analysis, to further investigate the phylogenetic signal in the data. In spectral analysis, support for a split (a split is any bipartition of the set of sequences and is thus equivalent to a branch of a tree) is related to the number of character state changes that correspond to that split (i.e., expected number of substitutions per site), whereas the conflict for a split is the sum of the support for the splits that conflict with it. For discussions of spectral analysis and its use see Lento *et al.* (1995), Page *et al.* (1998), Kennedy *et al.* (1999), and Kennedy and Spencer (2000). The program Spectrum is limited by the number of taxa that it can analyze. To reduce the number of taxa to 20 we excluded 1 of the outgroup taxa (the Red-footed Booby) and 3 of the shags and cormorants (the Japanese Cormorant, *P. capillatus;* the Chatham Island Shag, *P. onslowi;* and the Pitt Island Shag, *P. featherstoni*). These three shags and cormorants were selected because they are universally accepted as being very similar to their respective sister taxa (e.g., the sequence divergence of each pair of taxa is $\leq$0.1%; see Table 2). We computed the spectrum from distance matrices that had been calculated using the Tamura–Nei model to correct for superimposed changes (this model allows for unequal base frequencies and a transition/transversion bias with two transition classes; Tamura and Nei, 1993). In addition to computing the spectrum, the program Spectrum can calculate the support and conflict values for any bipartition of interest.

RESULTS

Our alignment gave a 383-bp fragment of 12S, whereas the overlapping ATPase 6 and 8 coding genes gave a 758-bp fragment. A partition-homogeneity test showed that there was no significant difference in phylogenetic signal in the ATPase and 12S sequences, and thus they can be analyzed as a single data set (100 replicates, $P = 0.62$). Of the 385 variable sites, 234 of the characters were parsimony informative. Both the significantly skewed tree-length distribution ($g_1 = -0.731$ from 10,000 random trees, $P \ll 0.01$; Hillis and Huelsenbeck, 1992) and a PTP test (1000 replicates, $P = 0.001$) showed that the data contain significant phylogenetic signal.

Equally weighted parsimony analysis found four trees. These four trees differed in the placement of *P. bougainvillii* and in the placement of *P. sulcirostris* and *P. varius* as sister taxa or not. Bootstrap analysis recovered one additional branch, that grouping *P. sul-*

TABLE 2

Tamura-Nei (1993) Distance Matrix: Percentage of Sequence Divergence between the Different Taxa

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 *Morus serrator*	—																							
2 *Sula sula*	14.4	—																						
3 *Phalacrocorax carbo*	18.0	18.7	—																					
4 *P. penicillatus*	16.8	17.8	6.5	—																				
5 *P. campbelli*	17.9	18.0	7.1	7.6	—																			
6 *P. capensis*	18.1	18.8	4.6	6.6	7.5	—																		
7 *P. onslowi*	18.1	18.4	7.1	7.9	0.7	7.4	—																	
8 *P. auritus*	17.1	18.8	6.7	7.7	6.2	7.3	6.0	—																
9 *P. aristotelis*	19.2	20.2	7.7	8.7	7.8	7.1	7.7	8.2	—															
10 *P. bougainvillii*	17.0	16.5	6.9	7.8	1.4	7.2	1.7	6.0	7.5	—														
11 *P. capillatus*	17.5	17.6	0.1	6.1	6.9	4.5	6.9	6.5	7.3	6.9	—													
12 *P. sulcirostris*	17.2	17.7	4.4	6.2	6.6	5.9	6.8	6.9	7.7	7.5	5.2	—												
13 *P. melanoleucos*	17.6	18.7	10.6	10.9	10.8	11.0	11.1	12.2	12.6	11.1	10.3	10.8	—											
14 *P. purpurascens*	17.5	18.1	7.3	7.7	0.9	7.1	1.0	5.7	7.7	1.3	7.0	7.0	11.0	—										
15 *P. magellanicus*	18.3	18.2	6.9	7.6	3.4	6.8	3.6	5.8	8.5	3.5	6.4	7.1	12.1	3.2	—									
16 *P. brasilianus*	17.6	18.5	6.4	7.4	5.3	6.7	5.3	2.2	7.1	5.7	6.1	6.5	12.0	5.1	4.9	—								
17 *P. pelagicus*	16.4	17.4	6.8	5.2	8.4	7.1	8.4	7.8	8.1	7.8	6.5	6.8	11.3	8.4	7.5	7.4	—							
18 *P. varius*	17.7	17.9	5.6	6.8	7.3	6.2	7.5	7.8	8.6	7.6	5.6	3.5	10.9	7.7	7.8	7.8	7.5	—						
19 *P. featherstoni*	18.5	18.6	6.3	7.3	7.1	5.7	7.4	7.8	8.5	6.7	6.0	5.1	11.5	7.4	7.3	7.2	7.9	5.4	—					
20 *P. urile*	17.7	19.1	7.1	5.7	8.9	7.2	8.8	8.3	8.1	8.4	6.8	7.3	12.4	8.8	8.1	7.9	2.5	8.0	8.0	—				
21 *P. gaimardi*	16.9	18.2	9.8	9.2	8.1	9.5	8.7	9.0	10.7	9.1	9.8	9.2	10.7	8.1	9.6	8.5	10.3	9.3	9.1	10.6	—			
22 *P. albiventer*	17.6	18.4	7.4	7.9	1.0	7.7	1.1	5.8	7.9	1.4	7.1	6.9	11.5	0.5	3.1	5.1	8.2	7.7	7.5	8.8	8.3	—		
23 *P. punctatus*	18.5	18.8	6.3	7.3	7.0	5.7	7.2	7.6	8.7	6.6	6.0	5.0	11.5	7.3	7.2	6.9	7.8	5.2	0.0	7.9	9.1	7.3	—	
24 *P. chalconotus*	17.9	18.5	6.9	7.8	0.6	7.3	0.0	6.1	7.7	1.6	6.7	7.0	11.0	0.9	3.5	5.4	8.5	7.6	7.5	8.9	8.7	1.0	7.4	—

cirostris and *P. varius*, in 56% of the replicates. Equally weighting parsimony analyses assumes that all characters have equal transformation costs, an assumption that is not necessarily the most realistic (Omland, 1997). As transitions occur more readily than transversions, the effect of homoplasy caused by multiple transitional changes at a site is lessened if transitions are relatively downweighted. With the optimality criterion set to maximum-likelihood the transition to transversion ratio estimated on the equally weighted parsimony trees was approximately 7:1. With the weight of transversions increased by this ratio we found two most-parsimonious trees (Fig. 2). As would be expected if the effect of homoplasy is being reduced or phylogenetic signal is accentuated, the consistency index (CI) and retention index (RI) are better for the trees from the weighted analysis than for those from the equally weighted analysis. The two most-parsimonious trees differ only in the placement of *P. bougainvillii*. Unlike the equally weighted parsimony analysis, *P. sulcirostris* and *P. varius* are sister taxa in both trees and have good decay index and bootstrap support (see Figs. 2a and 2b). Many of the branches have good bootstrap and decay index support. Two branches (those grouping *P. campbelli* + *P. chalconotus* + *P. onslowi* and *P. albiventer* + *P. purpurascens*) that have decay indices of only 1 have reasonable bootstrap support, suggesting that there is no signal in the data supporting alternative arrangements of those branches. Although *P. onslowi* was excluded from the analysis, spectral analysis confirms that the branch grouping *P. campbelli* + *P. chalconotus* (split M) has some support and no conflict (see Fig. 3).

In some circumstances maximum-likelihood provides a better estimate of the phylogeny, as it is better at dealing with unobserved substitutions than parsimony (Swofford *et al.*, 1996). The maximum-likelihood tree (Fig. 4) has the same topology as one of the weighted parsimony trees (Fig. 2a). With the exception of those branches that have low decay indices but reasonable bootstrap values in the weighted parsimony trees, the internal branches of the maximum-likelihood tree that are very short receive no bootstrap support in the weighted parsimony analysis (see Fig. 2b). The short internal branches that receive no bootstrap support receive either little or no support or high levels of conflict from the spectral analysis (thus some of the branches are not labeled on Fig. 4 as they are not shown on Fig. 3 because of their low levels of support).

We used Spectrum to evaluate the bipartitions that grouped Siegel-Causey's two subfamilies. Spectral analysis found no support (i.e., <0.001) and very high conflict for both subfamilies (the Phalacrocoracinae, split R on Fig. 1, had a conflict of 0.1711 and the Leucocarboninae, split S on Fig. 1, had a conflict of 0.1507). The positions of 10 of the 22 shags and cormorants in our maximum-likelihood tree, for example, are incongruent with the existence of these two subfamilies (see Figs. 1 and 4).

We tested for the presence of a molecular clock using

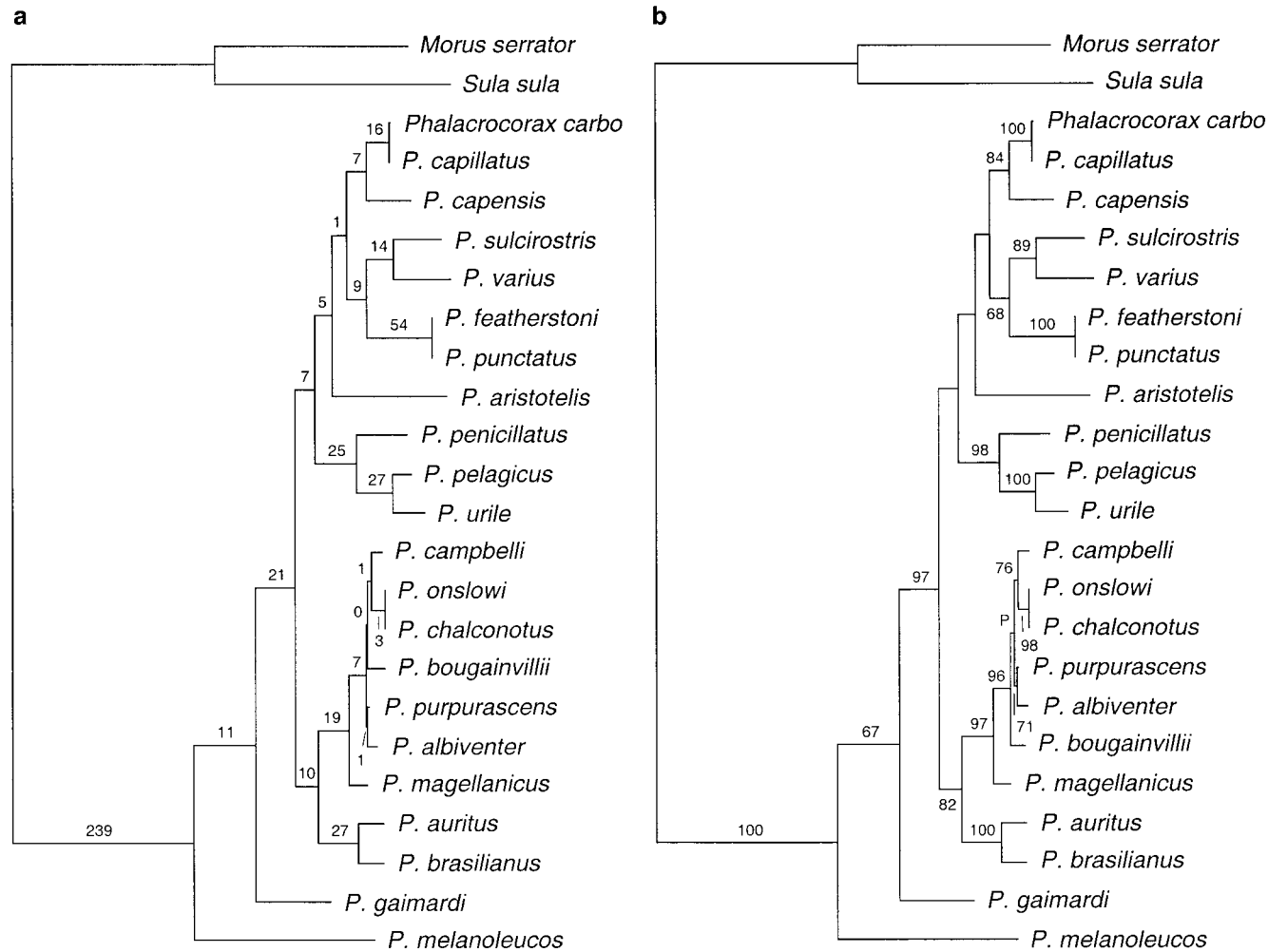

FIG. 2. The two weighted parsimony phylograms (tree length = 1779, CI = 0.736, RI = 0.727) generated from our sequence data. In this analysis transversions were weighted 7 times transitions (this ratio approximates the maximum-likelihood estimate on the equally weighted parsimony trees). Decay indices are shown on (a), and bootstrap values (≥50%) from 1000 replicates are shown on (b). The letter (P) relates to the split that groups *albiventer* + *purpurascens* + *chalconotus* + *onslowi* + *campbelli* (also see Fig. 3).

a likelihood difference test (Felsenstein, 1995) to ascertain whether it was valid to estimate divergence times from this data set. We computed the likelihoods for our maximum-likelihood tree both with and without imposing a molecular clock constraint and found that we could not reject the clock hypothesis (log likelihood difference = 25.152, χ^2_{22} = 33.924, $P > 0.05$). This result shows that it is appropriate to use our data to estimate divergence times. Because of the variety of sources of error associated with dating divergence times, however, the estimates must be treated as only course approximations (Hillis *et al.*, 1996). Friesen and Anderson (1997) showed, with reference to the fossil record, that a rate of change of about 0.2% per million years for transversions may be used to estimate approximate divergence times for members of the pelecaniforms. Although mitochondrial DNA does not necessarily evolve at a linear rate, transversions appear to have done so (Miyamoto and Boyle, 1989; Irwin *et al.*, 1991). We used the number of transversions calculated from all possible pairwise comparisons for each of the divergence points to estimate the divergence times mentioned in the Discussion. Because of possible errors in the estimates of the number of transversions and particularly in the calibration used for their rate of change, we emphasize that these dates should be viewed as only a general approximation of the relative dates of divergence.

DISCUSSION

Although they differed in some areas, van Tets' behavioral and Siegel-Causey's morphological taxonomies are fundamentally similar. We attempted to use sequence data to resolve the differences between the two taxonomies. Rather than supporting the similari-

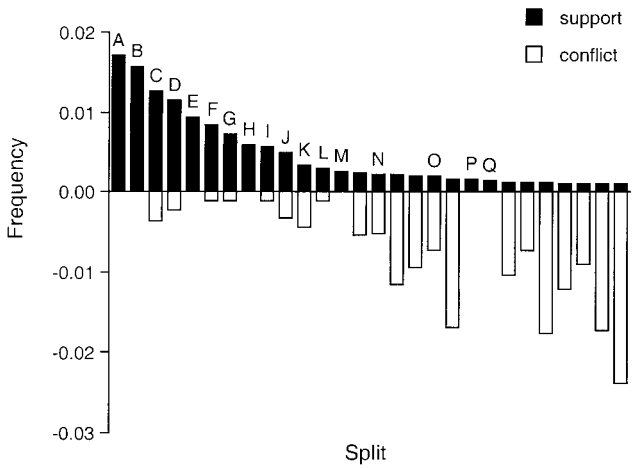

FIG. 3. The support/conflict spectrum. The splits are labeled alphabetically following Penny *et al.* (1999). The splits are ordered left to right by their (positive) support values (i.e., expected number of substitutions per site), with the (negative) conflict values normalized following Lento *et al.* (1995). Splits equating to terminal branches are not shown. (Some splits mentioned in the text are shown only on Fig. 1.)

ties and resolving the differences between the two taxonomies, however, our sequence-based phylogenies question several of the groups present in those taxonomies. Our sequence data thus suggest that the evolutionary history of the shags and cormorants is quite different from that implied by either the behavioral or the morphological taxonomies.

Our estimates of the phylogeny of the shags and cormorants are fairly robust to the method of phylogenetic reconstruction and weighting scheme used: the maximum-likelihood tree is one of the two weighted parsimony trees. These two trees differ only in the placement of the Guanay Shag (*P. bougainvillii*), which has an extremely short internal branch (see Fig. 4). The equally weighted parsimony trees differ additionally in the placement of the Little Black (*P. sulcirostris*) and Pied Cormorants (*P. varius*) as sister taxa or not, although bootstrap analysis did recover this pairing (with 56% support). The weighted parsimony trees grouped these two species with good decay index (14) and bootstrap support (89%). The monophyly of this pair of taxa is uncontroversial (Johnsgard, 1993), thus suggesting that the relative downweighting of transitions may provide a better estimate of the phylogeny. This finding reinforces the concept that some correction for multiple substitutions is better than none when estimating a phylogeny (Huelsenbeck, 1998). Given that the maximum-likelihood tree is one of the two weighted parsimony trees, the increased level of correction afforded by maximum-likelihood over weighted parsimony makes little difference for this data set (the two trees differ by a −Ln likelihood of only 0.318). Most of the branches of the weighted parsimony trees receive good bootstrap support, but four branches receive less than 50% bootstrap support (and low decay indices) and thus represent poorly supported parts of the phylogeny.

With the exception of grouping the Little Black and Pied Cormorants, the branches of the tree that are well supported in the weighted parsimony analysis were also well supported in the equally weighted parsimony analysis. The Little Pied Cormorant (*P. melanoleucos*), one of the microcormorants, is the most basal member of the Phalacrocoracidae in all of our analyses with reasonable decay index and bootstrap support in the parsimony analysis. Spectral analysis also showed good support (0.0072) and little conflict (0.0010) for placing the Little Pied Cormorant as the most basal of the shags and cormorants (split G, Fig. 4). Our estimates suggest that this lineage diverged from the other cormorants and shags about 12 million years ago. Although we were unable to obtain samples from any other members of the microcormorants, they are morphologically and behaviorally very similar (see Siegel-Causey, 1988; Johnsgard, 1993), and there is no reason to believe that they may not be a monophyletic group. The Red-legged Shag (*P. gaimardi*), one of the cliff shags (i.e., *Stictocarbo* sensu Siegel-Causey and van Tets), was consistently placed as the next most basal member of the family, with a high decay index and high bootstrap support in the parsimony analysis. The level of support for, and length (see Fig. 4) of, the branch grouping all of the shags and cormorants apart from Little Pied Cormorant and the Red-legged Shag (97% bootstrap support and a decay index of 21 for the weighted parsimony analysis, and very high support from spectral analysis for split C—though not supported by likelihood bootstrap analysis) strongly suggests that these two taxa represent the most basal lineages of this group. Our estimates suggest that this lineage diverged around 5 million years ago. The position of the Red-legged Shag suggests that the cliff shags do not form a monophyletic group.

As expected (see Siegel-Causey, 1988; Johnsgard, 1993), there is high decay index and bootstrap support for the Great (*P. carbo*) and Japanese (*P. capillatus*) Cormorants as sister taxa. The Cape Shag (*P. capensis*), one of van Tets' king shags and Siegel-Causey's guano shags, grouped with the Great and Japanese Cormorants (with 84% bootstrap support for weighted parsimony). As already mentioned, the weighted parsimony analysis gives high bootstrap support for grouping the Little Black and the Pied Cormorants, and both maximum-likelihood bootstrap and spectral analyses also support that grouping (split I, Fig. 4). Support for the sister taxa relationship of the Spotted (*P. punctatus*) and Pitt Island (*P. featherstoni*) Shags is very strong and there is some support for grouping the Spotted and Pitt Island Shags with the Little Black and Pied Cormorants. The sister taxa status of the

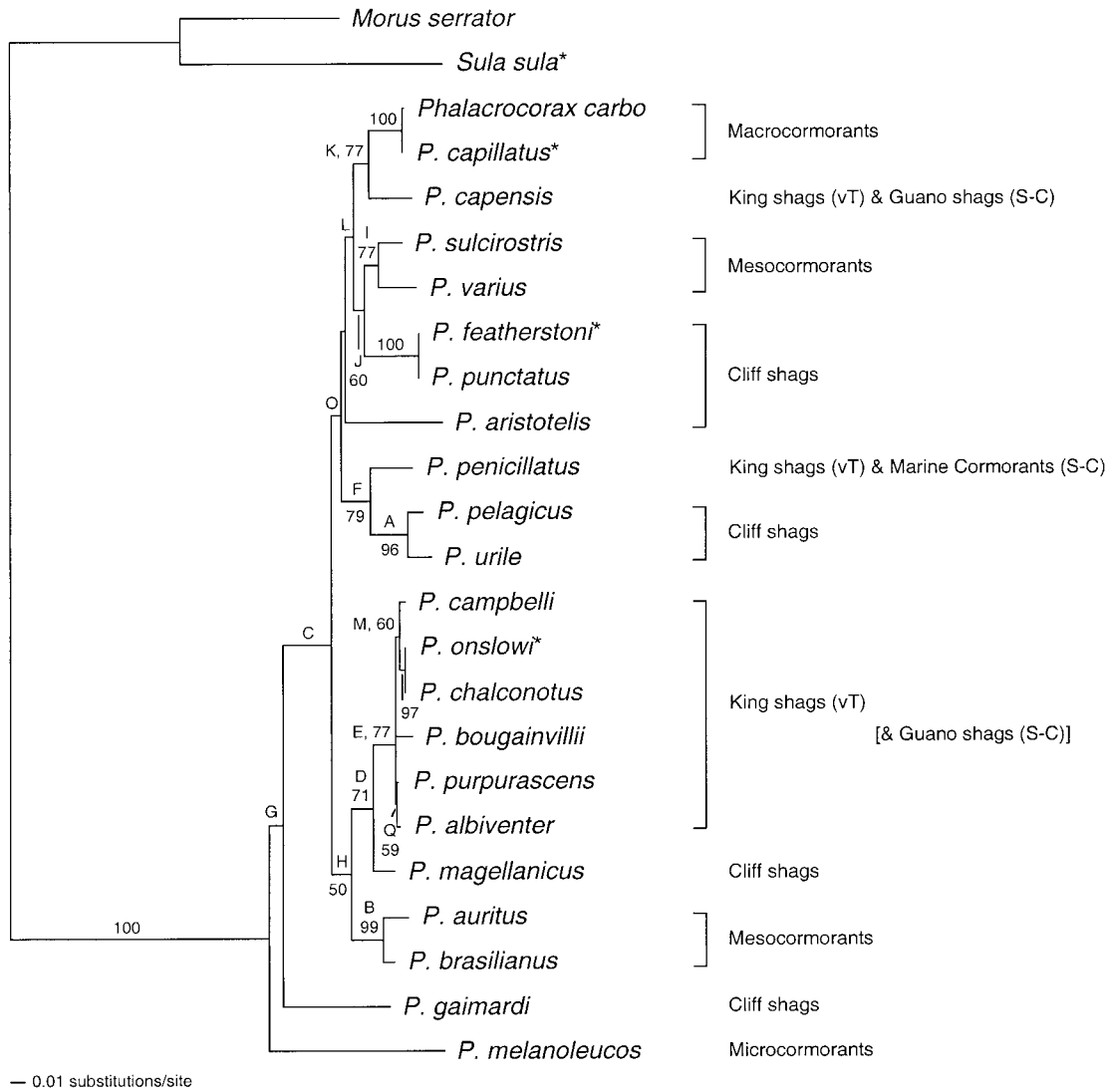

FIG. 4. The maximum-likelihood phylogram generated from our sequence data. The topology is the same as that in Fig. 2a. The branch lengths represent the estimated proportion of substitutions per site. One of the equally weighted parsimony trees was used as the starting tree for branch-swapping. The -Ln likelihood is 5166.13555. The letters associated with the branches refer to splits labeled in Fig. 3. The asterisked taxa are those not included in the spectral analysis; thus, the branches grouping them with their sister taxa were not evaluated. The group labels are annotated as (vT) or (S-C) if they differ between the two taxonomies.

Pelagic and Red-faced Shags (*P. pelagicus* and *P. urile*) is strongly supported by our data (split A, Fig. 4; 96–100% bootstrap support). There is also strong support for grouping Brandt's Cormorant with the Pelagic and Red-faced Shags (split F, Fig. 4; 79–98% bootstrap support). The relationships between these different groups (i.e., *carbo* + *capillatus* + *capensis*, *sulcirostris* + *varius* + *punctatus* + *featherstoni*, *aristotelis*, and *pelagicus* + *urile* + *penicillatus*) are, however, poorly supported with low decay indices and <50% bootstrap support. Spectral analysis finds some support but high conflict for this grouping as a whole (split O, Fig. 4).

The sister taxa status of the Double-crested and Neotropic Cormorants (*P. auritus* and *P. brasilianus*) is very strongly supported (split B, Fig. 4; 99–100% bootstrap support). There is also reasonable support for grouping this pair as sister taxa to the group of *magellanicus* + *bougainvillii* + *albiventer* + *purpurascens* + *chalconotus* + *onslowi* + *campbelli*, with a decay index of 10 and bootstrap support of 82% for weighted parsimony, as well as good support and no conflict from the spectral analysis (see split H, Fig. 4). Our estimates suggest that this lineage diverged about 3 million years ago. There is strong bootstrap, decay index, and spectral analysis support for the group of *magellanicus* + *bougainvillii* + *albiventer* + *purpurascens* + *chalconotus* + *onslowi* + *campbelli* (i.e., split D, Fig. 4)

TABLE 3

Evidence from the Sequence Data against the Monophyly of Some Hypothesized Groups

Group	Siegel-Causey's taxonomy	Split labeled in Fig. 1	Number of additional steps[a]	Spectral analysis	
				Support	Conflict
Mesocormorants	*Hypoleucos*	T	44	<0.0001	0.0731
Cliff shags	*Stictocarbo*	U	146	<0.0001	0.1809
King shags	Leucocarboninae − *Stictocarbo* + *Compsohalieus*	[b]	113	<0.0001	0.1308
Guano shags	*Leucocarbo*	V	52	<0.0001	0.1196

[a] Required for the weighted parsimony trees if each group was constrained to be monophyletic (the weighted parsimony trees have a length of 1779).
[b] Not shown in any figure. *Leucocarbo s.str.* of van Tets; see Table 1.

and strong support for the Rock Shag (*P. magellanicus*) being the most basal of this group (i.e., split E, Fig. 4). Because so few transversions have accrued between these taxa, this group (i.e., split D, Fig. 4) appears to have diverged within the last half million years or so. Within the remaining taxa (i.e., *bougainvillii* + *albiventer* + *purpurascens* + *chalconotus* + *onslowi* + *campbelli*), the position of the Guanay Shag is unresolved, but the relationships of the other taxa receive some support. The monophyly of the Imperial Shag (*P. albiventer*) and the Macquarie Island Shag (*P. purpurascens*) has a low decay index, but no apparent conflicting signal, and there is some bootstrap support for the group. Similarly, there are short internal branches and low decay index values for the relationships within the Campbell Island (*P. campbelli*), Stewart Island (*P. chalconotus*), and Chatham Island (*P. onslowi*) Shags but reasonable bootstrap support. Spectral analysis shows that these branches (i.e., splits Q and M, Fig. 4) are not strongly supported, but have no conflict.

The phylogenies estimated from our sequence data suggest that several of Siegel-Causey's (1988) and van Tets' (1976) groups are not monophyletic. The shags and cormorants (as diagnosed by either van Tets or Siegel-Causey; see Table 1 and Fig. 4), for instance, are intermingled with one another and thus do not form monophyletic groups (the comparisons between our sequence phylogeny and the behavioral and morphological phylogenies show that our sequence data does not support the monophyly of either the shags or the cormorants). Given the interspersed nature of the shags and cormorants within our molecular tree, our initial question regarding resolving the position of the marine cormorants becomes less compelling. We are, however, able to resolve the position of Brandt's Cormorant, the only marine cormorant for which we were able to obtain samples. In all of our estimates of phylogeny, there is strong support for grouping Brandt's Cormorant as the sister taxon to the Pelagic and Red-faced Shags (split F, Fig. 4). If the shags and cormorants were monophyletic groups, this finding would appear to favor the behavioral rather than the morphological taxonomy. Brandt's Cormorant does not, however, group with members of the king shags as the behavioral taxonomy suggests, but with two of the cliff shags. While the morphological and behavioral taxonomies differ mainly in the position of the marine cormorants, the differences between our phylogeny and the morphological and behavioral phylogenies suggest that several of their groups may not be monophyletic, and thus we reevaluate the support for each of these groups below.

Macrocormorants

The macrocormorants (*Phalacrocorax*) comprise the Great and Japanese Cormorants. Our phylogeny agrees with both the morphological and the behavioral taxonomies and places these two species as sister taxa (e.g., see Fig. 4). Interestingly, our phylogeny strongly supports placing the Cape Shag as sister taxon to the Great and Japanese Cormorants. Prior to van Tets' (1976) placement of the Cape Shag within the shags, it had been affiliated with several taxa, including the Indian Cormorant (*P. fuscicollis*), Socotra Shag (*P. nigrogularis*), Bank Cormorant (*P. neglectus*), Brandt's Cormorant, and the Great Cormorant (see Siegel-Causey, 1988). Siegel-Causey (1988) placed the Cape Shag in the guano shags (*Leucocarbo*), a subdivision of van Tets' king shags. Our sequence-based tree disagrees with both van Tets' (1976) and Siegel-Causey's (1988) taxonomies but agrees with one of the historical treatments (von Boetticher, 1937) in affiliating the Cape Shag with the Great Cormorant. We cannot comment on several of the historical treatments, as we were unable to obtain samples from the Indian and Bank Cormorants and Socotra Shag.

Mesocormorants

In our sequence-based phylogeny, the mesocormorants (*Hypoleucos*) do not form a monophyletic group (see Table 3), with the Double-crested + Neotropic

Cormorants and the Little Black + Pied Cormorants being placed in different parts of the tree (e.g., see Fig. 4). Biogeographically, the two pairs of taxa make sense, with support for grouping the Double-crested and Neotropic Cormorants from the Americas and grouping the Little Black and Pied Cormorants from Australasia (the Little Black Cormorant is also found in Indonesia). We were unable to obtain samples from the Indian Cormorant, which Siegel-Causey (1988) tentatively placed in the mesocormorants. If the Indian Cormorant is closely affiliated with any of the mesocormorants, biogeographically it is more likely to be affiliated with the Little Black and Pied Cormorants than with the Double-crested and Neotropic Cormorants.

Microcormorants

In our trees, the microcormorants (*Microcarbo*) form one of the two most basal lineages within the shags and cormorants. We were able to obtain samples from only the Little Pied Cormorant, but historically the microcormorants have always been viewed as a group (see Johnsgard, 1993), being morphologically distinct (Siegel-Causey, 1988), with small bodies and short bills. This group is so distinct that it is one of the few groups to have sometimes been given separate generic status by earlier taxonomists (e.g., Peters, 1931). Thus, in the absence of contradictory information, it seems reasonable to assume that the microcormorants are a monophyletic group. Although the sequence data provides support for the microcormorants being the most basal lineage within the shags and cormorants, without samples from other members of the group we cannot be certain that they are the most basal lineage. Adding other taxa from this group would break up the long terminal branch of the Little Pied Cormorant and ensure that its basal positioning was not because its relatively long terminal branch is attracted to the outgroup.

Cliff Shags

The cliff shags (*Stictocarbo*) are not supported as group (see Table 3) and appear to be polyphyletic (e.g., see Fig. 4). Of the cliff shags, the position of the European Shag (*P. aristotelis*) varies the most between our different analyses and it has a very short internal branch and long terminal branch (see Fig. 4). Spectral analysis shows that the European Shag is attracted to more than one part of the tree, with approximately equal support and high conflict for placing it near either the Neotropic and Double-crested Cormorants (i.e., split N, *aristotelis* + *auritus* + *brasilianus* + *magellanicus* + *bougainvillii* + *albiventer* + *purpurascens* + *chalconotus* + *onslowi* + *campbelli*) or the group that includes the Spotted and Pitt Island Shags (i.e., split O, Fig. 4). Similarly, there is support for placing the group of Brandt's Cormorant and the Pelagic and Red-faced Shags in several parts of the tree, hence the high level of conflict for their position in the weighted parsimony and maximum-likelihood trees (i.e., split O, see Fig. 4). The lack of bootstrap support and high conflict levels from the spectral analysis for some branches in our molecular analyses (see Figs. 2b and 4) indicate that our data are unable to resolve these particular relationships.

Murphy (1936) suggested that the Red-legged Shag was most closely related to the Spotted Shag of New Zealand (presumably because both are grey and because of their relative geographical proximity; see Siegel-Causey, 1987). Support for grouping the Red-legged Shag with the cliff shags has also come from both behavioral (van Tets, 1976; Siegel-Causey, 1987) and morphological (Siegel-Causey, 1988) sources. The Red-legged Shag's relatively well-supported position in our trees as the second-most basal branch within the shags and cormorants, however, indicates that it is not closely related to the Spotted Shag.

King Shags

The king shags of van Tets (1976) (*Leucocarbo s.str.*) are not a monophyletic group (see Table 3) because of the well-supported positions among other groupings of the Cape Shag and Brandt's Cormorant (e.g., see Fig. 4). Excluding the Cape Shag and Brandt's Cormorant, however, unlike in Siegel-Causey's (1988) phylogeny (see Fig. 1), the different blue-eyed shags do form a closely related monophyletic group (split E; if the Rock Shag is excluded, the genetic distances within the blue-eyed shags are $\leq$1.7%; see Table 2). Of the different genera that Siegel-Causey (1988) erected from van Tets' king shags, the guano shags (*Leucocarbo*) are not a monophyletic group (see Table 3), with the Cape Shag and Guanay Shags falling in different parts of the tree (e.g., see Fig. 4). As mentioned previously, our data group the Cape Shag with the Great and Japanese Cormorants and the Brandt's Cormorant with the Pelagic and Red-faced Shags.

The Rock Shag was generally perceived as a member of the blue-eyed shags (e.g., Murphy, 1936; Voisin, 1970, 1973) until both behavioral (van Tets, 1976; Siegel-Causey, 1986) and morphological (Siegel-Causey, 1988) information placed it with the cliff shags. Spectral analysis, bootstrap analysis, and decay indices, however, all strongly support the position of the Rock Shag (split D, Fig. 4) in our trees, well away from other cliff shag species, and, as was previously thought, it appears to be either a basal blue-eyed shag or the sister taxon to the blue-eyed shags. Our estimate of divergence for this group suggests that the Rock Shag and the other blue-eyed shags share a common ancestor within the last half million years or so.

Our phylogeny shows that the Guanay Shag belongs within the blue-eyed shags, but we are unable to resolve its exact position within that group. We get no

bootstrap support for the precise position of the Guanay Shag in our analyses. Spectral analysis, however, provides some support for the Guanay Shag's position in one of the weighted parsimony trees (split P, Fig. 2b). Although the support for this split is relatively low there is no support for any conflicting splits (i.e., no conflict for that split; see Fig. 3). Whereas Siegel-Causey's (1988) phylogeny (see Fig. 1) placed the Guanay Shag as sister taxon to the Cape Shag, our placement of the Guanay Shag with the blue-eyed shags agrees with both Murphy (1936) and Devillers and Terschuren (1978). Devillers and Terschuren (1978) argued that the external morphology of the Guanay Shag is little more different from that of the other South American blue-eyed shags than the Campbell Island Shag's is from the other New Zealand blue-eyed shags. Devillers and Terschuren (1978) also observed what they thought to be a hybrid Guanay and South American blue-eyed shag. Murphy (1936) suggested that Guanay Shag was a northern relative of the pan-antarctic blue-eyed shags and that it also resembled the Rock Shag. In discussing the Guanay Shag's foraging mode, Murphy (1936) suggests that to his knowledge only the Cape Shag has developed a similar method of flock-fishing on surface organisms. Murphy (1936) went on to suggest that, whereas the Guanay and Cape Shags are not closely related, their similar habitat and the interaction between them and that environment has led to such similarities. Convergences in osteology caused by convergent foraging mode could possibly explain why Siegel-Causey's (1988) phylogeny placed the Guanay and Cape Shags as sister taxa.

Marine Cormorants

As we were able to obtain samples from only one of the marine cormorants, we are unable to examine their monophyly. As Siegel-Causey's (1988) osteological analysis was the first to identify this group and given the disparity between our and Siegel-Causey's phylogenies, it is possible that sequence data would not support the monophyly of the marine cormorants. As noted previously, in our analyses there is strong support for placing Brandt's Cormorant as sister taxon to the Pelagic and Red-faced Shags (split F). All three species are distributed along the Pacific Coast of North America, with the distribution of the Pelagic Shag overlapping with those of both the Red-faced Shag and the Brandt's Cormorant (Johnsgard, 1993).

Taxonomy

Of the groups that van Tets' (1976) and Siegel-Causey's (1988) taxonomies support, our data support the monophyly of the macrocormorants and Siegel-Causey's blue-eyed shags (*Notocarbo*) and New Zealand blue-eyed shags (*Euleucocarbo*), but because of a lack of samples from certain taxa we cannot comment on the monophyly of the microcormorants or marine cormorants. Our data do not support the monophyly of several groups, including the shags, cormorants, mesocormorants, cliff shags, van Tets' king shags, and Siegel-Causey's guano shag's as discussed above. The king shags of van Tets (1976), for example, form a group in our analyses only if the Cape Shag and Brandt's Cormorant are excluded from the group. Whereas our trees support a monophyletic grouping of the blue-eyed members of the king shags (i.e., excluding the Cape Shag and Brandt's Cormorant), Siegel-Causey's (1988) phylogeny suggests that they are a paraphyletic group. Similarly, the cliff shags are not monophyletic because the Red-legged and Rock Shags do not fall within the group in our analyses. Excluding those two taxa, the group still does not appear to be monophyletic, but the branches that separate the Pelagic, Red-faced, European, Spotted, and Pitt Island Shags are not particularly well resolved. Because of the lack of support for these branches we cannot categorically state that these taxa are not a monophyletic group, but if they are a group, that group also needs to include Brandt's Cormorant.

Because we lack several taxa and resolution in parts of our phylogeny, it in itself does not warrant a revision of the taxonomy of the group. Given the lack of resolution and the levels of sequence divergence (see Table 2), we favor a conservative approach regarding the number of genera recognized. Until a more robust and complete phylogeny is available the use of the single genus, *Phalacrocorax*, appears sensible, as criteria for delimiting genera would be difficult to diagnose.

Sequence and Morphology

Our sequence-based phylogenies and Siegel-Causey's (1988) morphologically based phylogeny are dramatically different from one another. Although there is no reason to assume that sequence data is necessarily any less homoplasious than morphological data (Sanderson and Donoghue, 1989), it has been suggested that when sequence- and morphology-based phylogenies disagree with one another, the morphological characters may need to be reexamined (Hedges and Sibley, 1994; Siegel-Causey, 1997). Siegel-Causey (1988) stated that his morphological tree contained considerable evidence of convergence and reversal, some of which may be due to errors in the assessment of homology, but is more typically associated with characters that are probably adaptations for flight and feeding. Whereas Siegel-Causey (1988) was able to identify homoplasious characters by mapping them onto his phylogeny, other homoplasious characters within his osteological data may become apparent only when mapped onto an independently derived phylogeny (i.e., because parsimony may have incorrectly grouped some taxa because of the homoplasious characters). Thus, by mapping the morphological characters onto the se-

quence-based tree it may be possible to get a better indication of the level of homoplasy in that data set. When the number of changes required was calculated for Siegel-Causey's (1988) data on both the maximum-likelihood and the morphological trees, the number of changes altered for 63 of the 137 characters. Four of these characters appeared less homoplasious on the sequence tree, whereas the number of steps required increased by 3 or more for 34 of the characters. Although our tree is generally quite well supported, there are parts of the phylogeny that lack support, that may be inaccurate, and that thus accentuate the differences between the sequence and the morphological phylogenies.

Some external morphological characteristics that have traditionally been used to imply evolutionary relationships do appear to covary with phylogeny. Foot color, for example, fits our phylogeny well (see Fig. 5a), although the utility of soft body parts as taxonomic markers has been questioned, in part because of changes in coloration between live/fresh and dried specimens (see Murphy, 1936). The foot color of the majority of the shags and cormorants is some slight variation on black. The Little Pied Shag's feet are black (as are those of the other microcormorants), and thus black appears to be the ancestral foot color for this group. Different foot colors have evolved in three lineages, one of which is the bright red of the Red-legged Shag. A second foot color to evolve is the orange/yellow coloration of the Spotted and Pitt Island Shags, and the third different foot color to evolve is the pink or flesh color, which is shared by the Rock, Guanay, Campbell Island, Chatham Island, Stewart Island, Macquarie Island, and Imperial Shags. Foot color does not, however, fit Siegel-Causey's phylogeny well (see Fig. 5b). When mapped onto Siegel-Causey's phylogeny, foot color requires five evolutionary changes as opposed to the minimal three required by our phylogeny. According to Siegel-Causey's phylogeny foot color does not appear to be a good systematic character (see Kennedy *et al.*, 1993), whereas our molecular phylogeny suggests that foot color is a good systematic character.

Some other traditional morphological characteristics do not closely covary with phylogeny. The pink-footed shags are also often called the blue-eyed shags (e.g., see Murphy, 1936; del Hoyo *et al.*, 1992). The blue-eyed shags are named for the color of their eyelids or eye-rings, but the name is a misnomer: the Rock Shag has a red eyelid; the Guanay Shag has a green eye-ring; the Campbell Island, Chatham Island, and Stewart Island Shags' eye-rings are variations on purple; and the Macquarie Island and Imperial Shags' eye-rings are variations on blue (according to the species' accounts in Johnsgard, 1993). Some other shags and cormorants also have blue eye-rings (e.g., the Pied Cormorant), and eye-ring color appears to be quite variable. Thus, although the presence of blue eyes is not diagnostic of the blue-eyed shags, because their blue eyes covary with other morphological and ecological characters (see Murphy, 1936) that do allow a natural group to be diagnosed (i.e., the pink-footed shags), the term blue-eyed shag provides a useful name. Thus, unlike foot color, some external morphological characters that have been used to imply evolutionary relationships may not covary with phylogeny. As another example, the grey plumage of the Red-footed Shag and the Pitt and Spotted Shags appears to be have been derived independently in the two lineages.

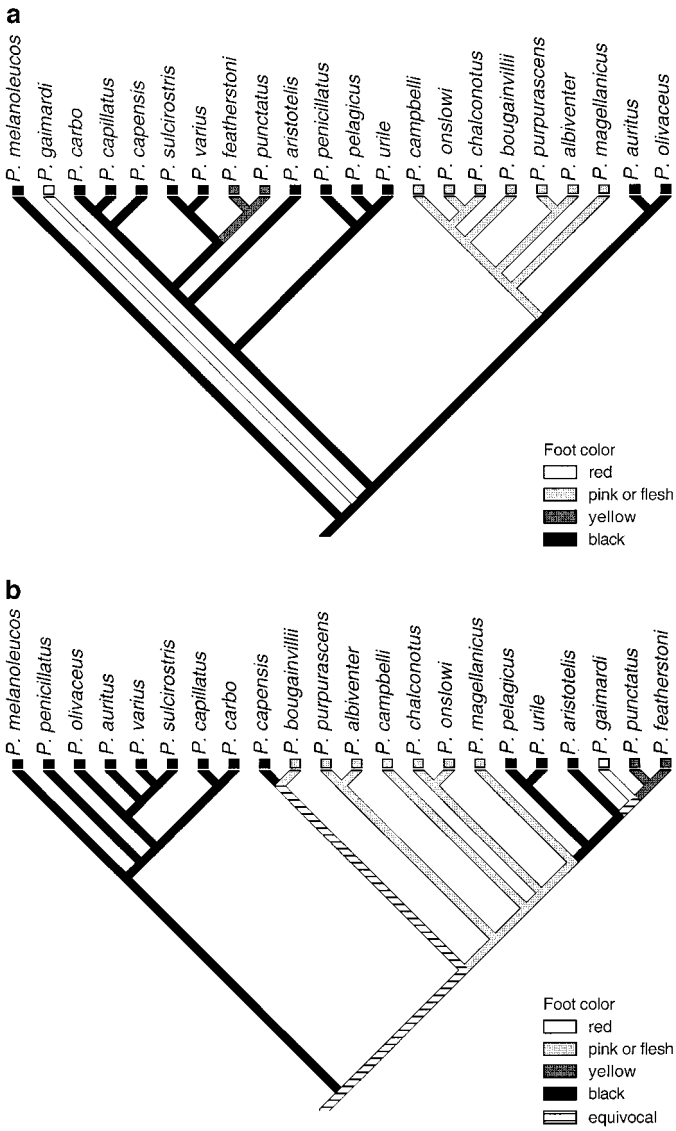

FIG. 5. The foot color of the shags and cormorants mapped onto (a) our maximum-likelihood tree topology (3 steps, CI = 1.0) and (b) Siegel-Causey's tree topology (5 steps, CI = 0.6). The information on foot color was taken from the species' accounts in Johnsgard (1993).

Sequence and Behavior

Our sequence-based phylogenies and the behaviorally based phylogeny (Kennedy *et al.*, 1996) are sub-

stantially different from one another. Using the taxa that the sequence and behavioral trees have in common (i.e., the two outgroup taxa, *P. carbo, P. auritus, P. brasilianus, P. varius, P. penicillatus, P. aristotelis, P. pelagicus,* and *P. urile*) we mapped the behavioral characters onto the topologies of the behavioral and sequence trees. Different numbers of steps were required for 12 of the 37 behavioral characters when mapped onto the sequence tree rather than the behavioral tree. Two of those characters required one fewer step on the sequence tree than on the behavioral tree, whereas the other 10 characters required one additional step when mapped onto the sequence tree. The bowing display (character 2 of Kennedy *et al.,* 1996), for example, appears to have been lost twice when mapped onto the behavioral phylogeny, whereas the sequence tree requires bowing to have been lost three times. It has been argued that complex characters, including behavioral displays, are more likely to be gained once and lost several times than to be independently gained in more than one lineage (Paterson *et al.,* 1995; Kennedy *et al.,* 1996; Omland, 1997). If the behavioral characters are assumed to have evolved just once, the number of losses required for several of the characters is similar for both the behavioral and the sequence trees. Nest-worrying (character 36 of Kennedy *et al.,* 1996), for example, requires a minimum of three steps on the behavior tree and a minimum of four steps on the sequence tree. On the behavioral tree those three steps must all be gains, whereas on the sequence tree the four steps can be either three gains and one loss or four gains. If, however, nest-worrying is constrained to have evolved just once, the behavioral tree requires the gain and four losses, whereas the sequence tree also requires the gain and four losses. Thus, if the behaviors are allowed to be more easily lost than gained, the sequence and behavior trees do not necessarily disagree about the level of homoplasy in the behavioral data. The homoplasy in the behavioral data is thus more likely to be caused by the loss of behaviors than by their convergent gain.

Biogeography

The well-supported portions of our phylogeny allow for speculation on the biogeographic origins of some of the shags and cormorants. The group of Brandt's Cormorant and the Red-faced and Pelagic Shags, for example, is unexpectedly well supported. Grouping Brandt's Cormorant and the Red-faced and Pelagic Shags makes biogeographic sense, given that these species all inhabit the north Pacific coasts of North America and Asia. Whereas the Pelagic Shag inhabits most of this range, the Red-faced Shag is restricted to Alaska and north of Japan and the Brandt's Cormorant is restricted to the North American coast (see Johnsgard, 1993). Brandt's Cormorant and the Pelagic Shag overlap in their ranges, but avoid competition for nest sites, as Brandt's Cormorants nest on the ground and Pelagic Shags nest on cliffs. Similarly, although their preferred prey species also overlap, the Brandt's Cormorant and Pelagic Shag decrease the effect of competition by utilizing different foraging microhabitats (Johnsgard, 1993). If cliff nesting evolved independently in several lineages (alternatively cliff nesting may have evolved once and subsequently changed to other forms of nesting), the shift to cliff nesting may constrain or force certain morphological and behavioral changes. Thus, those characters that have been used to group the cliff shags may represent adaptations to cliff dwelling rather than common ancestry. Alternatively, if cliff nesting evolved just once, the characters used to group the cliff shags may have evolved just once, but subsequently been changed in several lineages.

Another unexpected group in our sequence tree placed the Cape Shag with the Great and Japanese Cormorants (the behavioral and morphological taxonomies both placed the Cape Shag in the shags). Siegel-Causey's (1988) phylogeny places the Cape Shag as sister taxon to the Guanay Shag (as discussed above, our phylogeny places the Guanay Shag as a relatively northern representative of the blue-eyed shags; see Murphy, 1936) in a group that includes the Socotra Shag (*P. nigrogularis*). Biogeographically Siegel-Causey's grouping is difficult to accept, as the Cape Shag is restricted to the coastline of southwestern Africa, the Guanay Shag is found on the coast of South America (particularly the Pacific coast), and the Socotra Shag comes from the Persian Gulf (Johnsgard, 1993). As noted by Murphy (1936), rather than being closely related, the Cape and Guanay Shags may appear similar because of the similarity of their habitats. Thus, in some instances within the shags and cormorants, geographical proximity may be a better indicator of phylogenetic affinity than characters associated with independent radiations into similar ecological niches (e.g., nest types or foraging strategies). As with the taxonomy of this group, until a robust phylogeny with more of the extant taxa is available, global hypotheses about the biogeographic origin of the shags and cormorants will be difficult to evaluate.

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