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Data Set Incongruence and Correlated Character Evolution: An Example of Functional Convergence in the Hind-Limbs of Stifftail Diving Ducks

KEVIN G. MCCRACKEN,[1,4,5] JOHN HARSHMAN,[2] DAVID A. MCCLELLAN,[3] AND ALAN D. AFTON[4]

[1]*School of Forestry, Wildlife, and Fisheries, Louisiana State University, Baton Rouge, Louisiana 70803, USA; E-mail: kmccrack@darwin.helios.nd.edu*
[2]*4869 Pepperwood Way, San Jose, California 95124, USA*
[3]*Department of Biological Sciences and Museum of Natural Science, Louisiana State University, Baton Rouge, Louisiana 70803, USA*
[4]*U.S. Geological Survey, Louisiana Cooperative Fish and Wildlife Research Unit, Louisiana State University, Baton Rouge, Louisiana 70803, USA*

Abstract.—The unwitting inclusion of convergent characters in phylogenetic estimates poses a serious problem for efforts to recover phylogeny. Convergence is not inscrutable, however, particularly when one group of characters tracks phylogeny and another set tracks adaptive history. In such cases, convergent characters may be correlated with one or a few functional anatomical units and readily identifiable by using comparative methods. Stifftail ducks (Oxyurinae) offer one such opportunity to study correlated character evolution and function in the context of phylogenetic reconstruction. Morphological analyses place stifftail ducks as part of a large clade of diving ducks that includes the sea ducks (Mergini), *Hymenolaimus*, *Merganetta*, and *Tachyeres*, and possibly the pochards (Aythyini). Molecular analyses, on the other hand, place stifftails far from other diving ducks and suggest, moreover, that stifftails are polyphyletic. Mitochondrial cytochrome *b* gene sequences of eight stifftail species traditionally supposed to form a clade were compared with each other and with sequences from 50 other anseriform and galliform species. Stifftail ducks are not the sister group of sea ducks but lie outside the typical ducks (Anatinae). Of the four traditional stifftail genera, monophyly of *Oxyura* and its sister group relationship with *Nomonyx* are strongly supported. *Heteronetta* probably is the sister group of that clade, but support is weak. *Biziura* is not a true stifftail. Within *Oxyura*, Old World species (*O. australis, O. leucocephala, O. maccoa*) appear to form a clade, with New World species (*O. jamaicensis, O. vittata*) branching basally. Incongruence between molecules and morphology is interpreted to be the result of adaptive specialization and functional convergence in the hind limbs of *Biziura* and true stifftails. When morphological characters are divided into classes, only hind-limb characters are significantly in conflict with the molecular tree. Likewise, null models of synonymous and nonsynonymous substitution based on patterns of codon-degeneracy and chemical dissimilarity indicate that the nucleotide and amino acid changes postulated by the molecular tree are more plausible than those postulated by the morphological tree. These findings teach general lessons about the utility of highly adaptive characters (in particular those related to foraging ecology) and underscore the problems that convergence can pose for attempts to recover phylogeny. They also demonstrate how the concept of natural data partitions and simple models of evolution (e.g., parsimony, likelihood, neutrality) can be used to test the accuracy of independent phylogenetic estimates and provide arguments in favor of one tree topology over another. {Anatidae; Anseriformes; behavior; congruence analysis; cytochrome *b;* diving; functional morphology; foraging ecology; Oxyurinae.}

When phylogenetic estimates from different data sets concur, there is strong probabilistic evidence of phylogeny (e.g., Mickevich and Johnson, 1976; Cracraft and Mindell, 1989; Bledsoe and Raikow, 1990; Swofford, 1991). However, when phylogenetic estimates disagree, interesting lessons about patterns of evolution and the mechanics of phylogenetic estimation also can be learned (e.g., Poe, 1996; McCracken and Sheldon, 1998). Even so, when phylogenies are incongruent, systematists do not always pursue the matter. As a result, potentially corroborative information can be lost, and otherwise informative biological patterns can be overlooked. One such pattern is convergent evolution, which occurs when selective forces drive the independent fixation of similar adaptive traits in distantly related species. Although convergence

[5]Present address and address for correspondence: Department of Biological Sciences, 107 Galvin Life Science Center, University of Notre Dame, Notre Dame, Indiana 46556, USA. E-mail: kmccrack@darwin.helios.nd.edu

complicates phylogenetic analysis, it is not inscrutable, particularly when different sets of characters are tracking different aspects of history (Bull et al., 1993; Miyamoto and Fitch, 1995; Page, 1996; Slowinski et al., 1997). In many cases, convergent characters may be confined to one or a few anatomical units evolving under a functional regime, and thus evolving nonindependently. As such, convergent characters can be identified readily and discriminated from useful synapomorphies by use of functional criteria and comparative methods. Homologous characters, on the other hand, should be distributed in a stochastic pattern.

Stifftail Ducks

Stifftail ducks (Anatidae: Oxyurinae; sometimes Oxyurini) offer an opportunity to study phylogenetic incongruence and systematic methodology in the context of adaptation and functional morphology. Stifftails are easily distinguished from other waterfowl (Anseriformes: Anatidae) by their elongated, stiffened tail feathers; large, well-developed feet and swimming muscles set far back on the body; and proficient diving abilities (Raikow, 1970; Livezey, 1995a; Johnsgard and Carbonell, 1996). Stifftail ducks generally have been regarded as a monophyletic group of eight species (Delacour and Mayr, 1945; Raikow, 1970; Johnsgard, 1978; Livezey, 1986, 1995a; Johnsgard and Carbonell, 1996).

Stifftails traditionally have been split into four genera, three of which are monotypic: (1) musk duck (*Biziura lobata*), (2) black-headed duck (*Heteronetta atricapilla*), (3) masked duck (*Nomonyx dominicus*), and (4) five or six *Oxyura* species. *Biziura* shares several apparently derived morphological characters with other stifftails, including pointed tail feathers and well-developed legs and feet (Livezey, 1995a; Johnsgard and Carbonell, 1996). However, a plesiomorphic absence of plumage dichromatism and a range of autapomorphic characters (e.g., lek behavior, unique sexual displays, extreme sexual size dimorphism, divergent skeletal anatomy) confound our understanding of its relationship to other stifftails (McCracken, 1999). Because we consider the traditionally defined stifftails to be polyphyletic, we will sometimes, where there is possible ambiguity, distinguish between the "traditional" stifftails (all four genera) and the "true" stifftails (excluding *Biziura*). *Heteronetta,* which is from South America, is least similar to the other stifftails, behaviorally and anatomically. It shares traits with both surface-feeding dabbling ducks and stifftail diving ducks (Johnsgard and Carbonell, 1996). The rest of the group, as currently recognized (Livezey, 1995a; Johnsgard and Carbonell, 1996), consists of six or seven species of similar appearance that can be referred to collectively as "typical" stifftails (i.e., *Nomonyx, Oxyura*). The most divergent of these is *Nomonyx,* which inhabits tropical wetlands of Central and South America. Various authors have synonymized *Nomonyx* with *Oxyura* (Delacour and Mayr, 1945; Johnsgard, 1961; Johnsgard and Carbonell, 1996), but others have considered it a monotypic genus (Phillips, 1922–1926; Peters, 1931; Woolfenden, 1961; Livezey, 1986, 1995a). *Oxyura* can be subdivided into two geographically distinct groups: (1) two New World species, ruddy duck (*Oxyura jamaicensis*) from North America and Argentine lake duck (*O. vittata*) from South America; and (2) three Old World species, Australian blue-billed duck (*O. australis*), white-headed duck (*O. leucocephala*) from Eurasia, and maccoa duck (*O. maccoa*) from Africa. Two additional South American races, *O. j. ferruginea* and *O. j. andina,* generally have been regarded as subspecies of *O. jamaicensis* (Johnsgard, 1978; Johnsgard and Carbonell, 1996). Although the status of *O. j. andina* is not controversial, some authors believe *O. j. ferruginea* is a separate species (see Livezey, 1995a).

Phylogenetic Questions

Livezey's (1986) morphological estimate of waterfowl phylogeny depicted stifftails as monophyletic and as members of a larger clade of diving ducks that includes the sea ducks (Mergini) and pochards (Aythyini). This clade, in turn, was nested within a much larger clade (traditionally called Anatinae; Livezey's {1986} Anatinae

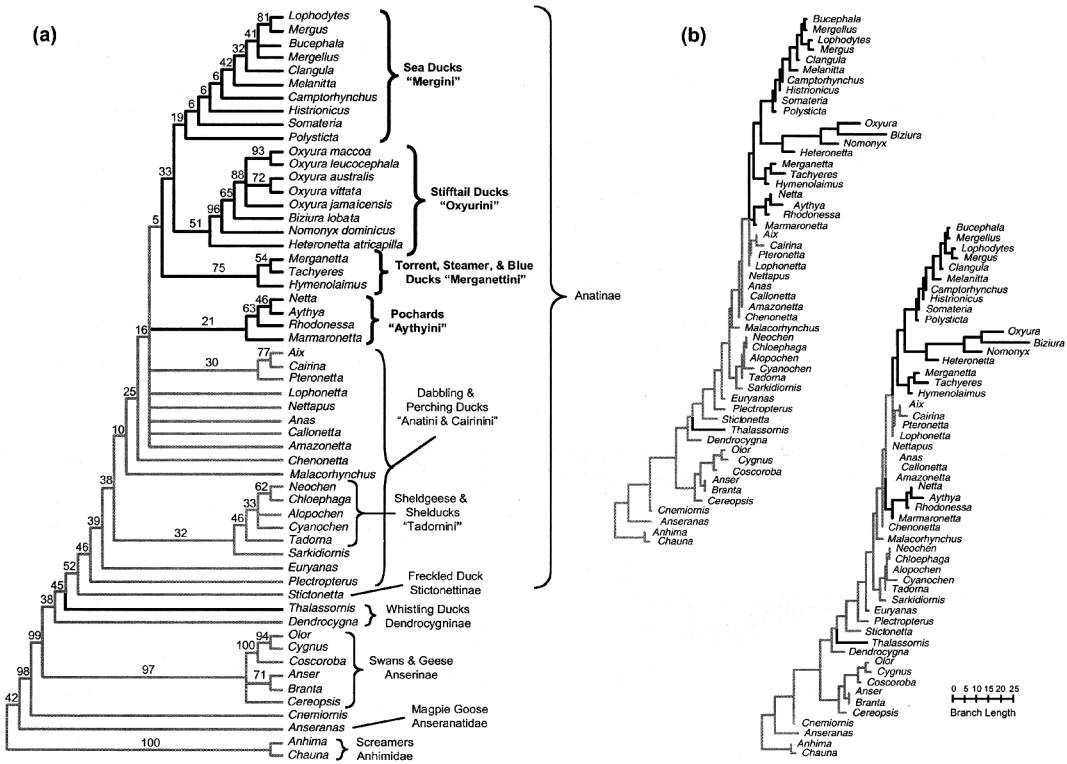

FIGURE 1. Most-parsimonious trees based on analysis of 122 informative characters from the expanded morphological data set. Diving duck lineages are depicted in black. (a) Strict consensus of 45,414 most-parsimonious trees (length = 319, CI = 0.552, RI = 0.833). Branching patterns within stifftails are identical to that shown in Figure 4. Traditional subfamilies and tribes mentioned in the text are labeled using the classification of Johnsgard (1978). Bootstrap consensus indices (1000 replicates) indicate support for nodes. (b) Two alternative reconstructions of the evolution of diving. Note that all trees include a clade composed of stifftails, sea ducks, and *Hymenolaimus–Merganetta–Tachyeres,* a topology that differs from that of Livezey (1986).

is less inclusive) that includes the dabbling ducks, perching ducks, and the shelducks and sheldgeese (Anatini, Cairinini, Tadornini; see Fig. 1a for the compositions of traditional tribes and subfamilies). Livezey's (1995a) analysis of stifftail phylogeny assumed these relationships by the invocation of a hypothetical ancestor, but in doing so failed to provide any further test of stifftail monophyly or relationships to other waterfowl. In contrast, six analyses of waterfowl phylogeny based on immunological distances, DNA hybridization distances, and mtDNA sequences placed stifftail ducks outside Anatinae (Bottjer, 1983; Madsen et al., 1988; Sibley and Ahlquist, 1990; Harshman, 1996; Sraml et al., 1996; Sorenson and Johnson, unpubl. 12S rDNA sequences). Three of these studies (i.e., Harshman, 1996; Sraml et al., 1996; Sorenson and Johnson, unpubl. 12S rDNA sequences) also found stifftails to be polyphyletic; the other three sampled only one stifftail species and cannot resolve this question.

Stifftail ducks thus present a series of interesting problems. First is the issue of the relationships of stifftails to other waterfowl: Are they the sister group of the sea ducks within Anatinae, as morphological analysis suggests, or are they outside Anatinae altogether, as molecular analyses claim? How many times did diving behavior evolve within waterfowl? On Livezey's (1986) preferred tree, diving is most-parsimoniously reconstructed as having evolved four times.

But Livezey's (1986) tree is not the most-parsimonious tree for his data set; a strict consensus of all most-parsimonious trees for Livezey's (1986) data displays very little resolution (Harshman, 1996), and the number of origins of diving can be estimated from two to six, depending on resolution of polytomies. An expansion and revision of Livezey's (1986) data set reduces the number of origins to either two or three (Fig. 1b). In any case, proficient diving abilities clearly evolved independently more than once in waterfowl. In particular, all studies agree that the white-backed duck (*Thalassornis leuconotus*) has evolved many convergent similarities to stifftails (Johnsgard, 1967; Livezey, 1995b; Harshman, 1996). There also is evidence that diving has evolved several times within Anatinae, the typical ducks (Johnson and Sorenson, 1998; Sorenson and Johnson, unpubl. 12S rDNA sequences; Harshman, unpublished analyses). If diving has evolved convergently on numerous occasions in other waterfowl groups, does this hold true for stifftails? Finally, which characters contribute to disagreement between molecular and morphological trees, and can analysis of incongruence guide us in choosing one tree topology over another?

To help answer these and other questions, we enlarged the data set of Harshman (1996) by sequencing most of the mitochondrial cytochrome *b* gene for five additional species of stifftails; the cytochrome *b* data now include all traditional stifftail species, omitting only the questionable species *O. j. ferruginea* and *O. j. andina*. Livezey (1995a) assumed stifftail monophyly and examined relationships within the group by using morphological characters. We have expanded this data set to add outgroups, thus allowing the data to be used to examine stifftail monophyly. Most of our analyses, of both molecular and morphological characters, included the eight traditional stifftail species and three outgroup species. For some analyses, we also added data for all available anseriform species. We present a detailed analysis of incongruence between the morphological data set and the morphological and molecular trees as competing hypotheses of morphological evolution (e.g., McCracken and Sheldon, 1998). For corresponding congruence analysis of the molecular data, we have applied McClellan's (2000) codon-degeneracy model as a null hypothesis of molecular evolution and mapped amino acid substitutions across alternative tree topologies, noting both the position and chemical nature of different kinds of amino acid substitutions in functionally discrete regions of the cytochrome b molecule. These observations, in turn, are compared with the patterns expected under effectively neutral conditions, thus drawing insights about the relative probability of accidental similarity and selective convergence. Finally, we discuss the results of mtDNA and congruence analyses in a larger functional context, draw insights from relevant behavioral information, and examine conceptual inferences pertinent to established methods of character selection and combined data analysis.

Materials and Methods

Collection, Amplification, and Sequencing

Samples for genetic analysis included tissues, extracted DNA, and published DNA sequences (Table 1). Most of the mitochondrial cytochrome *b* gene and part of the adjacent threonine tRNA gene (bp 14991–16064 in the chicken mitochondrial genome; Desjardins and Morais, 1990) were amplified by the polymerase chain reaction (PCR) (e.g., Gyllensten, 1989) from total genomic DNA preparations with use of generalized bird primers (L14990, H16065; Kocher et al., 1989; Helm-Bychowski and Cracraft, 1993). As a coding gene, cytochrome *b* changes rapidly at third-position sites, yet most of these substitutions are silent, resulting in highly conserved replacement of amino acids (Meyer, 1994). Consequently, it is a good choice for reconstructing evolutionary relationships among closely related taxa (Moore and De Filippis, 1997). Sequences for *O. vittata, O. australis, O. leucocephala,* and *O. maccoa* were obtained by dideoxy-sequencing double-stranded PCR products by using Sequenase T7 DNA polymerase (USB) (internal primers H15439, H15476; Kocher et al., 1989; Helm-Bychowski and Cracraft, 1993). Those for *Nomonyx* were obtained by using

TABLE 1. Species, geographic ranges, and sources of genetic material included in this study.

Species[a]	Geographic range	Locality, source
Musk duck *Biziura lobata*	Australia	Harshman (1996)[b,c]
Black-headed duck *Heteronetta atricapilla*	South America	Harshman (1996)[b]
Masked duck *Nomonyx dominicus*	South America	Bolivia, Dpto. Santa Cruz, LSUMNS 123431 (feather quill)
Ruddy duck *Oxyura jamaicensis*	North/South America	Harshman (1996)[b]
Argentine blue-billed duck *Oxyura vittata*	South America	Sylvan Heights Waterfowl, captive, LSUMNS B19175 (heart tissue)
Australian blue-billed duck *Oxyura australis*	Australia	South Australia, Cape Gantheaume Conservation Park (50 µl blood)
White-headed duck *Oxyura leucocephala*	Eurasia	Spain, Esther Signer, University of Leicester (extracted DNA)
Maccoa duck *Oxyura maccoa*	Africa	Sylvan Heights Waterfowl, captive (feather quill)

[a] *O. j. ferruginea* and *O. j. andina* samples not available. However, subsequent analysis of *O. j. ferruginea* (LSUMNS B34000) prior to final submission indicates that *O. j. ferruginea* is the sister group of *O. jamaicensis* (1.34% diverged; Hasegawa et al., 1985) with 99% bootstrap support.
[b] Cytochrome *b* gene sequences.
[c] In part from Sraml et al. (1996).

a Perkin-Elmer ABI 377 automated sequencer at the University of Michigan Museum of Vertebrate Zoology, Ann Arbor (gratis M. Sorenson). Homologous sequences for *Biziura, Heteronetta, O. jamaicensis,* and three outgroup species, including *Stictonetta naevosa, Cairina moschata,* and *Cygnus melanocoryphus,* as well as other sequences used in some analyses, were obtained from Kornegay et al. (1993), Harshman (1996), and Sraml et al. (1996). New sequences from this study have been deposited in Genbank (NCBI) under accession numbers AF119165–AF119169.

Phylogenetic Analysis of mtDNA Sequences

Sequences were aligned visually. Sequence divergence, base pair compositional bias, transition bias, and amino acid variation were analyzed prior to tree construction by using MEGA (Kumar et al., 1993) and PAUP* 4.0.0d64 (test version; D. L. Swofford). *Biziura, Heteronetta, Nomonyx,* and five *Oxyura* species (sensu Livezey, 1995a) were designated as ingroup taxa, and *Stictonetta, Cairina,* and *Cygnus* were included as outgroups. We estimated phylogenetic relationships of these 11 taxa, using unweighted parsimony, weighted parsimony, and maximum likelihood with PAUP* (test version 4.0.0d64; D. L. Swofford). Parsimony analyses employed branch-and-bound search algorithms, which converge on the most-parsimonious tree. For weighted parsimony analyses, transversions were preferentially weighted 5:1 over transitions in light of a 4.53–4.75:1 transition bias (ti:tv). The resulting tree did not differ topologically from trees in which transversions were weighted 100:1 over transitions. Ti:tv ratios and gamma shape parameters (α) for both parsimony trees were estimated simultaneously by using PAUP*'s (test version 4.0.0d64; D. L. Swofford) maximum likelihood score option. Maximum likelihood analyses employed heuristic searches with tree bisection and reconnection branch swapping, repeated 100 times, initiating each search with a random addition sequence to ensure unbiased sampling of tree space. Empirical base frequencies, a ti:tv bias of 4.75:1, and $\alpha = 0.2$ were defined a priori as parameters of the nucleotide substitution model. Ti:tv and α subsequently were reconfirmed iteratively by estimating one parameter and assuming it to estimate the other until both parameters stabilized. A molecular clock was not enforced. To make comparisons easy, all trees were

rooted on *Stictonetta,* although the true location of the root is unclear. To examine relationships of stifftails within the order Anseriformes, we constructed an expanded molecular data set by adding cytochrome *b* sequences from an additional 33 anseriform and 14 galliform species (Harshman, 1996) and performed a parsimony analysis of transversions only (all characters coded as purine or pyrimidine). For this expanded cytochrome *b* analysis, we used a heuristic search with tree bisection and reconnection branch swapping, repeated 100 times, initiating each search with a random addition sequence. Bootstraps were used to assess support for internal nodes for all analyses (Felsenstein, 1985; Hillis and Bull, 1993). When we refer to the molecular data set without qualification, we mean the 11-taxon, cytochrome *b* data set; the 58-taxon cytochrome *b* data set is referred to as the expanded molecular data set.

Analyses of Morphological Characters

Livezey's (1995a) cladistic analysis of stifftail morphology offers an opportunity to study patterns of morphological congruence. We first had to modify Livezey's (1995a) data set, because he used a hypothetical ancestral taxon to root his tree. Hypothetical ancestors assume both the monophyly of the ingroup and a particular set of relationships of ingroup to outgroups and among outgroups, and these were questions we wanted to test. We also wanted the morphological tree to be directly comparable with the molecular tree. To achieve this, we replaced the hypothetical ancestor of Livezey (1995a) with three real taxa, *Stictonetta, Cairina,* and *Cygnus,* using corresponding character states published in the literature (Livezey, 1986, 1991, 1996a; Appendix 1), and deleted *O. j. ferruginea* and *O. j. andina,* for which we had no sequence. We then analyzed this data set by using PAUP*'s (test version 4.0.0d64; D. L. Swofford) branch-and-bound search algorithm. As with the molecular trees, the morphological tree was rooted on *Stictonetta.* To gather further insight into patterns of morphological evolution across all anatid genera and guard against bias potentially induced by using only three outgroup species, we also combined our expansion and revision of Livezey's (1995a) stifftail data set with our revision of Livezey's (1986, 1989) data sets for anseriform genera to produce an expanded morphological data set that included all extant and recently extinct anseriform genera plus all the stifftail species. Merging the two data sets entailed some revisions in character coding, and some states were changed to reflect revised codings by Livezey (1991, 1996a, 1996b, 1996c, 1997; Appendix 2). Phylogenetic analysis of the expanded morphological matrix was done in two steps: an initial round of 500 random addition sequence replicates, each limited to finding five trees, followed by a single search, with no limit, using all trees from the first round as starting trees. Searches were heuristic, with tree bisection and reconnection branch swapping. When we refer to the morphological data set without qualification, we mean the 11-taxon data set, including our revisions of the characters scored by Livezey (1995a) and our three real outgroups in place of the hypothetical ancestor; the data set combining the morphological data set with our revisions of Livezey's (1986, 1989) genus-level data sets we call the expanded morphological data set.

Analyses of Combined Data

We constructed a combined 11-taxon data set by adding together all informative characters from the molecular and morphological data sets. When combining molecular and morphological data, the choice of equivalent characters is not obvious. We tried two simple approaches for the molecular portion of the data—the unweighted sequence, and a second analysis using transversions only (purines and pyrimidines) because transversions appear to be much more informative than transitions. In both cases, all characters were weighted equally. We analyzed the combined data by using PAUP*'s (test version 4.0.0d64; D. L. Swofford) branch-and-bound search algorithm and rooted the combined data trees on *Stictonetta.*

Analyses of Incongruence

Congruence analysis generally proceeds by one of two methods. Advocates of char-

acter congruence (e.g., Kluge, 1989; Kluge and Wolfe, 1993) combine all data into a single tree-building analysis under the principle of total evidence. An overall estimate of congruence, if desired, can then be obtained by comparing variation within and among data sets by using logic similar to that of the *F*-test (e.g., Omland, 1994; Farris et al., 1995). However, in certain instances, it is not desirable to combine data sets, e.g., if one data set is suspected of containing nonphylogenetic information, or in the case of gene versus species phylogenies, when different suites of characters are tracking different histories (Bull et al., 1993; Miyamoto and Fitch, 1995; Page, 1996). In such cases, taxonomic congruence (Mickevich, 1978) or gene tree parsimony (Slowinski et al., 1997), which maintain independent data sets and proceed by fitting one data set to another, are appropriate methods of analysis. In stifftails, incongruence between mtDNA and morphological estimates of phylogeny suggests that the data should be analyzed separately. Nevertheless, combination of incongruent data can reveal hidden patterns in the data, particularly if congruent signals are present in parts of both data sets (Barrett et al., 1991). Accordingly, we used the method of taxonomic congruence, but we also compare these results with the outcome of combined data analysis.

The question arises whether the mtDNA or morphological tree is more accurate. In this case, we were confronted with two alternative hypotheses of stifftail evolution. Even if we do not know a priori which (if either) tree is more accurate, it can be useful to observe the resulting patterns when one data set is mapped onto the tree generated by the other data set. Accordingly, we optimized our morphological data over the molecular tree and then optimized the molecular data (nucleotide and amino acid sequences) over the morphological tree, using PAUP* (test version 4.0.0d64; D. L. Swofford) and MacClade 3.04 (Maddison and Maddison, 1992).

To test null hypotheses that there are no differences in fit between trees, we performed a series of winning-sites tests (Kishino and Hasegawa, 1989). These tests proceed by scoring differences in the number of steps for each variable character (for morphological data) or differences in the log-likelihoods (for molecular data) between alternative tree topologies, and then comparing the distribution of scores with a *t*-distribution to obtain a test statistic. We performed one set of tests for the morphological characters and another for the molecular data (nucleotides and amino acids). We performed one-tailed tests whenever the data set being tested was the one used to produce one of the two trees, because no other tree can possibly explain that data better than the most-parsimonious or most likely tree. Kishino–Hasegawa (1989) tests, thus, are one-tailed unless otherwise specified in the text. For analyses in which the data set being tested was not the same data set used to produce either of the trees to be compared, we used two-tailed tests, e.g., the analyses of subsets of the anatomical characters. We investigated alternative tree topologies and calculated step differences for different arrangements of contentious nodes, using MacClade 3.04 (Maddison and Maddison, 1992). We chose to display characters in the figures by using DELTRAN, which favors parallelisms over reversals, because we believe that this optimization is most likely for most of the characters, but method of optimization has no effect on the statistical tests.

Livezey (1995a) included 92 morphological characters in his analysis. Fifty-two of these are informative for the eight stifftails plus three outgroups and belong to the following groups: 14 of the pectoral assemblage (4 sternal, 1 coracoidal, 7 humeral, 2 carpometacarpal), 10 of the pelvic assemblage (1 pelvic, 3 femoral, 3 tibiotarsal, 3 tarsometatarsal), 1 of the throat (1 tracheal), 4 of the natal integument, 18 describing color and pattern of the adult integument, and 5 describing structural features of the adult integument. We applied Kishino–Hasegawa (1989) tests to each category that had enough informative characters ($n = 4$) to allow a significant test statistic. Accordingly, the single informative throat character was omitted from group-wise tests.

Conducting a detailed analysis of congruence for the molecular data proved more challenging. Mapping nucleotide substitutions on the morphological tree and an-

alyzing patterns of synonymous and nonsynonymous substitution at different codon positions was straightforward. To draw more useful conclusions about the plausibility of substitutions hypothesized to have occurred, we applied McClellan's (2000) codon-degeneracy model of molecular evolution to competing hypotheses of stifftail evolution. As a null model based on discrete patterns of degeneracy within classes of codons, the codon-degeneracy approach emerges from inherent properties of the genetic code and functions independently of phylogenetic hypotheses. When the number of taxa in an analysis is small ($\leq$5), the codon-degeneracy model can be used to positively identify any subset of tree hypotheses that best fit null-expected synonymous substitution frequencies under neutral conditions (e.g., Kimura, 1983) and, to a lesser extent, those tree topologies that best fit expected nonsynonymous substitution frequencies under nearly neutral conditions. In congruence analysis, when the number of taxa typically is much greater than five, the model can be extended to discriminate among two or more alternative topologies. The model also can be applied to site-by-site comparisons of alternative estimates of amino acid substitution by comparing expected and observed profiles of chemical dissimilarity (e.g., Grantham, 1974), given the underlying codon-degeneracy model (McClellan, 1999; see also Xia, 1998; McClellan, 2000). The codon-degeneracy approach offers unusual predictive power and an exciting complement to traditional methods of congruence analysis that assess only character-fit or overall tree similarity. This information, in turn, can serve as a starting point for investigations of the relative selective advantages (or lack thereof) conferred by different kinds of amino acid replacements in different functional domains of protein molecules.

RESULTS

Sequence alignments show no evidence of insertions or deletions, nor evidence that we have amplified nuclear copies instead of the mitochondrial gene. The accidental amplification and inclusion of paralogous nuclear sequences presents a potential stumbling block to PCR-based studies involving mtDNA (Sorenson and Fleischer, 1996). The sequences in this study likely are of mitochondrial origin for several reasons. (1) The entire cytochrome *b* gene was amplified as a single continuous fragment, minimizing the chance of preferential amplification of smaller fragments of nuclear origin (Quinn, 1992; Smith et al., 1992; Kornegay et al., 1993). (2) Gene sequences were translated successfully into amino acid sequences without intervening stop codons or nonsense mutations. (3) Transition bias typical of avian mtDNA, but atypical of nuclear transpositions, was observed (Arctander, 1995). (4) Amino acid substitutions were highly conserved and those that have occurred appear to have a strong phylogenetic component.

Pairwise Sequence Divergence, Base Pair Composition, Transition Bias, and Amino Acid Variation

In the 11 sequences, 14.9% of sites (n = 156) are informative; of these, 80.1% (n = 125) are third-position sites, 15.4% (n = 24) are first-position sites, and 4.5% (n = 7) are second-position sites. Pairwise estimates of percent total genetic distance (Table 2), corrected for multiple hits by the method of Hasegawa et al. (1985), range from 4.72% between *O. vittata* and *O. australis* to 18.23% between *Heteronetta* and *Cairina,* the maximum observed for any species pair. Patterns of nucleotide compositional bias are similar to those found in mammals and other birds (Irwin et al., 1991; Kornegay et al., 1993; Nunn and Cracraft, 1996). Overall percent base pair composition (± SD) is as follows: G 14.8 ± 1.1%; A 26.6 ± 1.0%; T 23.9 ± 0.7%; and C 34.8 ± 0.7%. First positions are slightly C-rich (29.5 ± 1.2%) and low in T (22.6 ± 1.2%) and A (22.8 ± 0.5%). Second positions are more biased than first, being T-rich (40.8 ± 0.7%) and G-poor (13.0 ± 0.5%). The highest compositional bias is at third-position sites, which are rich in C (48.3 ± 1.3%) and A (37.1 ± 3.1%) but low in G (6.4 ± 2.9%) and T (8.3 ± 1.5%). A ti:tv ratio of 4.75:1, estimated by maximum likelihood on the maximum likelihood topology, is consistent with studies of other avian species (Edwards and Wilson, 1990; Krajewski and Fetzner, 1994). Pairwise calculations

TABLE 2. Percent cytochrome *b* gene sequence divergence (lower matrix) and pairwise transition:transversion ratios (upper matrix), corrected for multiple hits by the method of Hasegawa et al. (1985), among stifftail ducks and related waterfowl taxa.

Species	1	2	3	4	5	6	7	8	9	10	11
1. *Stictonetta naevosa*	—	2.92	2.40	3.07	3.32	2.48	2.81	2.70	2.33	2.12	2.32
2. *Cairina moschata*	16.05	—	3.18	4.43	3.31	2.54	2.51	2.57	2.10	2.33	2.10
3. *Biziura lobata*	14.71	15.85	—	2.35	1.81	2.55	1.79	1.86	1.73	1.67	1.67
4. *Cygnus melanocoryphus*	14.85	15.39	11.27	—	1.95	2.92	2.46	2.37	2.12	2.30	2.13
5. *Heteronetta atricapilla*	14.01	18.23	11.90	12.25	—	2.96	2.21	2.39	2.05	2.44	2.13
6. *Nomonyx dominicus*	15.03	14.86	13.47	14.86	13.01	—	4.32	3.96	3.70	3.36	3.08
7. *Oxyura jamaicensis*	14.78	16.43	11.92	13.82	11.12	10.54	—	8.68	8.79	6.01	5.21
8. *O. vittata*	13.41	15.24	11.54	12.88	11.32	11.18	5.18	—	5.37	4.51	3.12
9. *O. australis*	13.99	15.43	12.46	13.29	11.42	10.72	4.77	4.72	—	4.67	3.34
10. *O. leucocephala*	13.49	16.06	12.87	14.46	13.39	11.22	5.94	6.42	4.78	—	4.75
11. *O. maccoa*	14.87	15.52	13.03	14.12	13.56	11.98	7.32	5.30	5.05	6.12	—

of ti:tv ratios (Hasegawa et al., 1985) for all nucleotide positions ranged from 1.67:1 in distantly related taxa (*Biziura* and *O. maccoa*, *O. leucocephala*) to 8.79:1 in more closely related taxa (*O. jamaicensis* and *O. australis*) (Table 2). The ti:tv ratio for third positions alone was estimated to be 9.95:1. The distribution of nucleotide substitutions generally reflects a large number of synonymous substitutions and comparatively few nonsynonymous substitutions. In total, residues at 20 of 348 amino acid sites (5.75%) varied among translated sequences.

Phylogenetic Analyses of the 11-Taxon Cytochrome b *Data Set*

Unweighted parsimony that included all informative characters revealed a single most-parsimonious tree (length = 386, CI = 0.531, RI = 0.455; Fig. 2a). Stifftail monophyly (sensu Livezey, 1995a) is not supported, with the true stifftails consisting of *Heteronetta, Nomonyx,* and *Oxyura.* Within *Oxyura,* the Southern Hemisphere black-headed species (*O. australis, O. vittata,* and *O. maccoa*) form a clade. Weighting transversions 5:1 over transitions produced a tree (720 steps) differing in two important respects from the unweighted parsimony tree (Fig. 2b): *Heteronetta* is separated from the true stifftails, and the Southern Hemisphere black-headed *Oxyura* species no longer form a clade. A single best tree resulted from the maximum likelihood analysis (Fig. 2c). This tree differs from the weighted parsimony tree only in that *Heteronetta* is (as in the unweighted parsimony tree) the sister group of *Nomonyx–Oxyura.* The log-likelihoods (lnL) for the unweighted parsimony, weighted parsimony, and maximum likelihood trees are −4,018.68 (ti:tv = 4.53, $\alpha = 0.21$), −4,006.98 (ti:tv = 4.75, $\alpha = 0.21$), and −4,005.43 (ti:tv = 4.75, $\alpha = 0.21$), respectively. Log-likelihoods of the three trees do not differ significantly from each other in a Kishino–Hasegawa (1989) test (for unweighted parsimony vs. maximum likelihood, diff. lnL = 12.57, SD = 12.00, $t = 1.05$, $P = 0.15$; for weighted parsimony vs. maximum likelihood, diff. lnL = 1.54, SD = 2.98, $t = 0.52$, $P = 0.30$; for unweighted parsimony vs. weighted parsimony, two-tailed test, diff. lnL = 11.05, SD = 13.08, $t = 0.85$, $P = 0.40$; all trees evaluated by using parameters for the maximum likelihood tree). Monophyly of *Oxyura* is robustly supported with a bootstrap value >95% in all three analyses. *Nomonyx* is strongly supported as the sister group of

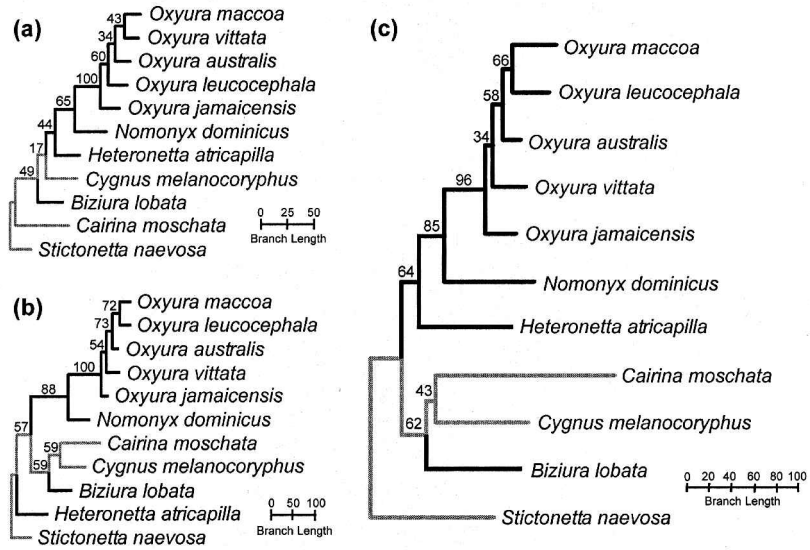

FIGURE 2. Unweighted parsimony, weighted parsimony, and maximum likelihood trees based on analysis of the 11-taxon molecular data set. Diving duck lineages are depicted in black. Bootstrap consensus indices (1000 replicates) indicate support for nodes. (a) Unweighted parsimony tree based on 156 informative nucleotide positions (length = 386, CI = 0.531, RI = 0.455). Log-likelihood (lnL) = −4,018.68 (ti:tv = 4.53, $\alpha = 0.21$). (b) Weighted parsimony tree based on 156 informative nucleotide positions (length = 720). Transversions were weighted preferentially 5:1 over transitions. lnL = −4,006.98 (ti:tv = 4.75, $\alpha = 0.21$). (c) Maximum likelihood tree based on 1045 nucleotide positions. lnL = −4,005.43 (ti:tv = 4.75, $\alpha = 0.21$).

Oxyura in two of the three analyses. *Heteronetta*'s relationship to this clade has weak support. A clade composed of *Biziura* and other stifftails is not even suggested, let alone supported, by any analysis.

Phylogenetic Analysis of the Expanded Cytochrome b *Data Set*

Unweighted parsimony analysis of transversions in the expanded 58-species cytochrome *b* data set gives results (Fig. 3) similar to those for the 11-taxon data set. *Biziura* is shown as the sister group of the swans and geese (Anserinae). *Heteronetta* is the sister group of *Nomonyx* and *Oxyura*. These three genera, the true stifftails, are the sister group to *Biziura*, Anserinae, and Anatinae. *Nomonyx* is the sister of *Oxyura*, and patterns within *Oxyura* are generally congruent with those revealed by the 11-taxon analyses (Figs. 2b, c). Bootstrap values show strong support for the *Nomonyx–Oxyura* clade and for monophyly of *Oxyura* but only weak support for other nodes within stifftails. *Stictonetta* is depicted as the first outgroup of the smallest clade encompassing all the traditional stifftails. Both *Biziura* and the true stifftails are shown to be outside Anatinae, and bootstrap support for this result is strong (80%).

Phylogenetic Analysis of the 11-Taxon Morphological Data Set

The morphological data set of 52 informative characters yields three trees (length = 100, CI = 0.730, RI = 0.794), one of which has the same ingroup topology as Livezey's (1995a) published tree (Fig. 4). Character 56 (black color of chin and throat), the sole character supporting the sister group relationship between *O. jamaicensis* and the rest of *Oxyura* in Livezey's (1995a) analysis, is informative only when Livezey's hypothetical ancestor is assumed. When our real outgroups are substituted, the node has no support.

Phylogenetic Analysis of the Expanded Morphological Data Set

The expanded morphological data set yields 45,414 most-parsimonious trees (length = 319, CI = 0.552, RI = 0.833, count-

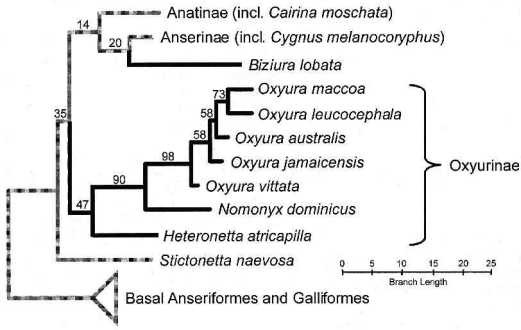

FIGURE 3. Relationships of stifftails within Anseriformes based on parsimony analysis of transversions at 252 informative positions in the expanded molecular data set (length = 924); the expanded cytochrome *b* data set includes sequences from 44 anseriform and 14 galliform species. lnL = −16,495.58 (ti:tv = 3.92, α = 0.33). Details irrelevant to relationships among traditional stifftails are condensed for clarity. Diving duck lineages are depicted in black. Bootstrap consensus indices (1000 replicates) indicate support for nodes.

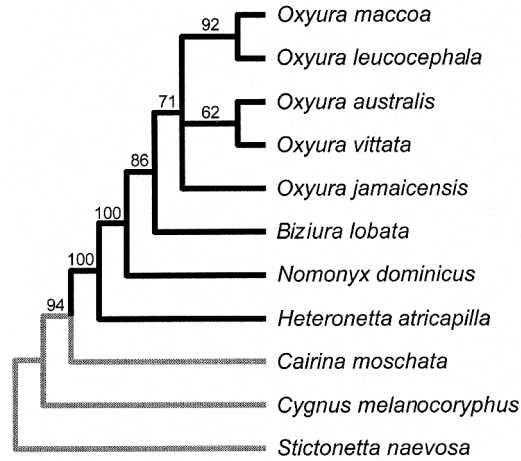

FIGURE 4. Strict consensus of three most-parsimonious morphological trees based on 52 informative morphological characters in the 11-taxon morphological data set (length = 100, CI = 0.730, RI = 0.794). Diving duck lineages are depicted in black. Bootstrap consensus indices (1000 replicates) indicate support for nodes.

ing informative characters only), of which we show the strict consensus (Fig. 1a). Within stifftails, topologies are identical, and bootstrap support similar, to those for the 11-taxon morphological trees. Stifftails are shown to be monophyletic and to be nested deeply within Anatinae as the sister group of sea ducks. Outside stifftails, the consensus shows differences from Livezey's (1986) preferred tree. In addition to the changes in basal relationships described by Livezey (1989), our trees unite three groups of diving ducks (tribes Mergini, Oxyurini, and Merganettini) into a single clade; Livezey (1986) showed Merganettini as sister group of the tribe Tadornini. Our consensus tree differs from the strict consensus of Harshman's (1996) reanalysis of Livezey's (1986, 1989) data in showing greater resolution. Few nodes, and none of those relevant to relationships among tribes of diving ducks, show bootstrap support >50%.

Phylogenetic Analysis of the 11-Taxon Combined Data

Combining the 47 informative transversions and 52 informative morphological characters in an unweighted parsimony analysis yields a single tree (length = 190, CI = 0.632, RI = 0.700; Fig. 5a). Topology within *Oxyura* matches the morphological tree (Fig. 4). Combining the 156 informative, unweighted sequence characters with the morphological characters also gives a single tree (length = 497, CI = 0.559, RI = 0.519; Fig. 5b). Topology within *Oxyura* matches the maximum likelihood tree (Fig. 2c). For both trees, *Biziura* is shown as the sister group of *Nomonyx* + *Oxyura*, a position intermediate between the morphological and molecular trees. Kishino–Hasegawa (1989) tests show that the molecular data alone fit the maximum likelihood tree significantly better than the combined data (transversions) tree (diff. lnL = 17.61, SD = 8.54, t = 2.06, P = 0.0198) but not better than the combined data (unweighted) tree (diff. lnL = 13.33, SD = 8.60, t = 1.55, P = 0.0608). The morphological data alone fit the morphological tree significantly better than the combined data (unweighted) tree (diff. = 8, SD = 4.13, t = 1.93, P = 0.0293) but not better than the combined data (transversions) tree (diff. = 5, SD = 4.10, t = 1.22, P = 0.1144).

Congruence Between Molecules and Morphology

Molecular and morphological trees are in substantial agreement regarding relation-

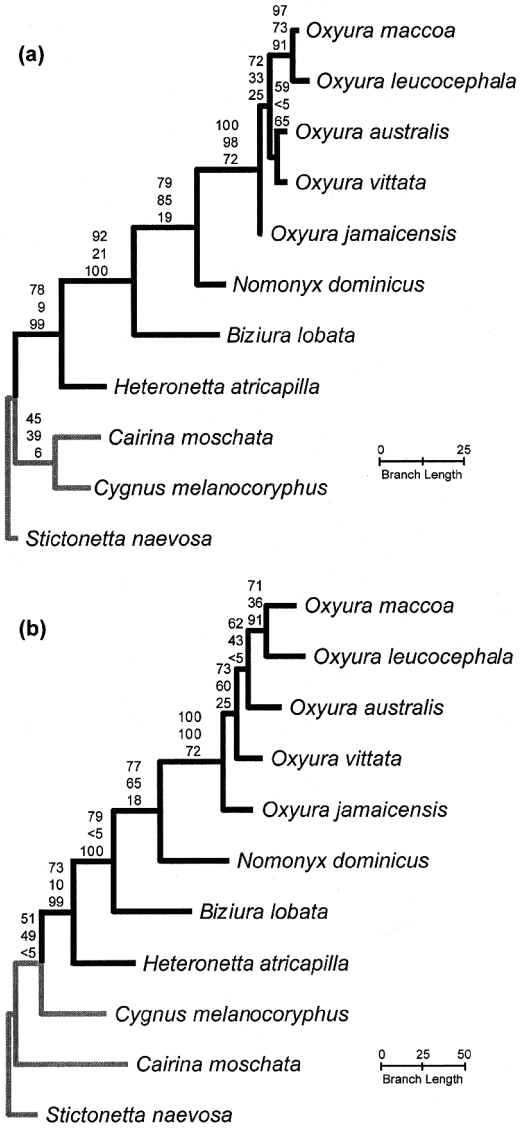

FIGURE 5. Most-parsimonious trees from the analysis of combined data, based on (a) 52 informative morphological characters and 47 informative cytochrome *b* transversions (length = 190, CI = 0.632, RI = 0.700) and (b) 52 informative morphological characters and 156 informative, unweighted cytochrome *b* positions (length = 497, CI = 0.559, RI = 0.519). Diving duck lineages are depicted in black. Bootstrap consensus indices (1000 replicates) indicate support for nodes from three data sets: top numbers, combined data; middle numbers, molecular data only; bottom numbers, morphological data only.

ships within *Oxyura* but disagree strongly with respect to relationships among genera. All analyses show *Oxyura* as monophyletic. Furthermore, they generally agree that *O. jamaicensis* is the sister group of the rest of *Oxyura*. The position of *Heteronetta* is ambiguous; the molecular weighted parsimony tree (Fig. 2b) disagrees with other analyses, although no position is strongly supported by any analysis.

Molecular and morphological analyses disagree strongly about the position of *Biziura;* all molecular analyses place it among outgroup taxa, and bootstrap support for *Nomonyx,* not *Biziura,* as the sister group of *Oxyura* is strong in all molecular analyses. Support for *Biziura* as the sister group of *Oxyura* is weak in the morphological analysis, but support for a clade composed of *Oxyura, Biziura,* and *Nomonyx* is strong. A series of Kishino–Hasegawa (1989) tests in which the position of *Biziura* is varied (Table 3) show that the morphological data reject any tree that does not join *Biziura, Oxyura,* and *Nomonyx,* but they do not reject separation of *Biziura* from *Oxyura.* The molecular data reject a sister group relationship between *Biziura* and *Oxyura* and also reject (or come close to rejecting, depending on the exact topology tested) a clade composed of *Biziura, Oxyura,* and *Nomonyx.*

Within *Oxyura,* all analyses except the molecular unweighted analysis show *O. leucocephala* and *O. maccoa* as sister groups. Unweighted parsimony (Fig. 2a), on the other hand, suggests that the Southern Hemisphere black-headed species (*O. australis, O. vittata, O. maccoa*) form a clade of their own, with white-headed Northern Hemisphere ducks (*O. jamaicensis, O. leucocephala*) branching basally.

Morphological Character Congruence

To study congruence between the morphological data and the molecular trees, we had to choose two topologies to compare. For a molecular tree, we chose the maximum likelihood topology (Fig. 2c), hereafter called the molecular tree, for several reasons. This topology differs from the expanded molecular data tree (Fig. 3) only in the position of *Biziura* and differs from the weighted parsimony tree (Fig. 2b) only in the position of *Heteronetta.* As explained in the Discussion, evidence from amino acid substitutions leads us to prefer a topology

TABLE 3. *P*-values for winning sites tests (Kishino and Hasegawa, 1989) of constrained and skeleton topologies. Skeleton topologies differ from the most likely (molecular) or most-parsimonious (morphological) trees only in the position of *Biziura*. Constrained topologies are the result of maximum likelihood or parsimony analyses with topological constraints invoked to require particular clades.

	Molecular data		Morphological data	
Position of *Biziura*	Constraint	Skeleton	Constraint	Skeleton
Sister of *Oxyura*	0.0099	0.0060	—[a]	—[a]
Sister of *Nomonyx* + *Oxyura*	0.0610	0.0437	0.2054	0.2054
Sister of *Heteronetta* + *Nomonyx* + *Oxyura*	0.1649	0.0929	0.0019	0.0046
Nested within outgroup	—[b]	—[b]	0.0041	0.0041

[a] Most-parsimonious tree.
[b] Most likely tree.

that makes *Heteronetta* a true stifftail. For a morphological tree, we modified the most-parsimonious morphological trees (Fig. 4), choosing the resolution within *Oxyura* that matched Livezey's (1995a) tree and the molecular tree. Because we were not interested in exploring the reasons for disagreements in outgroup topology, we changed the topology of the outgroup to match the molecular tree; this modified tree (length = 104, CI = 0.702, RI = 0.763) will be referred to below as the morphological tree (Fig. 6a).

When 52 informative morphological characters are parsimoniously mapped over the morphological and molecular trees, the morphological tree (Fig. 6a) is 20 steps shorter than the molecular tree (length = 124, CI = 0.589, RI = 0.611; Fig. 6b). A Kishino–Hasegawa (1989) test indicates that the data fit the morphological tree significantly better than the molecular tree (t = 3.48, P = 0.0005). This difference is accounted for by 34 characters, 27 of which have extra steps in the molecular tree and 7 of which have extra steps in the morphological tree (Table 4).

Two-tailed Kishino–Hasegawa (1989) tests performed on subsets of characters grouped according to anatomical region (e.g., pectoral, pelvic, etc.; Table 4) indicate that incongruence between the two trees can be attributed mostly to osteological characters of the pelvic region: pelvis, femur, tibiotarsus, tarsometatarsus (t = 11.00, $P < 0.0001$; Table 5). Character fits for the other anatomical subsets do not differ significantly between topologies (all P's > 0.18; Table 5). An examination of character evolution across taxa indicates that a majority of the extra steps in the molecular tree (Fig. 6b) are putative convergences between *Biziura* and the *Nomonyx–Oxyura* clade (putative synapomorphies of these three genera on the morphological tree).

Molecular Character Congruence

When 156 informative nucleotide substitutions are mapped parsimoniously over the molecular and morphological trees (as defined in the previous section), the molecular tree (length = 388, CI = 0.528, RI = 0.449) is 18 steps shorter than the morphological tree (length = 406, CI = 0.505, RI = 0.395). A two-tailed Kishino–Hasegawa (1989) test indicates that this difference is significant (t = 2.59, P = 0.0105). It is accounted for by substitutions at 58 sites, 38 of which have fewer steps in the molecular tree and 20 of which have fewer steps in the morphological tree. Substitutions at third positions account for 75.9% (n = 44) of the 58 sites. First- and second-position substitutions occur at 17.2% (n = 10) and 6.9% (n = 4) of the sites, respectively.

The synonymous substitution profile (McClellan, 2000) for the molecular tree (n = 237, χ^2 = 1.740) differs markedly from that observed for the morphological tree (n = 303, χ^2 = 18.619); the molecular tree's profile does not differ significantly from the expected substitution profile, but the morphological tree's profile does differ significantly (df = 2, α = 0.05; critical χ^2 = 5.991; Table 6). These differences are largely the result of an apparent excess of third-position synonymous transversions in the morphological tree. The observed nonsynonymous substitution profiles for the molecular tree (n = 30, χ^2 = 16.936) and the

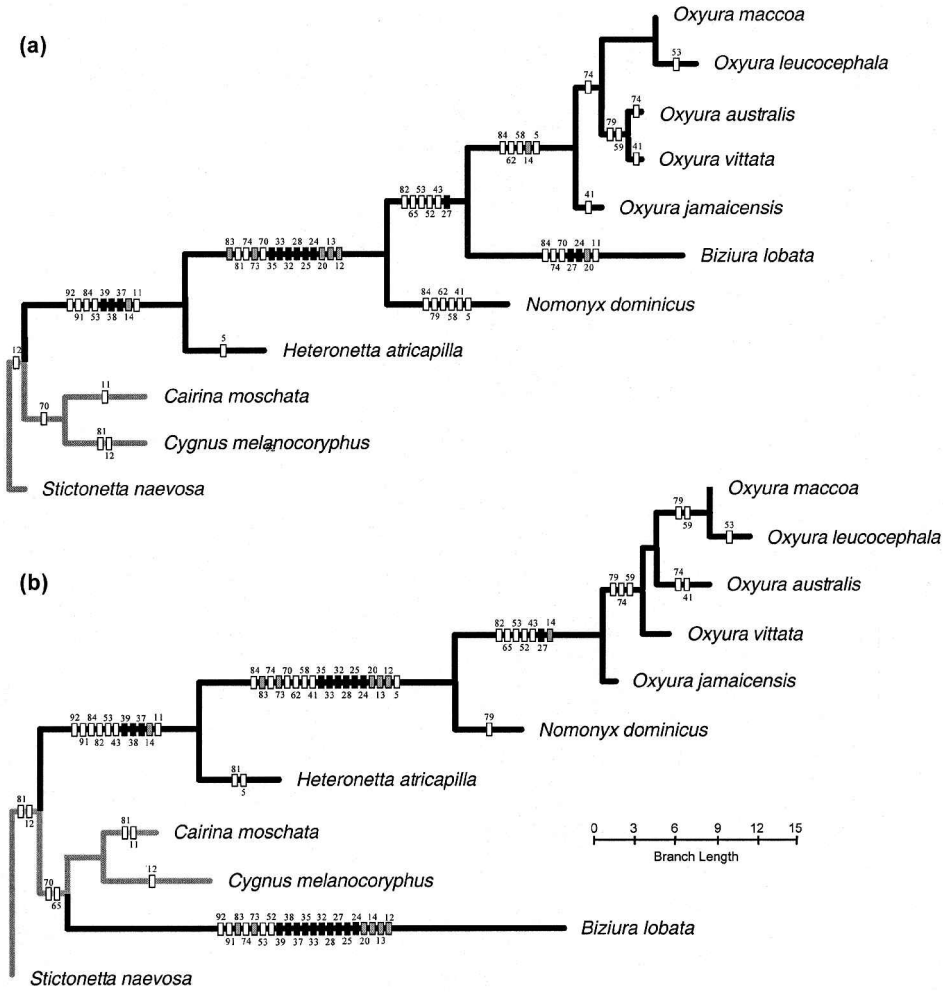

FIGURE 6. Morphological characters mapped onto the morphological and molecular trees. Branch lengths were calculated by mapping 52 informative morphological characters with the use of PAUP* 4.0.0d64 (test version; D. L. Swofford). (a) Morphological tree based on 52 informative morphological characters, with topology of the outgroup constrained to match the molecular tree (length = 104, CI = 0.702, RI = 0.763). (b) Molecular tree (Fig. 2c; length = 124; CI = 0.589; RI = 0.611). Diving duck lineages are depicted in black. Characters differing in number of steps between trees are depicted on the branches (see Table 4); character numbering as in Appendix 1 and Livezey (1995a). Black rectangles indicate hind-limb characters. Gray rectangles indicate other characters for which we have possible adaptive explanations related to diving.

morphological tree ($n = 60$, $\chi^2 = 34.313$) both differ significantly from the expected profile (df = 4, $\alpha = 0.05$; critical $\chi^2 = 9.488$); however, the fit of the molecular tree is better (Table 6).

To further examine nonsynonymous substitutions, we optimized the 20 informative amino acid characters onto both the molecular tree (length = 42, CI = 0.619, RI = 0.610; Fig. 7a) and the morphological tree (length = 47, CI = 0.553, RI = 0.488; Fig. 7b). Six amino acid positions require extra steps on the morphological tree, and one requires extra steps on the molecular tree (Table 7). A two-tailed Kishino–Hasegawa (1989) test indicates that amino acid substitutions fit the molecular tree significantly better than the morphological tree ($t = 2.46$, $P = 0.0282$). Of the eight additional nucleotide substitutions postulated by the morphological tree (two of the six amino acid substitutions require a minimum of two nucleotide substi-

TABLE 4. Groups of informative morphological characters that differ in number of steps between the molecular and morphological trees, anatomical descriptions, and Kishino–Hasegawa (1989) test scores. Steps were calculated by parsimoniously optimizing 52 informative characters over the molecular tree and morphological tree (Fig. 6).

Character no.	Description	No. of steps: mtDNA tree	No. of steps: Morph. tree	Step difference
Pectoral assemblage				
5	Sternum, rostrum, spina interna	2	3	−1
11	Coracoideum, extremitas sternalis	2	3	−1
12	Humerus, extremitas distalis humeri	4	3	1
13	Humerus, foramen pneumaticum	2	1	1
14	Humerus, corpus humeri	3	2	1
20	Carpometacarpus, trochlea carpalis	3	2	1
Pelvic assemblage				
24	Pelvis, ala ilii	3	2	1
25	Femur, cranial prominence	2	1	1
27	Femur, corpus femoris	3	2	1
28	Femur, fossa poplitea	2	1	1
32	Tibiotarsus, condylus medialis	2	1	1
33	Tibiotarsus, crista cnemialis cranialis	2	1	1
35	Tibiotarsus, tuberositas retinaculi m. fibularis	2	1	1
37	Tarsometatarsus, hypotarsus, crista medialis hypotarsi	2	1	1
38	Tarsometatarsus, corpus tarsometatarsi, facies dorsalis	4	2	2
39	Tarsometatarsus, corpus tarsometatarsi, margo lateralis	2	1	1
Trachea, syrinx, esophagus				
41	Trachea, saccus trachealis	2	3	−1
Natal integument				
43	Whitish flank spots	2	1	1
52	Pale supraorbital stripe	2	1	1
Definitive integument (color/pattern)				
53	Crown, black	4	3	1
58	Breast (also flanks), chestnut or maroon	1	2	−1
59	Breast, chestnut color	2	1	1
62	Dorsum (upper back, scapulars, rump)	1	2	−1
65	Contrasting pale supraorbital stripe	2	1	1
74	Dark cheek stripe (adult female)	6	5	1
79	Mantle, pyga, upper wing coverts	3	2	1
82	Pale superciliary stripe	2	1	1
84	Color of rhamphotheca	2	4	−2
91	Lower back, pyga	2	1	1
92	Upper tail coverts	2	1	1
Definitive integument (structure)				
70	Rectrices, modal number	3	4	−1
73	Rectrices, length and shape	2	1	1
81	Bill, shape	3	2	1
83	Molt of remiges	2	1	1

tutions each), seven occur in relatively unconstrained regions of cytochrome *b:* the transmembrane A, E, and H helices. Only one substitution lies in a constrained region, the extrinsic *ab* loop of the intermembrane region (Degli Esposti et al., 1993). A sign test rejects random distribution according to functional domain ($n = 8$, $z = 2.12$, $P < 0.0170$). Mean amino acid dissimilarity indices (D), based on chemical composition, polarity, and molecular volume (Grantham, 1974) for all eight substitutions (56.9 ± 26.9), likewise rank close to or below mean Grantham (1974) indices expected under completely neutral conditions (82.9 ± 48.1; McClellan, 1999). This latter trend is evident in Grantham (1974) profile plots for each of the two alternative tree topologies (Fig. 8a, b). In the complete absence of selection of any kind, amino acid substitution profiles are expected to adopt the shape of the dotted lines in Figure 8(a, b); these plots

TABLE 5. Kishino–Hasegawa (1989) tests for groups of anatomically distinct morphological characters.

Anatomical group	No. of characters	Score[a]	*t*-value	*P*-value[b]
Pectoral assemblage	14	4, 2, 8	0.81	0.4346
Pelvic assemblage	10	10, 0, 0	11.00	0.0001
Natal integument	4	2, 0, 2	1.73	0.1817
Definitive integument (color/pattern)	18	7, 3, 8	1.07	0.2980
Definitive integument (structure)	5	4, 1, 0	1.00	0.3739

[a]Score indicates the number of informative characters that best fit the morphological tree, the number that best fit the molecular tree, and the number that fit each tree equally parsimoniously.

[b]*P*-values indicate the probability of getting a more extreme *t*-value under the null hypothesis of there being no difference between the two trees (two-tailed test).

simply result from the enumeration of all possible (n = 190) single-step nonsynonymous substitutions multiplied by expected substitution profiles at each codon site (solutions to equations 16–20 in McClellan, 2000; Table 6 here). If there are strong functional constraints on amino acid sequence evolution, as is the case in most protein molecules, observed substitution profiles (solid lines) should never match expected profiles (dotted lines) but rather be skewed away from the expected profile towards near zero dissimilarity (i.e., the y-axis). For stifftail cytochrome *b*, we see exactly this kind of pronounced skew towards the y-axis (Fig. 8a, b), indicating strong purifying selection. The shapes of the curves, for both estimates of phylogeny, also are similar as is expected, yet the residual difference between any two observed profiles (Fig. 8c) serves as a useful indicator of the different kinds of changes inferred to have occurred under alternative historical branching patterns.

In the case of stifftails, examination of the residual plot (Fig. 8c) indicates that most additional amino acid substitutions required to form a clade composed of *Nomonyx, Biziura,* and *Oxyura* are of small chemical effect; 8% more replacements are observed in the D = 11–60 range, whereas 8% fewer are observed in the D = 61–150 range (Fig. 8c). The probability that these amino acid residues have become fixed by

TABLE 6. Analysis of congruence using the codon-degeneracy model (McClellan, 2000), including observed and expected synonymous and nonsynonymous nucleotide substitutions in the ingroup topologies of the molecular tree (Fig. 2c) and the morphological tree (Fig. 6a).

	Molecular tree[a]				Morphological tree[b]			
Substitution type	Obs.	Exp.[c]	$\frac{(Oij-Eij)^2}{Eij}$	χ^2	Obs.	Exp.[d]	$\frac{(Oij-Eij)^2}{Eij}$	χ^2
Synonymous								
1st pos. transition	15	16.84	0.20		17	21.79	1.05	
3rd pos. transition	172	177.74	0.19		203	226.83	2.50	
3rd pos. transversion	50	42.42	1.35	1.74	83	54.38	15.06	18.62
Nonsynonomous								
1st pos. transition	10	7.98	0.51		33	15.93	18.29	
1st pos. transversion	12	4.93	10.14		14	9.88	1.72	
2nd pos. transition	4	9.23	2.96		9	18.49	4.87	
2nd pos. transversion	4	4.62	0.08		4	9.24	2.97	
3rd pos. transversion	0	3.24	3.24	16.94	0	6.46	6.46	34.31

[a]Ingroup = *Heteronetta, Nomonyx,* and *Oxyura;* divergent basal taxa were omitted from the analysis.

[b]Ingroup = *Heteronetta, Nomonyx, Biziura,* and *Oxyura;* divergent basal taxa were omitted from the analysis.

[c]Expected number of substitutions based on a ti:tv ratio of 2:1 at fourfold degenerate sites (Kimura, 1980) and pooled sequence data of 1029 class 1 codons, 956 class 2 codons, 22 class 3 codons, and 233 class 4 codons (solutions to equations 13–20 in McClellan, 2000).

[d]Expected number of substitutions based on a ti:tv ratio of 2:1 at fourfold degenerate sites (Kimura, 1980) and pooled sequence data of 1189 class 1 codons, 1100 class 2 codons, 25 class 3 codons, and 274 class 4 codons (solutions to equations 13–20 in McClellan, 2000).

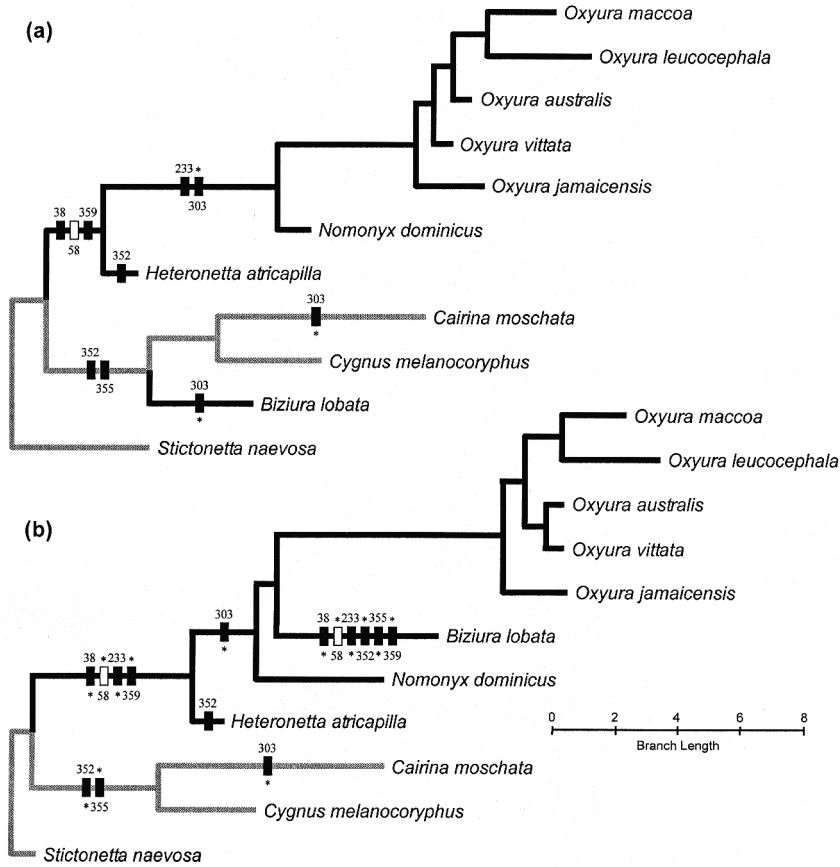

FIGURE 7. Amino acid characters mapped onto the molecular and morphological trees. Branch lengths were calculated by mapping amino acid substitutions at 20 informative sites with the use of PAUP* 4.0.0d64 (test version; D. L. Swofford). (a) Molecular tree (Fig. 2c; length = 42, CI = 0.619, RI = 0.610). (b) Morphological tree (Fig. 6a; length = 47, CI = 0.553, RI = 0.488). Diving duck lineages are depicted in black. Characters differing in number of steps between trees are depicted on the branches (see Table 6). Black rectangles indicate substitutions occurring in transmembrane regions of the molecule; white rectangles indicate substitutions inferred to have occurred in the intermembrane region. Asterisks show homoplasy.

adaptive convergence in primary protein structure thus can be construed as arguably low.

DISCUSSION

Homology has been regarded as the key to discovering the natural hierarchy of life since the time of Owen (1848), but it was not until Darwin (1859) formulated his theory of natural selection that our concept of homology acquired its current explanatory power. Darwin (1859) was among the first to propose that the usefulness of taxonomic characters is inversely related to the degree to which characters have responded to adaptive selection. However, it was outstanding morphologists such as Huxley (1860) and Haeckel (1866) in the 19th century, and Remane (1952) and Hennig (1966) in this century, who developed the criteria systematists use to distinguish useful taxonomic characters. These criteria largely were limited to the concepts of relative position and function of morphological features. Such criteria, however, also are equally applicable to molecules, particularly those composed of functionally discrete subunits such as cytochrome *b* (e.g., Irwin et al., 1991; Degli Esposti et al., 1993). Once functional units have been identified, the comparative method (Harvey and

TABLE 7. Amino acid substitutions that differ in number of steps between trees and best fit the molecular tree (Figs. 2c, 7a) and the morphological tree (Fig. 7b), including amino acid site, position in the cytochrome *b* molecule, observed substitution, and dissimilarity index (*D*).

Site[a]	Position	Substitution[b]	*D*[c]
Molecular tree			
38[d]	A helix	Ala ↔ Ile	94
58	*ab* loop	Ile ↔ Thr	89
233	E helix	Met ↔ Leu	15
352	H helix	Phe ↔ Leu[e]	22
355[d]	H helix	Ala ↔ Ile	94
359	H helix	Ile ↔ Thr	89
Morphological tree			
303	F helix	Ile ↔ Val	29

[a]Sites correspond to numbering of Degli Esposti et al. (1993), but positions in bird cytochrome *b* are actually two greater than these numbers because of insertions; e.g., position 38 is the 40th amino acid in bird cytochrome *b*.

[b]Underlined residues indicate ancestral states at the root of the 11-taxon tree, as inferred from the expanded cytochrome *b* data set.

[c]A function of amino acid composition, polarity, and molecular volume as calculated by Grantham (1974), based on a scale ranging from 5 (Leu ↔ Ile) to 215 (Cys ↔ Trp).

[d]Ala ↔ Ile requires two nucleotide/amino acid substitutions via Val or Thr intermediaries; respective dissimilarity indices equal 64 + 29 or 58 + 89. Total number of possible convergent nucleotide substitutions = 8; score does not include position 303.

[e]Ile is autapomorphic in *Heteronetta*.

Pagel, 1991; Brooks and McLennan, 1991) offers a useful and powerful tool for the critical study of homology and adaptation in both gene sequence and morphology.

We have used simple models of evolution (competing cladograms, parsimony, and maximum likelihood) and taxonomic congruence to compare molecular and morphological phylogenetic hypotheses quantitatively. Comparisons are relatively straightforward and rely on little more than the concept of the functional unit and emergent properties of the genetic code. But these simple comparisons have, we believe, enabled us to explain the incongruence between trees and data sets and to determine which phylogenetic hypothesis is more likely to be correct.

Explaining Incongruence Between Data Sets

The morphological and molecular data sets clearly are incongruent, and the major conflict is in the position of *Biziura*. The morphological tree (Fig. 4) places *Biziura* as sister group of *Oxyura*, and the molecular tree (Fig. 2c) nests it within the outgroup. Each data set significantly rejects the best tree for the other data set, and one or the other data set also rejects each intermediate position of *Biziura*, including the most-parsimonious position from combined data (Table 3). Each data set also rejects one of the two combined data trees (Fig. 5). At this point, we could accept one of the combined data trees, as argued by Kluge (1989) and Kluge and Wolf (1993), but this merely ignores strong conflict. The combined analyses put *Biziura* in a position supported by neither separate data set, but just the least objectionable compromise between them. Other authors have argued for separate analyses when data sets strongly support conflicting trees (de Queiroz, 1993), or when they are significantly heterogeneous (Bull et at., 1993), or when they represent different process partitions regardless of conflict (Miyamoto and Fitch, 1995). But how then do we reconcile the results of conflicting analyses? We could accept the strict consensus of trees from separate data sets (de Queiroz, 1993) as a conservative hypothesis (but see Barrett et al, 1991); unfortunately, the resulting tree in this case would be a polytomy except for relationships within *Oxyura*. Bull et al. (1993) suggested retaining both trees; although not explicitly a consensus method, this amounts to the same lack of resolution. Miyamoto and Fitch (1995) offered no guidance. These alternatives also are unsatisfying, because

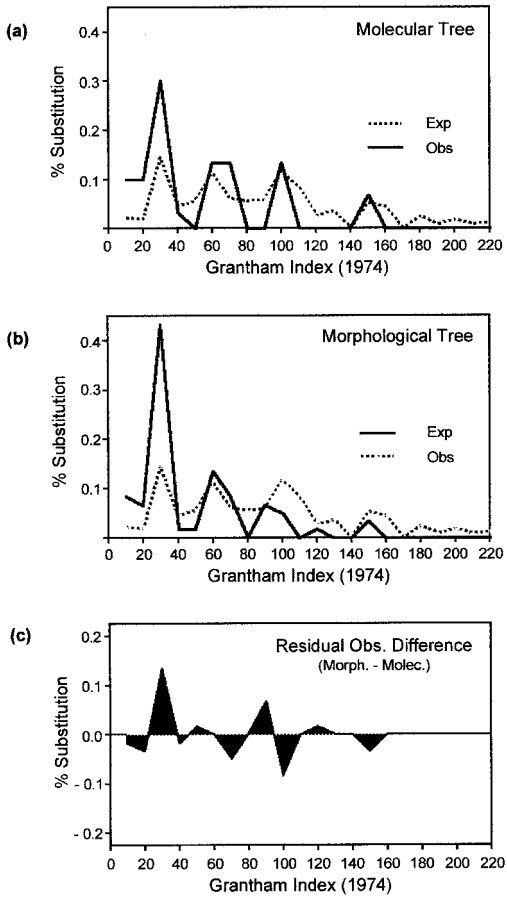

FIGURE 8. Observed and expected Grantham (1974) profiles for the (a) molecular tree, (b) morphological tree, and (c) the observed residual difference (i.e., observed morph. −observed molec.) between the two trees. Expected profiles are a function of the relative probability of all possible single-step nonsynonymous substitutions multiplied by expected substitution frequencies (i.e., solutions to eqs. 16–20 in McClellan, 1999). Areas under both observed and expected curves are equal to one.

we would prefer a single, well-resolved tree. By close examination of the conflicting data sets, we have attempted to determine the reasons for conflict, enabling us to choose one phylogenetic hypothesis over another.

When we divided the morphological characters into anatomical, and presumed functional, groups, only characters of the pelvic group differed significantly in fit between trees. Concentration of conflict in a small subset of the data, defined a priori, suggests that functional convergence is the explanation of incongruence between the morphological data and the molecular tree. Convergence in hind-limb characters is an expected result of adaptation for a diving habit. The simple division by anatomical groups was effective in detecting correlated convergence, even though some nonpelvic characters also show convergence (particularly those of the pectoral group), because pelvic characters were most strongly influenced by diving. If functional convergence had acted strongly in several of our a priori partitions, rather than just one, the test would have been ineffective, but we would be no worse off than when we started. We still would have unexplained conflict between data sets, with no way to choose between hypotheses.

In contrast, incongruence between the molecular data and the morphological tree has no obvious mechanism and can best be explained by the morphological tree being an incorrect estimate of phylogeny. Synonymous changes reconstructed on the molecular tree fit McClellan's (2000) null model quite well, but synonymous changes reconstructed on the morphological tree are a poor fit (Table 6). Reconstructed nonsynonymous changes on both trees are not evolving neutrally (Table 6), which is expected, given the conserved amino acid sequence of cytochrome *b*.

Amino acid changes (Fig. 7, Table 7) significantly reject the morphological tree. If this were a case of adaptive convergence, we might expect some changes of large effect, either in highly constrained regions of the molecule or between amino acids with high dissimilarity, as measured by the Grantham (1974) index. However, neither of these occur. Changes are strongly skewed toward unconstrained regions, and frequency plots of the Grantham (1974) indices are skewed toward changes of small effect, which do not differ noticeably between trees (Fig. 8). Furthermore, there are no suggested functional reasons for sequence convergence between *Nomonyx* and *Oxyura,* or between *Biziura* and Anserinae.

Relationships Among Stifftails

The traditional stifftails appear to be polyphyletic. The true stifftails (Oxyurinae)

include *Oxyura, Nomonyx,* and *Heteronetta* but not *Biziura.* Kishino–Hasegawa (1989) tests using molecular data significantly reject a clade composed of *Biziura, Nomonyx,* and *Oxyura.* The DNA sequence data do not significantly reject *Biziura* as the sister group of the true stifftails, but the amino acid data are strongly suggestive. Unfortunately, our *Heteronetta* sequence is lacking two of the potentially informative amino acid characters (positions 38 and 58). But position 359 is a unique synapomorphy of true stifftails, position 355 is a unique synapomorphy of *Stictonetta* and true stifftails, and position 352 is a synapomorphy of *Stictonetta, Nomonyx,* and *Oxyura,* with *Heteronetta* being autapomorphic. Thus, amino acid evidence suggests that *Stictonetta* is the sister group of true stifftails. The molecular data place *Biziura* as sister group of Anatinae plus Anserinae (not shown) or as sister group of Anserinae (Fig. 3), depending on details of analysis, but no relationship is strongly supported, and there are no strong amino acid characters.

Within true stifftails, *Nomonyx* and *Oxyura* are sister groups. Livezey (1986, 1995a) recognized *Nomonyx* as a separate genus because his preferred topology made a monophyletic *Oxyura* that included *Nomonyx* impossible. Our topology makes it possible to lump the two genera, but we still recommend retention of *Nomonyx,* to recognize the large genetic distance between *Nomonyx* and *Oxyura.*

Within *Oxyura,* morphological and molecular data are congruent, and the combined data trees, as well as separate analyses, show strong support for three nodes. First, the monophyly of *Oxyura* is very strongly supported by most analyses, contradicted by none, and also supported by three unique amino acids, at positions 228, 233, and 333. Second, *O. jamaicensis* is strongly supported to be the sister group of the remaining species in the genus. Third, support is strong for a sister group relationship between *O. leucocephala* and *O. maccoa* in most analyses, and the two species also share a unique amino acid at position 182.

Resolution is lacking on only one question: whether *O. australis* is the sister group of *O. vittata* or of *O. leucocephala* and *O. maccoa.* We slightly prefer the second alternative. First, it appears on the maximum likelihood tree (Fig. 2c), which we consider the best single analysis, as well as on two other analyses of the molecular data (Figs. 2b, 3). Second, it appears on the combined data (unweighted sequence) tree (Fig. 5b), and shows hidden congruence between data sets, in that this clade appears on neither of the trees from separate data sets (Figs. 2a, 4), and its bootstrap value is greater than the bootstrap value for that clade in either separate analysis (Fig. 5b). Finally, this arrangement is geographically more parsimonious. The origin of stifftails is unambiguously optimized as South American; the second topology requires only one dispersal to the Old World, whereas the first topology requires two dispersal events (see Fig. 9). Nevertheless, neither topology is strongly supported by the data, in any combination, and we consider this node still unresolved.

Combining Data

There are good reasons why separate analyses of the molecular and morphological data are appropriate. The two data sets strongly support different trees (de Queiroz, 1993), and they surely belong to different process partitions (Bull et al., 1993; Miyamoto and Fitch, 1995). We also have greater expectation of character independence between data sets than within data sets (Lanyon, 1993), and we have reason to believe that one data set, the morphological one, is positively misleading (Bull et al., 1993).

Nevertheless, we also have performed combined analyses of the two data sets. Even though the data sets conflict strongly, that conflict is localized; it involves, almost entirely, the position of *Biziura.* In other parts of the tree, notably within *Oxyura,* the data sets are congruent and reinforce each other. Bootstrap values for the same nodes, compared among analyses of the combined data set and separate data sets, make useful indices of local conflict and congruence (Fig. 5). If the two data sets conflict at a node, the bootstrap value for the combined data is lower than that for the single data set that supports the node; for example, the

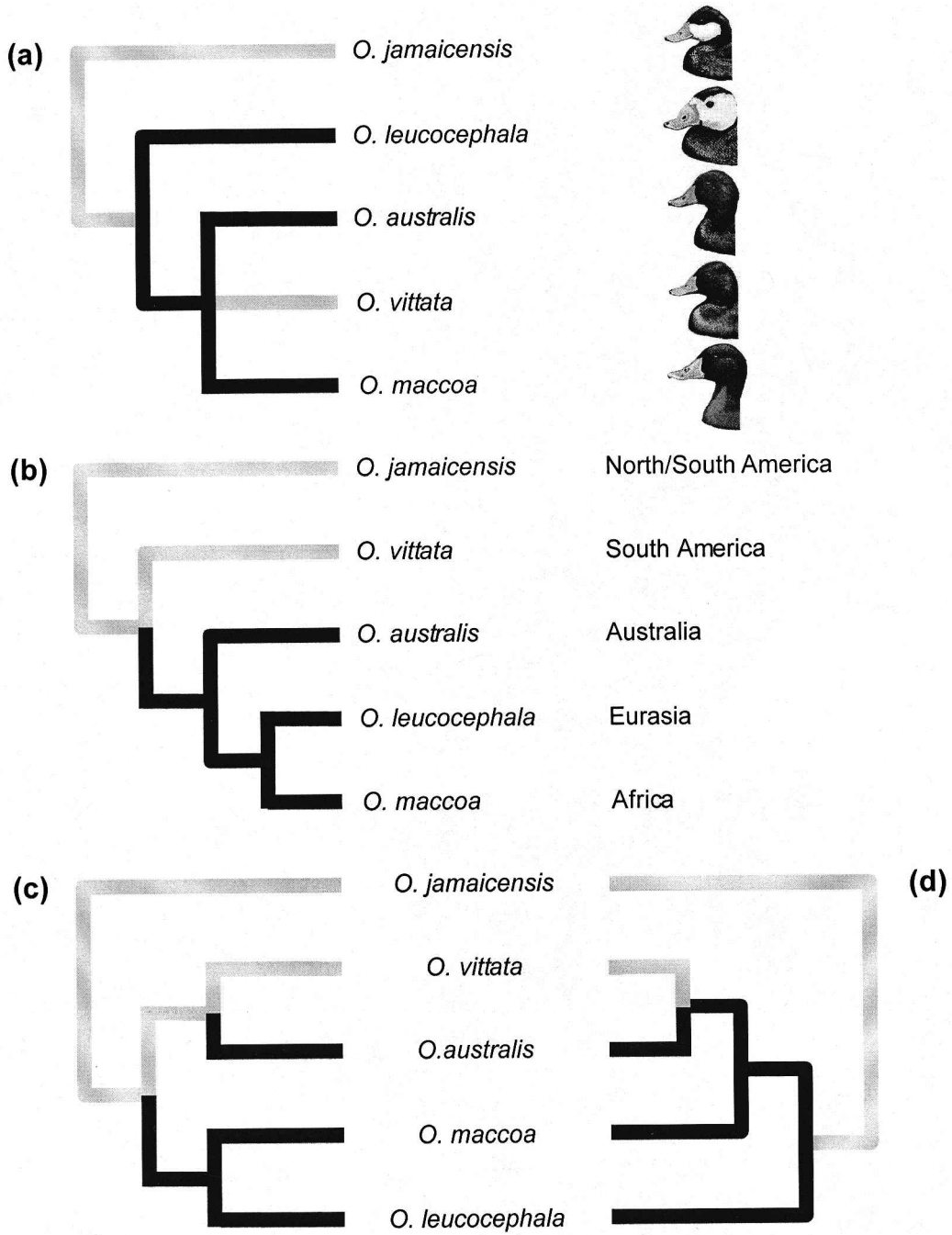

FIGURE 9. Phylogenetic analyses of *Oxyura,* including six informative social–sexual and precopulatory displays (Johnsgard and Carbonell, 1996:79), assuming a root at *O. jamaicensis.* (a) Tree supported by "dab-preening", "choking", "swimming shake", and "sousing" displays, with male head color also indicated. (b) Tree supported by "bill-flicking" and "inflated esophagus" displays, with geographic ranges also indicated. (c, d) Two most-parsimonious trees based on combined behavioral and morphological data. New World lineages are depicted in gray, Old World lineages in black.

monophyly of traditional stifftails is supported by a bootstrap value of 78% in the combined (transversions) data set (Fig. 5a), which is much lower than the value of 99% for the morphological data alone. On the other hand, if two separate data sets are congruent at a node, the bootstrap value for the combined data is higher than that for any one data set; for example, a sister group relationship between *O. leucocephala* and *O. maccoa* is supported by a bootstrap value of 97% for combined (transversions) data, greater than the value for either transversions alone (73%) or morphological data alone (91%). Hidden character congruence can result in the appearance of well-supported clades on a combined data tree that do not appear on trees from either of the separate data sets. This possibility was introduced by Barrett et al. (1991) using hypothetical data, but we apparently have real examples in our data. The most interesting of these is the clade composed of *O. vittata, O. australis, O. leucocephala,* and *O. maccoa,* which has a respectable bootstrap value of 72% for combined (transversions) data (Fig. 5a), but does not appear at all on the morphological tree (Fig. 4) or the transversions tree (not shown, but identical in topology to Fig. 3). Another clade of interest is *O. australis, O. leucocephala,* and *O. maccoa* on the combined (unweighted) data tree (Fig. 5b). Its bootstrap value is not high, but interestingly, the clade does not appear at all on the tree from either separate data set (Fig. 2a, Fig. 4). Presumably, the common signals are individually weak and easily obscured by noise but reinforce each other when data are combined.

Additional Behavioral and Morphological Data

Social and sexual display behavior has been described for *Biziura* (e.g., Frith, 1967; Marchant and Higgins, 1990; McCracken, 1999). However, there is little or no evidence for a sister group relationship between *Biziura* and other stifftails. *Biziura,* in particular, lacks the variety of ritualized display behaviors that set *Oxyura* apart from other waterfowl groups. For example, 23 complex social, sexual, and precopulatory displays shared by one or more *Oxyura* (Carbonell, 1983; Johnsgard and Carbonell, 1996:79; including each of the informative displays depicted in Fig. 9) are completely absent in *Biziura.* The six displays (Johnsgard and Carbonell, 1996:79) that *Biziura* previously was reported to share with one or more *Oxyura* likewise display no clear homology or are plesiomorphic (McCracken, 1999); one exception might include "neck/throat expansion", which occurs in each of the traditional stifftails. Displays previously reported to be homologous (Johnsgard and Carbonell, 1996) include "tail-cocking", "tail up and down", and "foot kicking". However, each of these movements is a key element of a *Biziura* advertising display ("paddle–plonk–whistle–kick") that bears little visual or acoustic resemblance to any of the convulsive display activities performed by *Oxyura* (McCracken, 1999; *Oxyura* displays described by Carbonell, 1983; Marchant and Higgins, 1990; Johnsgard and Carbonell, 1996; McCracken, unpubl. data). "Wing shaking/lifting" and "head stretched forward" displays occur in many anatids and are unlikely to be diagnostic for any group. Displays of *Oxyura,* on the other hand, show a number of potential synapomorphies (see Fig. 9). Those for *Nomonyx* are yet to be described, but the few observations that are available suggest that *Nomonyx* displays are similar to those of *Oxyura* (Johnsgard and Carbonell, 1996). *Heteronetta* shares only three displays with *Biziura,* "wing shaking/lifting", tail-cocking", and "neck/throat expansion" (Johnsgard and Carbonell, 1996); however, the first of these obviously is plesiomorphic, and the homology of the second display is questionable.

Some additional behavioral information and other morphological data also support the idea that *Biziura* is outside Anatinae. In general, the repertoire of *Biziura,* albeit ostentatious, is of limited diversity (McCracken, 1999), and in this respect is similar to those of other basal waterfowl (e.g., whistling ducks, swans, and geese). Dabbling ducks, sea ducks, and other members of the Anatinae typically possess a great variety of displays derived from comfort movements (e.g., Lorenz, 1941; Johnsgard, 1965; McKinney, 1961, 1965, 1975) that are not evident in *Biziura.* Monochromatic

plumage also points toward a relatively basal origin. Subsequent evolution of plumage dichromatism is synapomorphic for *Heteronetta, Nomonyx,* and *Oxyura* and convergently derived in Anatinae.

Several behavioral and morphological similarities point towards a sister group relationship between *Stictonetta* and *Heteronetta.* Both species have similarly abducted hind-limbs and are heavily wing-loaded like diving ducks, yet they are curiously inefficient divers (Weller, 1968; Raikow, 1970; McCracken, pers. obs.). Might their morphology reflect a preadaptation for diving or a partial loss of diving? Other putative synapomorphies shared by these two species, but not by *Biziura, Nomonyx,* or *Oxyura,* include very short tail coverts, unusually wide, flat bills, and basally red bills in breeding males (Marchant and Higgins, 1990; Livezey, 1995a; Johnsgard and Carbonell, 1996). *Stictonetta* also possesses an unusually long penis similar to the type found in *Oxyura* (Marchant and Higgins, 1990). If correspondingly long penes also are present in *Heteronetta* and *Nomonyx,* this character might be a useful synapomorphy for a clade composed of *Stictonetta* and the true stifftails; *Biziura* does not have an unusually long penis (Forbes, 1882; Marchant and Higgins, 1990; McCracken, pers. obs.). Fullagar et al. (1990) likewise suggested that *Stictonetta* and *Heteronetta* share homologous vocalization patterns. The "toad-call" of *Heteronetta,* in particular, resembles the "axle-grind" display performed by *Stictonetta* (Fullagar et al., 1990; see also McKinney, 1992); both displays are of short duration, performed in the presence of males and females, and often occur in aggressive encounters. The elaborate advertising calls of *Biziura* also have been reported to bear some resemblance to the "axle-grind" and "toad-call" (Fullagar et al., 1990). Some similarities are clearly evident in immatures that have not learned their calls (McCracken, 1999). However, these and other vocalizations performed with an outstretched head or inflated neck may be plesiomorphic for several groups of anatids. There currently are too few observations of *Stictonetta* and *Heteronetta* to adequately assess the homology of displays performed by these species and *Oxyura.*

Sexual Displays of Oxyura

Sexual display behavior within *Oxyura* is a potential source of additional character data, and we have found six elements of sexual display to be informative (Carbonell, 1983; Johnsgard and Carbonell, 1996:79). Males of *O. australis, O. vittata,* and *O. maccoa* perform complex "sousing" (or dunking) sequences, as well as "dab-preening", "choking", and "swimming shake" displays; *O. jamaicensis* and *O. leucocephala* do not exhibit these. *O. jamaicensis* and *O. vittata* also share another unique display, "bill-flicking". Finally, *O. jamaicensis, O. vittata,* and *O. australis* share another potentially homologous display, "inflated esophagus". Unfortunately, these characters are of limited utility because their states in *Nomonyx* are unknown, and thus the primitive state in *Oxyura* cannot be determined. We nevertheless have made some use of the characters by limiting the analysis to *Oxyura* alone and by assuming *O. jamaicensis* to be the sister group of the remaining species. Under these conditions, the first four characters support a clade (Fig. 9a) that also appears on the molecular unweighted parsimony tree (Fig. 2a). The last two characters are incompatible with the first four but compatible with each other. Together they support, in both cases by loss of the behavior in question, a tree (Fig. 9b) identical to the molecular weighted parsimony and maximum likelihood topologies (Fig. 2b, c) and the combined data (unweighted) topology (Fig. 5b). If the behavioral and morphological data are combined, there are two equally parsimonious topologies, one (Fig. 9c) identical to the morphological tree (Fig. 4) and the other (Fig. 9d) not previously seen; the strict consensus of these two trees (not shown) preserves only one node. If the behavioral characters are added to either of the two combined data sets, the resulting topologies are unchanged (Figs. 5a, b). To improve the utility of behavioral characters for stifftail systematics, behavioral studies of *Nomonyx* and *Heteronetta* are needed.

Relationships of Stifftails to Other Ducks

Previous analyses of cytochrome *b* sequences have strongly supported the conclusion that stifftails (both *Biziura* and true

stifftails) are not members of Anatinae (Sraml et al., 1996; Harshman, 1996). Our expanded cytochrome *b* data set, incorporating all species of traditional stifftails, offers further confirmation (Fig. 3). By greatly shortening the most relevant branches, our analysis renders less tenable any hypothesis that long branch attraction (Felsenstein, 1978) has misled previous analyses. Three other types of molecular data also exclude stifftails from Anatinae: immunological distances based on serum proteins (Bottjer, 1983); single-copy, whole-genome DNA-hybridization distances (Madsen et al., 1988; Sibley and Ahlquist, 1990), and 12S rDNA sequences (Sorenson and Johnson, unpubl. data). Consilience among four quite different types of data, subject to radically different evolutionary regimes, is powerful evidence for their common conclusion. In contrast, analysis of the expanded morphological data set (Fig. 1) offers only weak support for monophyly of Anatinae, with or without stifftails.

Multiple Origins of Diving

Although most waterfowl dive in some circumstances, only those that habitually dive for food are called diving ducks. Diving ducks clearly do not form a single clade. The expanded morphological data tree (Fig. 1) postulates either two or three evolutionary origins of diving, depending on resolution of the polytomy that includes pochards. But we already have shown that there are at least two more origins of diving, once each for *Biziura* and true stifftails. If adaptive convergence has caused morphological analyses to unite the traditional stifftails, and to place them within Anatinae, perhaps convergence has confused relationships among other diving ducks.

Are the three tribes of diving ducks within Anatinae—Mergini, Aythyini, and Livezey's (1986) Merganettini—monophyletic? Support for sea ducks and pochards from morphological characters is weak, as shown by bootstrap values (Fig. 1a), and support for all three groups largely is composed of characters that have evolved more than once in diving ducks (see below). There also is strong molecular evidence against monophyly of *Hymenolaimus–Merganetta–Tachyeres. Tachyeres* is nested within the genus *Anas* with strong support from both cytochrome *b* and ND2 (Johnson and Sorenson, 1998). *Hymenolaimus* is outside *Anas,* and not closely related to *Tachyeres,* but is within the dabbling ducks, with strong support from cytochrome *b* (Harshman, unpublished analysis). *Merganetta* does not appear to be a member of the dabbling ducks (Sorenson and Johnson, unpubl. 12s rDNA sequences). Monophyly of pochards was confirmed by Sorenson and Fleischer (1996). However, there are insufficient DNA sequence data to test the monophyly of sea ducks.

Do sea ducks and pochards represent a single origin of diving? Bottjer (1983) and Madsen et al. (1988), despite limited taxon samples, investigated the question. Both included representatives of sea ducks, pochards, and dabbling ducks, and both united the latter two exclusive of the first. Neither study included *Hymenolaimus, Merganetta,* or *Tachyeres.* Cytochrome *b* shows pochards as nested within a clade of nondiving ducks, with moderate bootstrap support (Harshman, 1996; Harshman, unpubl. analyses).

Molecular data suggest at least eight origins of diving in waterfowl: *Thalassornis,* true stifftails, *Biziura,* pochards, sea ducks, *Hymenolaimus, Merganetta,* and *Tachyeres.* However, the addition of further data may bring the total number of origins of diving to ten or more. Molecular data suggest that another diving duck, *Salvadorina,* for which we have no morphological data, is not the sister group of any other diver (Sorenson and Johnson, unpubl. 12s rDNA sequences), and the monophyly of sea ducks remains unconfirmed.

Morphological Convergence in Diving Ducks

Unrecognized homoplasy can confound phylogenetic analyses. However, given a topology, multiple origins of similar character states form the raw material of comparative analyses and can be used to test hypotheses of adaptive evolution (Brooks and McLennan, 1991; Harvey and Pagel, 1991). The diving habit appears to exert strong selection on waterfowl anatomy, and many characters have evolved along the same paths in several groups of diving ducks.

Livezey (1986) recognized convergence in 10 morphological characters, and we recognize 14 more, for a total of 24 characters with particular states that appear to have evolved at least twice in separate groups of diving ducks (Table 8). Other characters not coded by Livezey (1986) also have evolved more than once in diving ducks, among them a hallux lobe (Livezey, 1995a, 1996b), and elongated, stiffened tail feathers, the character responsible for the term "stifftail" (Livezey, 1995a).

For some characters, there is a clear functional explanation. Most pelvic region characters can be explained by increased muscle attachment surface or mechanical advantage for muscles used in the swimming power stroke, or by decreased attachment surface or mechanical advantage for muscles used in walking or standing (Raikow, 1970). Characters describing closure of pneumatic foramina are related to high bone density, an obvious advantage in diving. Other characters have been less completely analyzed, but we still can suggest functional hypotheses. Most pectoral characters probably are related to the high wing-loading of diving ducks, a consequence of their short wings and high bone density. The unusual tail-feathers of stifftails (also convergently evolved in *Merganetta* and *Histrionicus*) are used as rudders (Raikow, 1970; McCracken, pers. obs.). Other diving ducks like pochards, sea ducks, and *Tachyeres* lack stiffened tail feathers and rely more on their feet plus movements of the head, neck, or wings in the case of sea ducks, to steer themselves underwater (Bent, 1962; Kortright, 1967; Raikow, 1970). For still other characters, no hypothesis suggests itself, but their observed taxonomic distribution clearly suggests that upon close investigation these characters also will be found to be adaptations to diving. The mapping of characters onto a topology generates a set of predictions to be tested by functional morphologists.

Despite the amount of convergence that has taken place, no one character state is universal in diving ducks. A few transformations have happened a minimum of six times (e.g., characters 37, 56, 75), while many have happened only twice. Although we have not done so, it would be useful to investigate whether some character states were positively or negatively correlated with each other, or with membership in particular subguilds of the diving guild, e.g., feeders in swift-flowing streams, shallow and deep water divers, or fish-eaters. For example, *Biziura* is ecologically convergent on the subguild of large-bodied marine ducks, represented by *Somateria* and *Polysticta* in the Northern Hemisphere and *Tachyeres* in South America, but otherwise an unoccupied niche in Australian waters (McCracken, 1999). We suggest that morphological convergence among these groups will be revealed upon close examination; as a start, all four genera have large, well-developed mandibles capable of crushing hard-shelled prey items. Future work also might investigate the distribution of diving-associated characters among foot-propelled divers outside Anseriformes: loons (Gaviidae), grebes (Podicipedidae), coots (*Fulica*), finfoots (Heliornithidae), cormorants (Phalacrocoracidae), and anhingas (Anhingidae), as well as several extinct groups (e.g., Dabelow,1925; Stolpe, 1932; Storer, 1960; Owre, 1967).

Conclusion

We have argued that the traditional stifftail ducks are polyphyletic and that *Biziura* and the true stifftails adopted a diving habit convergently. Adaptive convergence related to diving appears to explain much of the conflict between molecular and morphological data throughout waterfowl. Our work also suggests some lessons of general utility in systematics. While diving-related characters clearly must be synapomorphic for some groups, and thus of some phylogenetic utility, we advise caution in their use, particularly in the acceptance of groups supported only or mostly by diving-related characters. We also should be careful in our use of highly adaptive characters in general. Characters that play an important role in foraging ecology may be particularly troublesome.

Some deny that there are such things as natural partitions in data (Kluge, 1989; Kluge and Wolf, 1993; Kluge, 1997), but we suggest that partitions are widespread, not just between morphological and molecular

TABLE 8. Waterfowl morphological characters (Livezey, 1986, 1995a) believed to have evolved convergently in two or more clades of diving ducks, including character state, possible adaptive explanation, taxa, and character state description. Tribal names and assumed intratribal relationships for groups other than stifftails correspond to those defined in Figure 1.

Character no.[a]	State[b]	Explanation[c]	Taxa[d]	Description
1 (83)	c	1	*Biziura, Nomonyx + Oxyura*	two synchronous wing molts
6 (40)	d	?	Aythyini, Mergini (−)	bulla ossea of trachea symmetrically enlarged, fenestrated
24	b	2	*Hymenolaimus*, Mergini (−)	humerus: proximo-anconal region with a deep, trench-like depression
26 (12)	c	2	*Thalassornis, Biziura*, Oxyurinae, *Tachyeres*, Mergini	humerus: attachment surface for anterior articular ligament elevated, angled medially
28 (13)	b	3	*Aythya, Polysticta + Somateria*	humerus: pneumatic fossa closed except for small central opening
28 (13)	c	3	Mergini (−), *Thalassornis*, (*Malacorhynchus*)	humerus: pneumatic fossa closed completely
28 (13)	d	3	*Biziura, Nomonyx + Oxyura*	humerus: pneumatic fossa closed but perforated by numerous small holes
29 (14)	b (c)	2	*Biziura*, Oxyurinae	humerus: attachment site of m. latissimus dorsi posterioris in line with outer edge of pectoral attachment (c: on raised ridge)
30 (15)	b	2	*Oxyura, Bucephala + Mergellus*	humerus: distal portion of anconal surface of bicipital crest produced medially with distinct proximal cup-like depression
34	b	2	*Biziura, Nomonyx + Oxyura*	humerus: pit for attachment of m. flexor carpi ulnaris reduced or obsolete
37 (20)	b (c)	2	*Biziura, Nomonyx + Oxyura*, Aythyini (−), *Hymenolaimus*, *Merganetta, Tachyeres*, Mergini (−)	carpometacarpus: distal end of internal rim of carpal trochlea without prominent swelling (c: deeply excavated)
47 (23)	b	2	*Biziura, Hymenolaimus*, *Merganetta, Tachyeres*, (*Cyanochen*)	carpometacarpus: distal portion of internal rim of carpal trochlea distinctly thickened
52 (25)	b	4	*Thalassornis*, *Biziura, Nomonyx + Oxyura*, Mergini (−)	femur: depth of trochanter no greater than depth of head
55 (27)	b	5	*Oxyura, Aythya, Merganetta*, Mergini (−), (*Malacorhynchus*)	femur: shaft moderately curved
55 (27)	c	5	*Thalassornis*, *Biziura, Melanitta, Bucephala*	femur: shaft strongly curved, subangular
56 (28)	b	5	*Biziura, Nomonyx + Oxyura, Aythya, Hymenolaimus*, *Merganetta, Tachyeres*, Mergini	femur: popliteal fossa deep, typically pitted
64 (32)	b	5	*Thalassornis, Biziura, Nomonyx + Oxyura, Aythya*, Mergini	tibiotarsus: anterior extent of internal and external condyles subequal
65 (33)	b	5	*Thalassornis, Biziura, Nomonyx + Oxyura, Aythya*, Mergini	tibiotarsus: inner cnemial crest continued distally along anterior surface of shaft by distinct ridge, well beyond proximal end of fibular crest
67 (35)	b	?	*Biziura, Nomonyx + Oxyura*	tibiotarsus: external ligamental prominence produced laterally, ridge-like

TABLE 8. Extended

Character no.[a]	State[b]	Explanation[c]	Taxa[d]	Description
69 (36)	b	5	*Heteronetta*, *Aythyini* (−), *Hymenolaimus*, *Merganetta*, *Tachyeres*, Mergini	tarsometatarsus: anterior of two ligamental passages between trochlea for digits III and IV exposed to anterior view
70 (37)	b	?	*Biziura*, Oxyurinae, (*Anseranas*)	tarsometatarsus: internal calcaneal ridge of hypotarsus greatly exceeds other calcaneal ridges in posterior extent
75 (38)	b (c)	5	*Thalassornis*, *Biziura*, Oxyurinae, *Netta* + *Aythya*, *Tachyeres*, Mergini	tarsometatarsus: internal ridge of shaft less prominent anteriorly than internal ridge, associated with moderate twisting of shaft (c: internal edge depressed below level of shaft, shaft strongly twisted)
76 (39)	b	?	*Biziura*, Oxyurinae	tarsometatarsus: external margin of shaft straight, trochlea for digit IV internally deflected
78	c	3	*Thalassornis*, *Biziura*, Oxyurinae, *Tachyeres*, Mergini (−)	sternum: pneumatic foramen closed
80 (3)	b	6	*Biziura*, *Merganetta*, *Tachyeres*, *Mergus*	sternum: lateral profile of carina reduced, ventral margin essentially straight for posterior half
115	b	5	*Biziura*, Oxyurinae, Mergini, (*Coscoroba* + *Cygnus* + *Olor*)	pelvis: body of pubis convex dorsally
119 (24)	b (c)	5	*Thalassornis*, *Biziura*, *Nomonyx* + *Oxyura*	pelvis: anterior terminus of shield coincident (c: well caudad) to acetabula

[a]Character numbers correspond to those described by Livezey (1986); numbers in parentheses indicate corresponding character numbers of Livezey (1995a) and the 11-taxon morphological data set (Appendix 1).

[b]Character states in parentheses indicate ordered characters, with the second state nested in the first.

[c]Possible adaptive explanations: 1 = biannual replacement of feathers, diving species incurring greater feather wear; 2 = hypothesized to be related to high wing-loading and short wings of divers; 3 = reduction in skeletal pneumaticity, overall increase in bone density facilitates diving efficiency; 4 = decreased attachment surface or mechanical advantage for muscles used in walking; 5 = increased attachment surface or mechanical advantage for muscles used in diving; 6 = reduction in cross-section of the body increases underwater swimming efficiency; ? = unknown.

[d]Taxa in parentheses are not divers; a minus sign in parentheses indicates that one or more genera in a group do not have the character state.

data, but within both types of data. We have found study of these partitions crucial in understanding our data, and in understanding the evolutionary history of stifftails and other waterfowl. McClellan's (2000) codon-degeneracy model proceeds from quite simple assumptions, fits the inferred synonymous partition of our cytochrome *b* data very well, and shows the nonsynonymous partition to be highly conserved. We thus advocate more exploration of the model's possibilities. Statistical tests (Kishino and Hasegawa, 1989) of partitions within the morphological data also support the hypothesis of adaptive convergence in traditional stifftails.

Our results further suggest that the practice of combining all available data in a single analysis (Kluge, 1989; Kluge and Wolf, 1993) is ill-advised when characters are clearly nonindependent and functionally correlated. In this case, combining the data yields what we believe, based on good evidence, to be an incorrect tree. Such an outcome is expected when correlated adaptive convergence, affecting many characters, has obscured the phylogenetic signal in one of the data sets. On the other hand, even when data sets conflict strongly, they may not conflict on every point. Our data disagree strongly about the position of *Biziura*, because of functional convergence in mor-

phology, so combined data tell us nothing useful regarding its placement. However, there is no apparent functional convergence within true stifftails. Here, the combined data reinforce each other, particularly within *Oxyura*, where some nodes that are ambiguous with separate data sets become strongly supported when data are combined. We therefore propose that data should be analyzed both separately and in combination. Understanding patterns of congruence and incongruence, within and between data sets, is the best road to understanding phylogeny.

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APPENDIX 1. ELEVEN-TAXON MORPHOLOGICAL DATA MATRIX

Character descriptions and numbering follow Livezey (1995a) with the following exceptions. Symbols used for characters 2, 3, 5, 6, 7, 10, 12, 13, 16, 21, 22, 27, 30, and 83 conform with state descriptions of Livezey (1986), but the ingroup states themselves are the same as those used by Livezey (1995a). Character 24 corrects an apparent typographical error in Livezey's (1995a) matrix. The following multistate characters are ordered, and the rest are unordered: 12, 14, 20, 24, 27, 38, 70, 74, and 84. This differs slightly from Livezey's (1995a) ordering.

	Character No.									
Species	1 1234567890	1111111112 1234567890	2222222223 1234567890	3333333334 1234567890	4444444445 1234567890	5555555556 1234567890	6666666667 1234567890	7777777778 1234567890	8888888889 1234567890	99 12
Stictonetta naevosa	?dababaaab	baaaaabaaa	bbaaaaaaaa	aaaaaaaaaa	a??bb????a	??aa?a?aaa	daaa???aaa	b?aaa?????	a?babaaaaa	aa
Cairina moschata	?eababaaab	abaaabbaaa	bbaaaaaaaa	aaaaaaaaac	a??a?ab?aa	?aab?b?aab	daaa???a?c	a?aaa?????	a?baaaaaaa	aa
Biziura lobata	ccbaaacbbc	bcdbaabaac	aabcbbcbbc	bbbbbabcba	abbbbbbbaa	abcaaabaab	daaabababdc	bbbdbbaaab	bbcaaaabaa	bb
Cygnus melanocoryphus	?aaaaabaab	baaaaaaaaa	abaaaaaaaa	aaaaaaaaaa	a??bb????a	??ab?b?aaa	d?aa???aac	a?aaa?????	b?baaaaaaa	a?
Heteronetta atricapilla	bdabcbaaab	ababababa	bbaaaaaaaa	aaaaabbcba	abaaabaabb	babbabbaab	baaaaaaaaa	aaaaaaaaaa	aabbbaaaaa	bb
Nomonyx dominicus	bdabbbaaab	acdbabbaab	bbabbaabaa	abbababcba	bbaaabbbaa	aabbacbbab	cbaaaaaaab	abbbaaaabb	baccabaabb	bb
Oxyura jamaicensis	bgabbbaaab	acdcbabbab	bbabbabbaa	abbababcba	babbbbbbaa	abccbabbab	dbabbaaacb	abbbaaaaab	bbccaaaaaa	bb
O. vittata	bgabbbaaab	acdcbabbab	bbabbabbaa	abbababcba	bbbbbbbbaa	abcbababbb	dbaabaaacb	abbcaaaacb	bbccaaaaaa	bb
O. australis	bgabbbaaab	acdcbabbab	bbabbabbaa	abbababcba	abbbbbbbaa	abcbabbbbb	dbaabbaacb	abbdaaaacb	bbccaaaaaa	bb
O. leucocephala	bgabbbaaab	acdcbabbab	bbabbabbaa	abbababcba	??bbbbbbaa	abdcacbbab	dbbabaabbb	abbcaabbab	bbccaaaaaa	bb
O. maccoa	bgabbbaaab	acdcbabbab	bbabbabbaa	abbababcba	?bbbbbbbaa	abcbabbbab	dbbabaabbb	abbcaabaab	bbccaabaaa	bb

APPENDIX 2. DIFFERENCES BETWEEN THE EXPANDED MORPHOLOGICAL DATA SET AND LIVEZEY'S (1986) DATA SET

Revisions were based on Livezey's (1991, 1995a, 1996a, b, c, 1997) character descriptions. Characters included in Livezey's (1995a) data set but not Livezey's (1986) data set were coded as in Appendix 1, with other taxa coded as missing data. Ordering is as in Livezey's (1986) data for characters included in that matrix and as given in Appendix 1 for other characters.

Character[a]	Taxa	State revision	Reference
7	*Stictonetta*	a → b	Livezey, 1996c
16	*Coscoroba, Cygnus, Olor, Anser, Branta, Cyanochen*	a → c	Livezey, 1996a, c
21	*Tadorna, Alopochen, Neochen, Chloephaga, Cyanochen*	e → a/e	Livezey, 1996c
41	*Plectropterus*	a → b	Livezey, 1996c
41	*Sarkidiornis*	b → c	Livezey, 1996c
79 (2)	*Pteronetta*	e → d	Livezey, 1991
79 (2)	*Netta*	c → c/f	Livezey, 1996b
81 (4)	*Neochen*	b → a	Livezey, 1996c, 1997
82 (5)	*Nettapus*	a → b	Livezey, 1991
82 (5)	*Nomonyx, Oxyura*	d → b	Livezey, 1995a
101	*Tadorna, Alopochen, Neochen, Cyanochen, Tachyeres*	a → b	Livezey, 1996c, 1997
105	*Anser*	a → a/b	Livezey, 1996a
111	*Cygnus, Olor*	b → a	Livezey, 1996a

[a]Numbers in parentheses indicate corresponding characters in Livezey (1995a).