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Combined Support for Wholesale Taxic Atavism in Gavialine Crocodylians

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Abstract.— Morphological and molecular data sets favor robustly supported, contradictory interpretations of crocodylian phylogeny. A longstanding perception in the field of systematics is that such significantly conflicting data sets should be analyzed separately. Here we utilize a combined approach, simultaneous analyses of all relevant character data, to summarize common support and to reconcile discrepancies among data sets. By conjoining rather than separating incongruent classes of data, secondary phylogenetic signals emerge from both molecular and morphological character sets and provide solid evidence for a unified hypothesis of crocodylian phylogeny. Simultaneous analyses of four gene sequences and paleontological data suggest that putative adaptive convergences in the jaws of gavialines (gavials) and tomistomines (false gavials) offer character support for a grouping of these taxa, making Gavialinae an atavistic taxon. Simple new methods for measuring the influence of extinct taxa on topological support indicate that in this vertebrate order fossils generally stabilize relationships and accentuate hidden phylogenetic signals. Remaining inconsistencies in minimum length trees, including concentrated hierarchical patterns of homoplasy and extensive gaps in the fossil record, indicate where future work in crocodylian systematics should be directed. [Aves; combined evidence; Crocodylia; fossil; gavial; phylogeny.]

The 23 extant species of Crocodylia and their close fossil relatives compose a monophyletic group of morphologically conservative archosaurs (Benton and Clarke, 1988). The clade is generally considered to be the relictual extant sister group of Aves (birds) and includes four primary lineages: Gavialinae (gavials), Crocodylinae (crocodiles), Alligatoroidea (alligators and caimans), and Tomistominae (false gavials) (see Fig. 1 for taxonomic definitions). Various systematic data sets have been applied to crocodylian phylogeny, but certain relationships remain contentious (reviewed by Poe, 1996; Brochu, 1997).

Incongruence is most evident at the base of Crocodylia, where the affinities of Gavialinae are contradictory (Fig. 1). Traditional morphological studies suggested that the lone extant gavial, *Gavialis gangeticus*, is characterized by a suite of primitive traits and is the extant sister taxon to all other Crocodylia (Fig. 1a). This result was first proposed by Duméril (1806) and has been corroborated by recent numerical cladistic analyses (Norell, 1989; Brochu, 1997; Buscalioni et al., 2001). The morphological hypothesis conflicts with biochemical and molecular trees that group *G. gangeticus* with the only living false gavial, *Tomistoma schlegelii* (Tomistominae). According to these trees, Crocodylinae and Alligatoroidea are successively more distantly related to the *G. gangeticus* + *T. schlegelii* clade (Fig. 1b; Densmore, 1983; Densmore and Owen, 1989; Densmore and White, 1991; Gatesy and Amato, 1992; Hass et al., 1992; Aggarwal et al., 1994; White and Densmore, 2001; Harshman et al., 2003 [this issue]).

Morphological and molecular hypotheses differ only in the phylogenetic placement of Gavialinae (Fig. 1). Historically, similarities in the long, narrow rostra of *G. gangeticus* and *T. schlegelii* were interpreted as striking examples of adaptive convergence (Fig. 1a) and often were dismissed as phylogenetic evidence (Langston, 1965; Hecht and Malone, 1972; Tarsitano et al., 1989). Although the narrow-snouted condition has evolved multiple times in Archosauria (Clark, 1994), the precise pattern of evolution within Crocodylia has been debated (Brochu, 2001). From a molecular systematic perspective, the slender jaws of gavialines and tomistomines are best interpreted as homologues (Fig. 1b).

The *Gavialis* problem represents one of the more longstanding discrepancies between morphological and molecular approaches to systematics (Densmore, 1983; Buffetaut, 1985; Norell, 1989; Tarsitano et al., 1989; Poe, 1996; Brochu, 1997; Hillis and Wiens, 2000). In part, the difficulty lies in distinguishing convergent ecological specializations from uniquely evolved traits, but another aspect of the conflict involves establishing a consistent phylogenetic root at the base of Crocodylia. Pinpointing a root would seem to be straightforward from a morphological perspective. Skeletal characters can be scored from well-preserved close relatives of Crocodylia from the Cretaceous (e.g., Norell and Clark, 1990; Clark and Norell, 1992), and these fossil crocodyliform outgroups strongly implicate Gavialinae as the basalmost branch of Crocodylia (Fig. 1a).

Because of the geometry of lineage splitting and extinction within Archosauria, no near relatives of Crocodylia are living (Benton and Clark, 1988). In this case, outgroup comparisons using molecular data might be more problematic than outgroup analysis of morphological evidence (Norell, 1989). First appearances of the four major lineages of extant crocodylians range from the late Cretaceous to the Eocene, but Crocodylia split from its extant sister group in the Triassic, approximately 230 million years ago (Fig. 1b; Carroll, 1988). The inevitable result of this 160-million-year gap is that for many genetic loci birds are highly derived relative to crocodylians. Such long outgroup branches could hinder the estimation of ancestral states and the rooting of trees based on molecular evidence (Felsenstein, 1978; Wheeler, 1990).

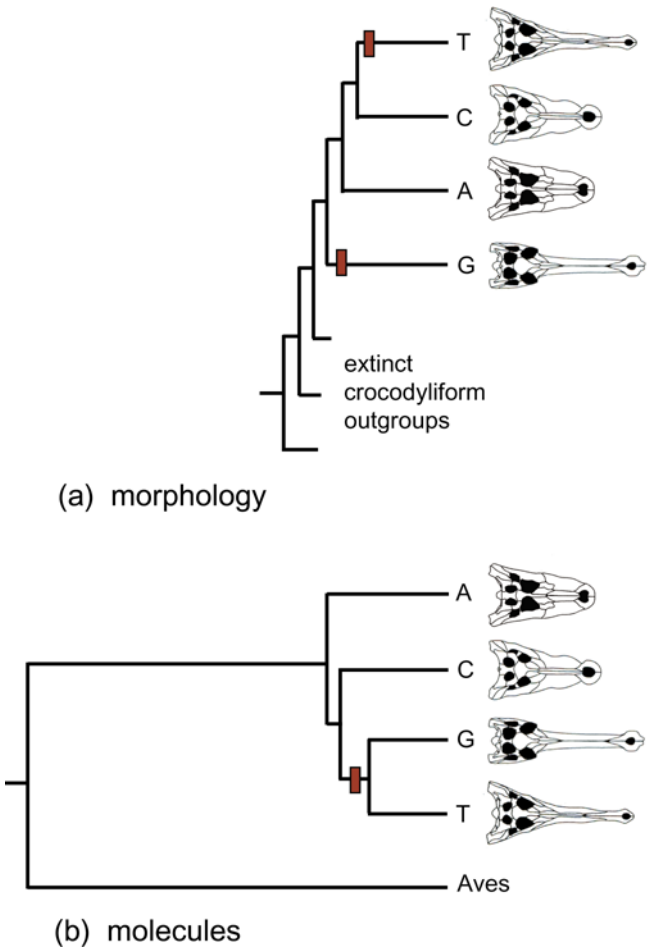

FIGURE 1. Hypotheses of systematic relationships among major clades of crocodylians; topologies are based on gross anatomical characters (a) and molecular information (b). Extinct crocodyliform taxa that diverged from Crocodylia in the Cretaceous can be utilized as outgroups in morphological studies. Because of extinction on the crocodylian stem lineage, the closest extant outgroup of Crocodylia is Aves. Red bars mark the evolution of the longirostrine, narrow-snouted condition in gavials and false gavials. Branch lengths are roughly proportional to time. Taxonomy is slightly modified from that of Brochu (2003): Crocodylia = last common ancestor of *Gavialis gangeticus*, *Alligator mississippiensis*, and *Crocodylus rhombifer* and all of its descendents; Tomistominae (T) = *Tomistoma schlegelii* and all taxa closer to it than to *G. gangeticus* or *C. rhombifer*; Crocodylinae (C) = *C. rhombifer* and all taxa closer to it than to *T. schlegelii*; Alligatoroidea (A) = *A. mississippiensis* and all taxa closer to it than to *G. gangeticus* or *C. rhombifer*, Gavialinae (G) = *G. gangeticus* and all taxa closer to it than to *T. schlegelii* or *C. rhombifer*.

Unfortunately, the majority of previously collected molecular data for Crocodylia is not amenable to comparisons with distantly related outgroup taxa. Thus, published trees based on immunological reactions, allozymes, unmapped restriction fragments, and DNA fingerprints have not included birds and have been rooted at the midpoint using a variety of distance-based tree-building algorithms (Densmore, 1983; Densmore and Dessauer, 1984; Densmore and Owen, 1989; Densmore and White, 1991; Hass et al., 1992; Aggarwal et al., 1994). Because these topologies were not rooted using the outgroup criterion (Maddison et al., 1984; Nixon and Carpenter, 1993), it has been impossible to rigorously assess incongruence between the biochemical and gross anatomical evidence (see Poe, 1996; Brochu, 1997; Brochu and Densmore, 2001). Preliminary cladistic studies of mitochondrial (mt) 12S ribosomal (r) DNA sequences supported the topology favored by the phenetic molecular analyses (Fig. 1b; Gatesy and Amato, 1992; Hass et al., 1992; Gatesy et al., 1993; Poe, 1996; Brochu, 1997), and additional DNA data recently have corroborated this result (White and Densmore, 2001; Harshman et al., this issue). However, the molecular topology implies a suspiciously large number of evolutionary character reversals, i.e., taxic atavisms (Stiassny, 1992), that are concentrated in Gavialinae (Densmore, 1983; Hass et al., 1992).

Here, sequence data for four genes, ~3,000 nucleotides from the mt and nuclear (nu) genomes, were utilized to make more extensive molecular comparisons between crocodylians and birds. Instead of simply noting topological similarities and differences between molecular and paleontological results, a combined evidence approach (Miyamoto, 1985; Kluge, 1989; Nixon and Carpenter, 1996) was applied to crocodylian phylogeny (Poe, 1996; Brochu, 1997; Brochu and Densmore, 2001). The goals of this study were to (1) partition conflicts and common support among molecular and gross anatomical data sets within the context of all relevant characters, 2) determine the influence of fossil evidence on rooting position and clade stability, 3) discern secondary signals in different systematic data sets (Trueman, 1998; Brochu, 1999), 4) erect hypotheses of morphological evolution based on the minimum length topologies, and 5) suggest future avenues of systematic work within Crocodylia.

MATERIALS AND METHODS

Molecular Data

Our approach was to utilize a battery of DNA sequences that could be aligned between distantly related organisms. Segments of four genes, mt 12S rDNA (~240 base pairs), mt 16S rDNA (~400 base pairs), mt cytochrome *b* (cytb, ~240 base pairs), and nu recombination activating protein 1 (RAG-1, ~2,000 base pairs), were sequenced from 15 taxa. All extant genera of Crocodylia and exemplars from the two basal clades of Aves were represented in the sample.

Blood samples were acquired from zoological parks, DNA was extracted from fresh tissues (Gatesy and Amato, 1992), and DNA vouchers from all species were deposited in the frozen collections at the American Museum of Natural History. "Universal" mt primers (Kocher et al., 1989; Irwin et al., 1991; Simon, 1991) and crocodylian-specific nu RAG-1 primers were used in polymerase chain reaction (PCR) amplifications and sequencing (Table 1). Most mt data were collected using single-stranded PCR protocols and the dideoxy sequencing method (Gatesy and Amato, 1992; Gatesy et al., 1993). Alternatively, double-stranded PCR products were processed on an ABI automated sequencer

TABLE 1. Oligonucleotide primers used in this study.

Primer Position no.[a]	Sequence (5′ to 3′)
12SA 850	AAACTGGGATTAGATACCCCACTAT
12SB 1270	GAGGGTGACGGGCGGTGTGT
16SA 2290	CGCCTGTTTACCAAAAACAT
16SB 2860	CCGGTCTGAACTCAGATCACGT
cytbA 14605	CCATCCAACATCTCAGCATGATGAAA
cytbB 14920	CCCTCAGAATGATATTTGTCCTCA
RAGL1 450	ACTCGATTTTGTCACAATTG
RAGL2 459	TGTCACAATTGCTGGAGTAT
RAGL3 1227	AAGGCTGTTTGCATGACTTTGTT
RAGR1 1262	ATAGCTTCCAGCTCATCTGCTTG
RAGR2 1268	TGCATTATAGCTTCCAGCTCATC
RAGR5 2462	AGCAAAGTTTCCATTCATCCTCAT

[a]Numbers refer to positions in the *Bos taurus* mtDNA sequence of Anderson et al. (1982; NCBI J01394) or in the *G. gangeticus* RAG-1 sequence of Groth and Barrowclough (1999; NCBI AF143725).

(Gatesy and Arctander, 2000). Polymorphisms and bases that could not be scored clearly were coded as IUPAC ambiguities.

Five higher level taxa and associated species were sampled for DNA characters: Alligatoroidea: *Caiman crocodilus, Caiman latirostris, Melanosuchus niger, Paleosuchus palpebrosus, Paleosuchus trigonatus, Alligator sinensis*, and *Alligator mississippiensis*; Crocodylinae: *Crocodylus rhombifer, Crocodylus intermedius, Crocodylus cataphractus*, and *Osteolaemus tetraspis*; Gavialinae: *Gavialis gangeticus*; Tomistominae: *Tomistoma schlegelii*; Aves: *Gallus gallus* and *Struthio camelus*. New data for 43 gene fragments were combined with 17 published sequences. All mtDNA data for *G. gallus* were from Desjardins and Morais (1990; NCBI X52392), and RAG-1 sequences for *G. gangeticus, A. mississippiensis, G. gallus*, and *S. camelus* were from Groth and Barrowclough (1999; NCBI AF143725, AF143724, AF143730, and AF143727 respectively). The 12S rDNA sequences for *Caiman crocodilus, C. latirostris, M. niger, P. palpebrosus, P. trigonatus, A. sinensis, A. mississippiensis, Crocodylus rhombifer, G. gangeticus*, and *T. schlegelii* were from our previous publications (Gatesy and Amato, 1992; Gatesy et al., 1993) and were deposited into GenBank with new sequences from the present study (NCBI AY239124–AY239176).

Sequence Alignment

Multiple sequence alignment can be interpreted as the determination of primary homology as defined by DePinna (1991). Because DNA sequences are simple linear strings of four discrete nucleotides, primary homology can, in part, be derived algorithmically. In this study, we took the following approach to sequence alignment. We aligned each genic region with the parsimony based multiple alignment program MALIGN (Wheeler and Gladstein, 1994) over a wide range of alignment parameters. For each gene, we then chose the alignment that gave the shortest cladogram given a weight of 1 for each individual nucleotide insertion, deletion, or substitution (Gatesy and Arctander, 2000). Assuming that individual base pair insertions/deletions (indels) are interpreted as independent pieces of phylogenetic evidence, this alignment implied the fewest character transformations to explain the differences among orthologous sequences. This framework was the most consistent with the combined approach advocated by Kluge (1989) in which all character transformations are given equal weight (Kluge, 1997; Frost et al., 2001). In part, our procedure also was consistent with the rationalizations of Wheeler and colleagues (Wheeler and Gladstein, 1994; Wheeler, 1995, 1996; Giribet and Wheeler, 1999; Phillips et al., 2000). As for these authors, our goal was to find alignments that were simplest within the context of the character weighting scheme that was employed in phylogenetic analysis (in this case, equal weighting). However, instead of minimizing *scaled character incongruence among data partitions* (Wheeler, 1995), our procedure simply minimized the *number of character transformations* necessary to explain differences among orthologous sequences.

Because of the vagaries of pairwise multiple alignment, optimal alignments (those that return the shortest cladogram) for a gap weight of 1 and a nucleotide substitution weight of 1 may be derived from alignments at gap cost/substitution cost ratios other than 1. Therefore, we tested a variety of alignment parameters. Eleven gap: substitution cost ratios were tested for each of the mt rDNA data sets. The cost parameters were as follows: (gap cost = the cost for opening a gap, followed by extragap cost = the cost for extending an initial gap, followed by the cost for a nucleotide substitution) 1/1/1, 1.5/1.5/1, 2/2/1, 3/3/1, 4/4/1, 5/5/1, 1.5/1/1, 2/1/1, 3/2/1, 4/3/1, 5/4/1. Other MALIGN parameters were contig, score 4, treea, treeswap, atbr, tbr, build, keepaligns 10, keeptrees 10, time, iter, and phylotime. For the protein-coding genes, adjacent gaps were consolidated by eye using SeqApp (Gilbert, 1992), so that coding regions were not disrupted by frameshift mutations. Phylogenetic analyses were conducted for the alternative alignments.

Morphological Data

Morphological characters primarily were those analyzed by Brochu (1997). Subsequent to the publication of that paper, Brochu made adjustments to his original data set that were incorporated here: (1) ten extinct taxa were added to the matrix; (2) two characters, presence of pneumatic cavity in prefrontal pillar (165) and surangular truncated dorsally (166), were added; (3) two other characters, prominent ridge on palatine (99) and shape of lacrymal (106), exhibited more intraspecific variation than previously thought and were deleted; and (4) several character codings in *Tomistoma cairense* were corrected (see *Systematic Biology* web site). Stratigraphic occurrences of taxa were taken from Brochu (1997, 2001). The morphological data set utilized here includes taxa slightly different from those used by Harshman et al. (this issue), which affected some character state optimizations.

Combined Matrix

The morphological data set of 164 characters was merged with the sequence data in a combined matrix of 14 extant taxa (Aves was constrained to be monophyletic), 54 extinct taxa, and 3,104 characters. Morphological characters were included for all 68 taxa. Molecular data were included for each of the 14 extant taxa and were coded as missing in all 54 extinct taxa. (The matrix is available at the *Systematic Biology* web site: systbiol.org/info/issues.html.)

Some published data sets were excluded from the combined matrix for a variety of reasons. First, Densmore and White (1991) and Aggarwal et al. (1994) scored the presence and absence of restriction endonuclease fragments as alternative character states. Character correlation and misrepresentation are unavoidable in restriction fragment data that are not mapped, and there are no straightforward corrections for these effects (see Swofford and Olsen, 1990; Siddall, 2001). Second, Densmore (1983) presented allozyme matrices for alligatoroids and for crocodylines + *G. gangeticus* + *T. schlegelii*. Alleles were coded separately for each of these two presumed groups. Therefore, even though the same loci were examined for both "groups," the character codings were incompatible between matrices. Third, Densmore (1983), Densmore and Owen (1989), and Hass et al. (1992) explored phenetic analyses of immunological distances among extant crocodylians. Specific hypotheses of character homology (Patterson, 1982) cannot be inferred from these data (Brower et al., 1996). Fourth, Brooks and O'Grady (1989) summarized systematic evidence for helminth parasites of crocodylians and used these coevolutionary data to infer relationships within Crocodylia. In our study, *extrinsic* phylogenetic evidence from worms was not merged with the *intrinsic* morphological and molecular characters from crocodylians. Fifth, preliminary analyses of partial mt ND6, $tRNA^{glu}$, and cytb sequences were presented by Brochu and Densmore (2001) and White and Densmore (2001), but the sequence data were cited as White and Densmore (in review) and were not published before submittal of this manuscript.

Phylogenetic Analyses

Simultaneous parsimony analyses of primary homology statements were executed in PAUP* (Swofford, 1998). Searches of the combined 68-taxon matrix were heuristic with minimally 100 random taxon addition replicates and tree bisection–reconnection branch swapping. Characters were unordered, individual gaps in sequence alignments were treated as a fifth character state (Giribet and Wheeler, 1999), all character transformations were equally weighted (Kluge, 1997), and branches with a minimum length of 0 were collapsed ("amb-" option). All topologies were rooted on the Aves outgroup branch, and strict consensus trees (Schuh and Polhemus, 1980) were derived from optimal topologies found in each search.

Additional parsimony analyses of separate data partitions were executed to assess the phylogenetic impacts of morphological characters, nuDNA sequences, mtDNA sequences, outgroup taxa, fossils, and sequence gap characters. Searches were branch and bound (Hendy and Penny, 1982) or heuristic. Molecular data sets also were analyzed using likelihood methods to determine the stability of the parsimony results to an explicit model-based approach (Felsenstein, 1981). "Optimal" models of molecular evolution were chosen using likelihood ratio tests (Goldman, 1993; Whelan and Goldman, 1999) implemented in ModelTest (Posada and Crandall, 1998). Model parameters then were imported into PAUP,* and heuristic searches were executed.

For the combined crocodylian data set, there were thousands of optimal topologies, branches with a minimum length of 0 were collapsed, and many extinct taxa were distributed along stem lineages of crown taxa. Currently, there are no automated algorithms for determining whether a given character transformation is unequivocally optimized to a particular internal branch that connected the extant crocodylian taxa. Therefore, unequivocal character changes, hypothesized here, were derived from comparisons of different optimizations (e.g., Acctran and Deltran) on minimum length trees, character mapping on the strict consensus tree, and selected constrained parsimony searches. Characters were optimized onto cladograms using the "list of apomorphies" and "show reconstructions" commands of PAUP* (Fitch, 1971; Swofford, 1998).

Data Set Incongruence/Conflict

The Wilcoxon signed rank test (WSRT; Templeton, 1983; Larson, 1994) implemented in PAUP* was used as a heuristic to compare differential character support for alternative *a priori* molecular and morphological hypotheses (Fig. 1). In some analyses of the fossil data, thousands of equally parsimonious trees were recovered. In these instances, a single minimum-length topology was used in the signed rank test. Because of difficulties in interpreting WSRT results in such cases, no threshold for significance was specified, but very low P values were taken as an indication of conflicting character support for at least some of the optimal topologies.

The incongruence length difference (ILD) is the minimum number of extra character steps required from several systematics data sets when these partitions are analyzed simultaneously compared with the sum of character steps when the data sets are analyzed separately (Mickevich and Farris, 1981). Randomizations of the original data sets were used to estimate the extremity of empirical ILDs (Farris et al., 1994, 1995). Because of missing molecular data, extinct taxa were excluded. Only informative characters were considered, 999 randomizations were analyzed, and PAUP* tree searches were branch and bound. A P value of 0.05 was taken as the threshold for significant conflict.

Group Stability/Support

In cladistic analysis, competing phylogenetic hypotheses are judged according to their length; trees that

imply the fewest character transformations are preferred (Farris, 1983). Given this optimality criterion, the support for a given grouping of taxa has been equated with the difference in length between the shortest trees that lack the group of interest and the shortest trees that include the group of interest. Bremer (1994) defined this difference in character steps as branch support (BS). For BS, the criterion for group stability/support is the same as the criterion for tree choice. Here, BS and recent elaborations of this index (Baker and DeSalle, 1997; Gatesy et al., 1999; Wilkinson et al., 2000) were employed in novel combinations to measure the stability of relationships supported by the combined crocodylian data matrix.

This data set was characterized by extensive missing data. Some extinct taxa were scored for as few as 25 of the 835 informative characters, so our analysis of tree stability focused on relationships among extant taxa. To measure BS for relationships among living species within the context of the fossil data, double decay analyses (Wilkinson et al., 2000) were executed. Backbone constraint trees (Swofford, 1993) that defined relationships among extant taxa were used to estimate the minimum number of extra character steps required to disrupt these relationships. The constraint trees did not include any of the extinct taxa, but all extinct taxa were utilized in analysis. The phylogenetic placements of extinct taxa relative to the extant taxa were not fixed, so fossils were allowed to "float" in constrained tree searches (Wilkinson et al., 2000). Only the differential costs of contrasting relationships among extant taxa, irrespective of the positions of extinct taxa, were noted. These length differences, double decay BS, were calculated for all groupings of extant taxa supported by the 68-taxon data set. PAUP* searches were as described above.

Robustness/stability of systematics results to the addition of fossils was determined by comparing BS scores for groups supported by the matrix of 14 extant taxa with double decay BS for these relationships in the context of all 68 extant and extinct taxa. For a particular grouping of extant taxa, an increase in group stability with the addition of fossils is indicated when the difference between double decay BS with fossils and BS without fossils is positive. A decrease in stability is indicated when this difference is negative. A rearrangement of relationships with the addition of fossils is indicated when this difference is negative and equal/greater in absolute magnitude relative to the BS score without fossils.

Double decay BS scores were partitioned to measure the contributions of different data sets to nodal stability. For the 68-taxon matrix, partitioned branch support (PBS; Baker and DeSalle, 1997) for three data sets (mtDNA, nuDNA, and morphology) was calculated. PBS scores for relationships among the 14 extant taxa were determined within the context of all 68-taxa (double decay PBS). The double decay PBS procedure was analogous to standard PBS analysis but utilized backbone constraint trees as in double decay analysis (Wilkinson et al., 2000). Double decay PBS derived from searches of extinct plus extant taxa was compared with PBS derived from searches of extant taxa only to assess the influence of fossils on the distribution of character support among data sets.

These indices measure support in terms of extra character steps. Stability to the removal of character data was used as an alternative index of clade robustness. Character jackknife (JK) analyses (Penny and Hendy, 1986; Farris et al., 1996) were executed for several data sets. In each JK replicate, 50–90% of parsimony-informative characters in the original data set were deleted, and the PAUP* search of the perturbed matrix was branch and bound. One thousand JK replicates were executed, and JK percentages were calculated for all groups supported by the original data set. Because of extensive missing data in extinct taxa, JK analyses were done for extant taxa only. JK analyses within a likelihood framework were based on random deletions of 50% of all characters. For maximum likelihood analyses, 100 heuristic JK replicates were executed with taxon addition "as is" and tree bisection–reconnection branch swapping.

Hidden Stability/Support

The interaction of different data sets in simultaneous analysis often implies hidden character support (Barrett et al., 1991; Chippindale and Wiens, 1994; Olmstead and Sweere, 1994). For a particular set of data partitions and a particular group, hidden support can be defined as increased character support for the group of interest in the simultaneous analysis of all data partitions relative to the sum of support for that group in the separate analyses of each partition. Hidden support in different data sets can be quantified with a variation of BS, partitioned hidden branch support (PHBS; Gatesy et al., 1999).

PHBS was calculated for relationships among extant taxa supported by the combined matrix to quantify secondary phylogenetic signals in the mtDNA, nuDNA, and morphological character sets. For a particular relationship, PHBS is a measure of the difference between character support from an individual data set within the context of the combined evidence analysis (PBS) and character support in the separate analysis of that individual data set (BS). A positive PHBS score for a particular data set indicates a secondary phylogenetic signal for the relationship of interest that emerges in simultaneous analysis of diverse data sets (Gatesy et al., 1999). PHBS for relationships among extant taxa was measured in the context of all 68 taxa using backbone constraint trees for the extant species as in the double decay analyses (double decay PHBS) and without extinct taxa (PHBS) to assess the influence of fossils on hidden support.

RESULTS AND DISCUSSION

Sequence Alignments

Optimal alignments, given an equal weighting of all character transformations, were found at the following parameters (gap cost/extragap cost/substitution cost). For mt 12S rDNA sequences, 1.5/1.5/1 produced a cladogram of 290 steps. For mt 16S rDNA sequences, 2/1/1 yielded a cladogram of 512 steps. With an equal

weighting of all character transformations, alignments at the high end of the gap-cost range predictably implied much longer cladograms for the rDNA data sets. The protein coding mt cyb and nu RAG-1 showed little alignment ambiguity. The cytb alignment had no internal gaps, and the RAG-1 alignment had a single 3-base pair gap in the *G. gallus* outgroup sequence.

Incongruence Among Morphological and Molecular Data Sets

Mitochondrial DNA and nuDNA were partitioned to measure differences in character support provided by these two linkage groups (Miyamoto and Fitch, 1995). The mt and nu data implied divergent base compositions, transition: transversion ratios, and branch lengths (Figs. 2a, 2b). Despite these profound differences in rate and mode of sequence evolution, mtDNA and nuDNA sequences each favored the same basic scheme of relationships (ILD = 0 extra steps). Both data sets supported a *G. gangeticus* + *T. schlegelii* group and a *G. gangeticus* + *T. schlegelii* + Crocodylinae clade, congruent with previous molecular hypotheses (Fig. 1b). The only difference between systematic results for nu and mt loci was a lack of resolution among three crocodyline taxa (*Crocodylus intermedius* + *C. rhombifer*, *C. cataphractus*, and *Osteolaemus tetraspis*) in the nuDNA analysis (Fig. 2b).

The mtDNA data set of 418 informative sites required 58 extra character steps to accommodate the traditional morphological tree (WSRT, $P < 0.0001$), and 13 additional character steps were necessary to fit the nuDNA data set of 257 informative sites to this topology ($P < 0.001$). Unrooted analyses of the DNA data sets, where the bird sequences were removed, overwhelmingly supported ((Alligatoroidea + Crocodylinae) (*G. gangeticus* + *T. schlegelii*)); no rooting of this network was consistent

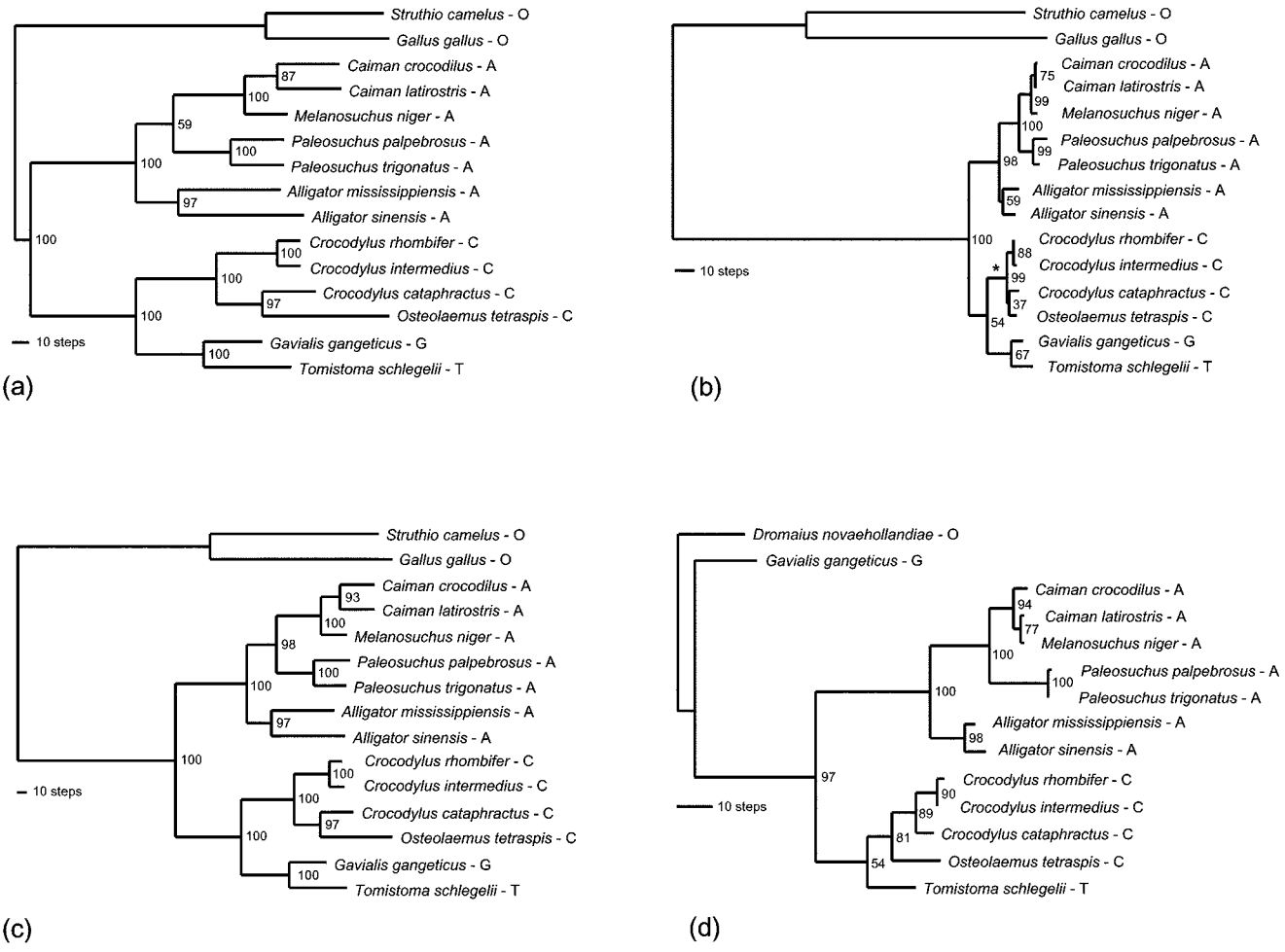

FIGURE 2. Minimum-length topologies for extant taxa that were supported by different data sets. (a) Mitochondrial DNA. (b) One of two optimal cladograms for nuDNA. (c) Combined DNA. (d) Gross anatomical characters. The 50% character removal JK percentages are at internal nodes. BS scores for trees a and b are in Figure 4, and BS for tree d is in Figure 3b. The asterisk in tree b marks the alternative position of *Crocodylus cataphractus* in the second minimum-length topology for the nuDNA data set. Maximum likelihood analyses of the mtDNA and combined DNA data sets produced topologies that were identical to the cladograms shown (a and c). For the nuDNA, maximum likelihood rooted the ingroup topology on the branch that joins *Tomistoma* and *Gavialis*. Branch lengths are proportional to the number of character steps inferred by parsimony (Acctran optimization). Higher level groups are as in Figure 1.

with the traditional hypothesis of relationships (Fig. 1a). Thus, removal of distant outgroup taxa did not reconcile the DNA data sets with the morphological topology (WSRT for mtDNA, $P < 0.0006$; for nuDNA, $P < 0.005$).

Separate analysis of the morphological data set favored the traditional scheme of relationships (Fig. 1a). A *T. schlegelii* + Crocodylinae clade (double decay BS = +15) and a *T. schlegelii* + Crocodylinae + Alligatoroidea group (double decay BS = +8) were robustly supported (Fig. 3b) and inconsistent with the molecular perspective (Fig. 1b). This topology was obtained whether fossils were excluded or included (Figs. 2d, 3b). The complete morphological data set of 160 informative characters required 19 extra character steps to fit the molecular hypothesis (WSRT, $P < 0.02$), and for the extant taxa, congruence between morphological and molecular data partitions was rejected (ILD = 17 extra steps, $P = 0.001$; $P = 0.009$ excluding outgroup taxa). When fossils were included, the ILD for molecules versus morphology increased to 24 extra character steps.

High JK support scores for incompatible groups also suggested sharp discrepancies between morphological and molecular partitions (Figs. 2c, 2d). The combined DNA data set strongly supported *G. gangeticus* + *T. schlegelii* and *G. gangeticus* + *T. schlegelii* + Crocodylinae (JK with 50% character removal = 100%), but both of these clades were contradicted by the *T. schlegelii* + Crocodylinae + Alligatoroidea group favored by the morphological analysis (JK = 97%). Furthermore, *Crocodylus cataphractus* + *Osteolaemus tetraspis* (JK = 97%) was supported solidly by the molecular characters, but this clade was inconsistent with a monophyletic *Crocodylus* (JK = 89%) in the morphological tree (Figs. 2c, 2d).

In summary, topologies derived from the unlinked mtDNA and nuDNA data sets were congruent, but morphological and molecular data sets showed extensive character conflicts and robustly supported contradictory trees. Incongruence between molecular and morphological character sets was not limited to the position of the root; exclusion of distant outgroup taxa did not rectify the character conflict between data sets.

Simultaneous Analysis

Extant and extinct taxa.—The combined parsimony analysis of molecules and morphology for all 68 taxa yielded 2,592 optimal trees (minimum length = 2,262); when sequence gaps were treated as missing data, the same set of topologies was recovered. The strict consensus of these trees (Fig. 3a) was well resolved and consistent with previous molecular results (Fig. 1b). The stability of relationships among basal crocodylian taxa was assessed by determining double decay BS for *G. gangeticus* + *T. schlegelii* and for *G. gangeticus* + *T. schlegelii* + Crocodylinae to the exclusion of other extant taxa in the analysis. Double decay BS scores were high for these relationships, +18 and +39 extra steps, respectively. Seven traditionally recognized groupings of extant taxa (Jacarea, *Paleosuchus*, Caimanae, *Alligator*, Alligatoroidea, Crocodylinae, and New World *Crocodylus*) also were solidly supported, with double decay BS scores ranging from +16 to +52 (Figs. 3a, 4). Some extinct taxa that grouped within Crocodylia in the analysis of morphological data (*Borealosuchus* spp. and *Pristichampsus*) were placed as the closest outgroups to Crocodylia in the simultaneous analysis of molecules and morphology. Thus, according to the combined evidence, the taxonomic content of the crown group was more restricted than in trees based solely on morphological data. Overall, 11 clades emerged in simultaneous analysis that were not supported by separate analysis of the morphological data (Fig. 3).

The traditional hypothesis of crocodylian relationships (Fig. 1a) was incompatible with the combined evidence. Constraining the combined matrix to fit this topology demanded 61 additional character steps relative to the minimum length (WSRT, $P < 0.0001$), and the gross anatomical data set required 24 extra character steps to fit the combined evidence hypothesis (WSRT, $P < 0.019$). In spite of this conflict, the overall agreement among data sets was overwhelming in the simultaneous analysis. Double decay PBS scores were positive for the nuDNA, mtDNA, and morphological characters for 8 of the 11 groupings of extant taxa, and both nuDNA and mtDNA provided positive support at all 11 nodes (Fig. 4). The position of the outgroup root was supported by all three character sets; double decay PBS scores were uniformly positive for Alligatoroidea and for *G. gangeticus* + *T. schlegelii* + Crocodylinae, the two basalmost clades of Crocodylia. Double decay PBS was negative for the morphological partition at only two nodes, *Crocodylus cataphractus* + *Osteolaemus* (−3) and *T. schlegelii* + *G. gangeticus* (−9).

As in other studies where diverse systematic data sets for Crocodylia were compared and integrated, widespread agreement between morphology and molecules was the rule, with only a few conflicts at particular nodes. Previous simultaneous analyses of Crocodylia yielded mixed results; the support for competing hypotheses (Fig. 1) shifted with changes in the relative numbers of molecular and morphological characters (Poe, 1996; Brochu, 1997; Brochu and Densmore, 2001). Brochu (1997:501–502) noted that "one would expect the addition of a large sequence data set to tip the balance in favor of the molecular tree." This was exactly the case here. Nearly 3,000 aligned nucleotides were analyzed in this study as opposed to the ~250 bases of Brochu (1997), and in contrast to the results of Brochu (1997), our minimum length trees conformed to previous molecular hypotheses of crocodylian phylogeny (Fig. 1b).

Influence of fossils.—Certain fossils are expected to preserve ancestral morphologies that have been radically altered in extant taxa and might allow more precise hypotheses of homology in divergent anatomical systems. By including fossils, more characters and taxa (especially primitive taxa with unique combinations of morphological character states) can be utilized in phylogenetic analysis (Gauthier et al., 1988; Donoghue et al., 1989; Novacek, 1992). In some empirical studies, inclusion of extinct taxa

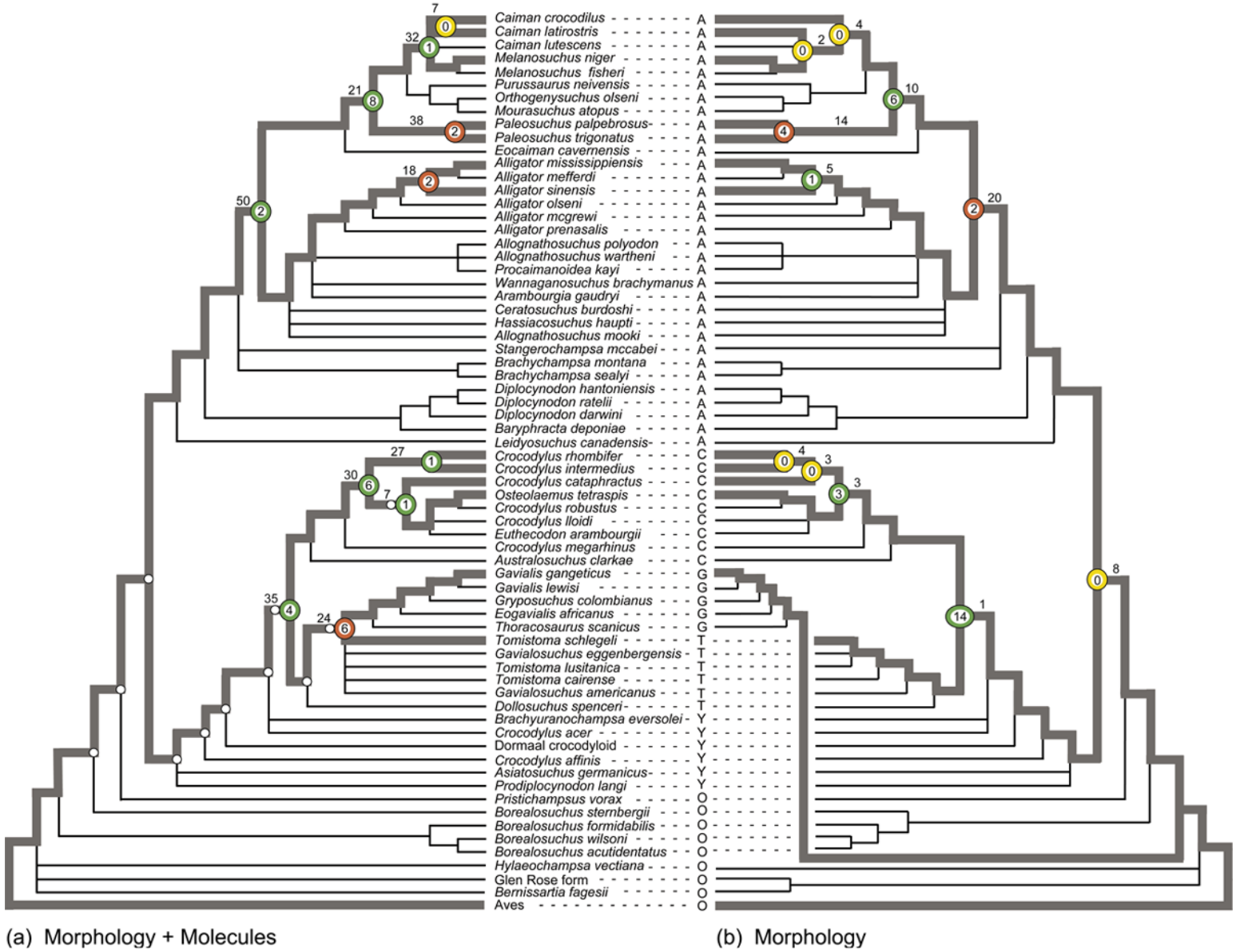

FIGURE 3. Strict consensus of optimal topologies for extinct and extant taxa: morphology plus molecules (a) and morphology (b). Minimum tree lengths were 2,262 steps for the combined evidence matrix and 491 steps for the morphological data. Thick branches connect the 14 extant taxa. BS scores from analyses of extant taxa only are above internal branches. The effects of fossils on BS are indicated at nodes within colored circles. Increases (green), decreases (red), and lack of change (yellow) in double decay BS relative to BS are shown. Minimally 100 random taxon addition replicates were utilized in each constrained heuristic search, but given the complexity of the combined matrix and the potential for multiple islands of equally parsimonious trees (Maddison, 1991), these BS scores could be lower than indicated. Small white circles at internal nodes mark clades in the combined analysis tree that strictly conflict with the morphological tree. A = Alligatoroidea; C = Crocodylinae; G = Gavialinae; T = species recognized as tomistomines in the morphology tree; Y = other crocodylians; O = outgroups to Crocodylia in the combined analysis. Branch lengths are not proportional to the numbers of character steps.

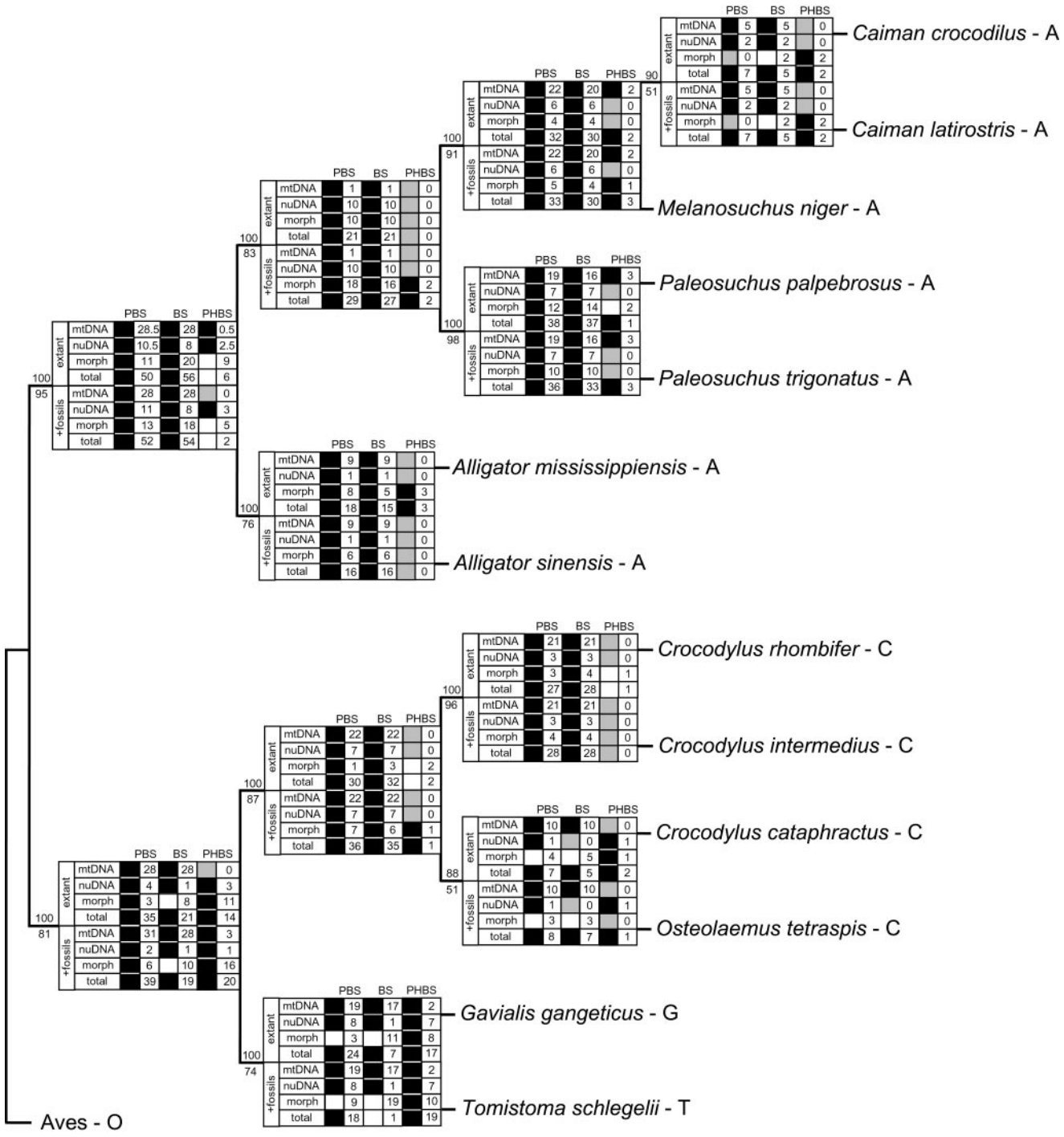

FIGURE 4. The distribution of character support among data sets. At each node, the following information is listed for analyses of extant taxa and extant plus extinct taxa (+ fossils). PBS = PBS for three data sets, mtDNA, nuDNA, and morphology (morph), in simultaneous analysis of extant taxa and double decay PBS for these partitions in simultaneous analysis of extant plus extinct taxa; BS = BS in the separate analyses of individual data partitions for the extant taxa and double decay BS in the separate analyses of individual data partitions for extant plus extinct taxa; PHBS = PHBS for each data set in the simultaneous analysis of extant taxa and double decay PHBS in the simultaneous analysis of extant plus extinct taxa; total = the sum of the PBS, BS, or PHBS scores with or without extinct taxa. Solid, open, and shaded boxes to the left of the values indicate positive scores, negative scores, and scores of 0, respectively. The 50% character removal JK percentages for the combined matrix of extant taxa are above internodes, and the 90% character removal JK percentages are below internodes. Higher level groups are indicated as in Figure 1.

has resulted in rearrangment of phylogenetic relationships supported by analyses of extant taxa alone (e.g., O'Leary and Geisler, 1999). However, the importance of fossils in simultaneous analyses of morphological and molecular characters has been examined in only a few test cases (e.g., Eernisse and Kluge, 1993; Shaffer et al., 1997; Horovitz, 1999; O'Leary, 1999; Giribet et al., 2001).

For the crocodylian matrix, inclusion of extinct taxa did not overturn any relationships supported by the analysis of extant taxa but did alter the character support for most groups. Simultaneous parsimony analysis of extant taxa yielded a single minimum-length topology that was robustly supported and perfectly congruent with the combined evidence analysis of extinct plus extant taxa (Figs. 3a, 4). For 9 of 11 nodes, 50% character removal JK scores were 100%, 90% character removal JK scores were >70%, and BS was high (+18 to +50; Fig. 4). With the inclusion of fossils, stability (in terms of extra character steps) increased at seven nodes (range = +1 to +8), decreased at only three nodes (range = −2 to −6), and was unchanged at one node. The extinct taxa generally solidified systematic relationships among extant taxa and effected a net increase of +13 extra steps in nodal stability (Fig. 3a).

With fossils, BS for the controversial grouping of extant crocodylines, *G. gangeticus*, and *T. schlegelii* jumped from +35 to +39. In part, this additional character support was due to the inclusion of extinct outgroups. These fossil crocodyliform taxa often could be scored for traits that were highly derived in distant extant outgroups; 42% of the morphological characters were coded as inapplicable in the exemplar of Aves coded by Brochu (1997). In many cases, the unique information from fossils established or overturned the polarity of character transformations at the base of Crocodylia. For example, character 78, occlusal pattern of dentary teeth, is inapplicable in modern birds that lack teeth. When only extant taxa were considered, it was not clear whether occlusion lingual to maxillary teeth (most alligatoroids) or occlusion in line with maxillary teeth (crocodylines, *T. schlegelii*, and *G. gangeticus*) was primitive. When close outgroups, such as *Hylaeochampsa* and *Bernissartia,* were included, parsimony reconstructions showed that alligatoroids expressed the primitive state. In the context of the fossil evidence, there were three distinct states for character 78, and two transformations in the character were unequivocally optimized to the internode that joined extant crocodylines, *T. schlegelii*, and *G. gangeticus*

For the combined matrix of extant and extinct taxa, numerous morphological synapomorphies were assigned to this internode (e.g., characters 3, 12, 13, 41, 43, 78, 89, 103, 110, 119, 120, 122, 127, 145, 154, 159, and 162). Several of these characters (13, 41, 120, 122, 127, 154, and 162) are shared by crocodylines and tomistomines but not gavialines. When only extant taxa were considered, these traits were equivocally optimized and could be interpreted in two equally parsimonious ways: (1) as parallel gains in Crocodylinae and *T. schlegelii* or (2) as a gain in the common ancestor of Crocodylinae + *T. schlegelii* + *G. gangeticus* with a subsequent loss in *G. gangeticus* (Hass et al., 1992). The placement of key fossils along the stem lineage of Crocodylinae + *T. schlegelii* + *G. gangeticus* made optimizations of many morphological traits unequivocal and consistent with the second interpretation (Fig. 5). Thus, within the combined evidence framework, some of the strong morphological support for Crocodylinae + *T. schlegelii* (double decay BS = +15) was reinterpreted as unequivocal, albeit homoplastic, synapomorphies for a more inclusive grouping of Crocodylinae + *T. schlegelii* + *G. gangeticus*.

The greatest decrease of character support with the addition of fossils occurred at the *G. gangeticus* + *T. schlegelii* node, a drop in BS of six character steps. For this group, the conflict from the gross anatomical partition was more pronounced with fossils (double decay PBS = −9) than without fossils (PBS = −3; Fig. 4). A more complete sampling of taxa uncovered additional homoplasy, revealed uncertainties in character optimizations, and ultimately overturned hypotheses of homology that were based solely on the extant biota. Early representatives of Crocodylia and fossil outgroups had a strong impact on characters 22, 36, 80, and 88 (Fig. 6). For example, among extant crocodylians, only *T. schlegelii* and *G. gangeticus* have rectangular dorsal midline osteoderms, as opposed to the square/equant osteoderms of other taxa (character 36). The acquisition of rectangular osteoderms was an unequivocally optimized synapomorphy for *T. schlegelii* + *G. gangeticus* in the analysis of extant taxa, but fossils showed that this state was very broadly distributed among early alligatoroids, a basal crocodyline, and close outgroups to Crocodylia (Fig. 6). The combined evidence topology implied that square/equant osteoderms instead were independently derived from rectangular osteoderms within Alligatoroidea and Crocodylinae.

Improved sampling of Gavialinae and Tomistominae, taxa with single extant representatives, also influenced nodal stability. For example, among living crocodylians, the superior edge of the coronoid slopes strongly anteriorly in jacarean alligatoroids, *T. schlegelii*, and *G. gangeticus* (character 54). In the analysis of extant taxa, this condition provided unequivocal support for Jacarea (*Caiman* + *Melanosuchus*) and for *T. schlegelii* + *G. gangeticus*, but a fossil gavialine, *Gryposuchus colombianus*, and an extinct tomistomine, *Gavialosuchus americanus*, express the alternative state, superior edge of coronoid almost horizontal (Brochu, 1997). With these additional observations, the optimization of character 54 was rendered equivocal at the internode that joined *T. schlegelii* and *G. gangeticus* (Fig. 6).

The fossil taxa had diverse effects. Overall, 10 morphological traits were interpreted as unequivocal synapomorphies for *G. gangeticus* + *T. schlegelii* in the analysis of extant taxa. Six of these characters (22, 36, 45, 54, 80, and 145) did not unambiguously support this group when fossils were included, but three novel morphological synapomorphies (43, 88, and 95) did emerge in the simultaneous analysis of extant plus extinct taxa. These reinterpretations of character evolution reduced BS for *G. gangeticus* + *T. schlegelii* (Figs. 4, 6) and again showed that assessments of phylogenetic signal, in the

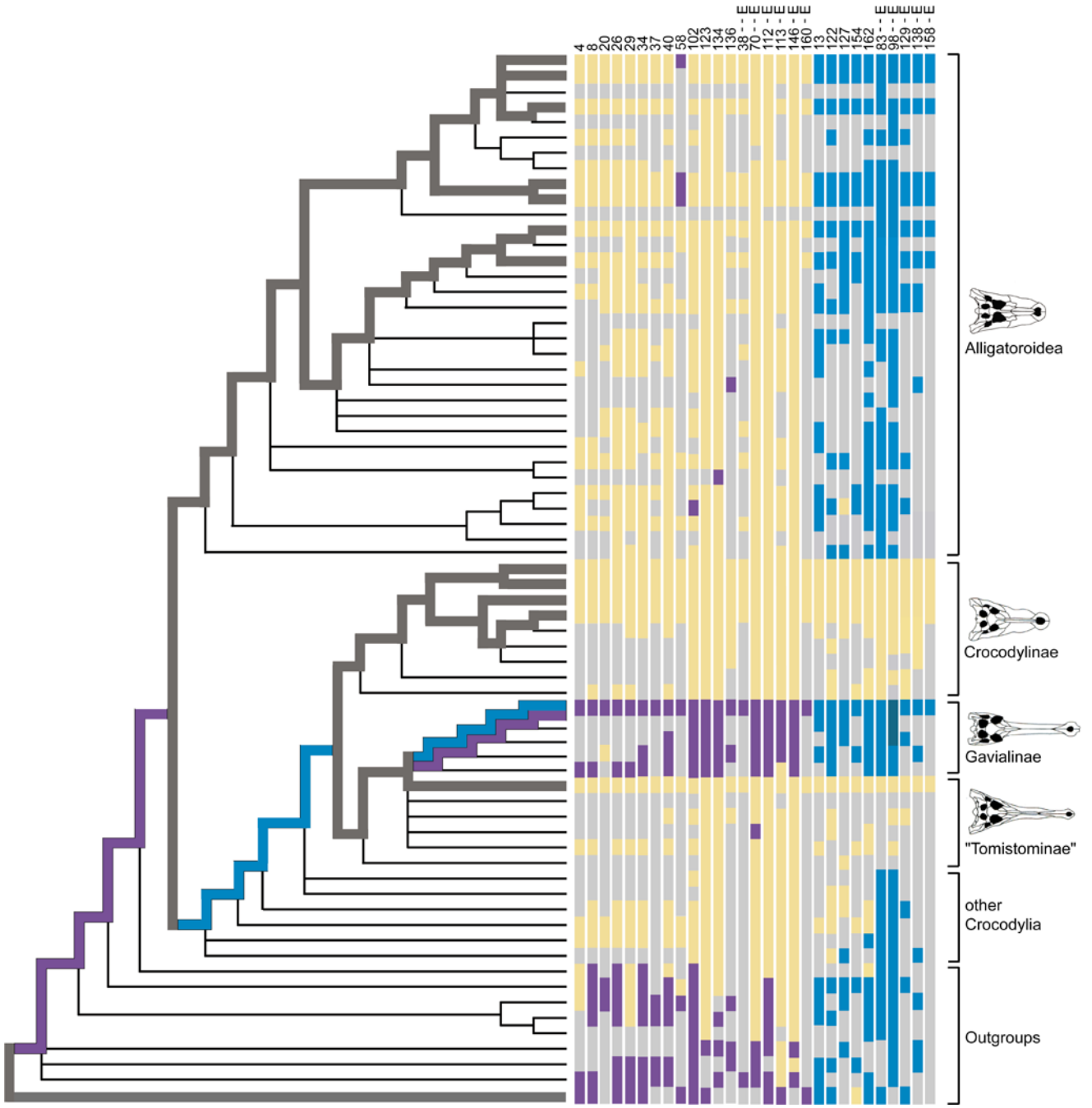

FIGURE 5. Potential taxic atavisms in gavialines mapped onto the strict consensus tree for the combined matrix. The phylogenetic distributions of 29 characters are shown. Numbering of characters at the top is from Brochu (1997). E = characters that may be reversals to primitive states in gavialines, but character optimizations were equivocal. Character states expressed in *Gavialis gangeticus* that can be interpreted as reversions to states observed in outgroup taxa are colored purple. Alternative character states are tan, and missing data are light gray. In parsimony reconstructions, these traits changed on the stem lineage of Crocodylia and reversed back to the primitive condition in gavialines (purple branches). Character states expressed in *G. gangeticus* that can be interpreted as reversions to states in hypothetical ancestors along the stem lineage of Gavialinae + Tomistominae + Crocodylinae are colored blue. Alternative character states are tan, and missing data are light gray. In parsimony reconstructions, these traits changed on the stem lineage of Gavialinae + Tomistominae + Crocodylinae and reversed back to the primitive condition in gavialines (blue branches). Dark blue = character state in *G. gangeticus* that was interpreted as a derivative of the state expressed in *Tomistoma schlegelii* (lighter blue) in some optimizations. For each of the 29 taxic atavisms, the character state expressed in *G. gangeticus* was gained and/or lost at most three times according to parsimony reconstructions. Seven other characters were consistent with taxic atavism but implied more complicated patterns of evolution and are not shown (characters 25, 28, 35, 41, 51, 120, and 128). Group membership is as in Figure 3.

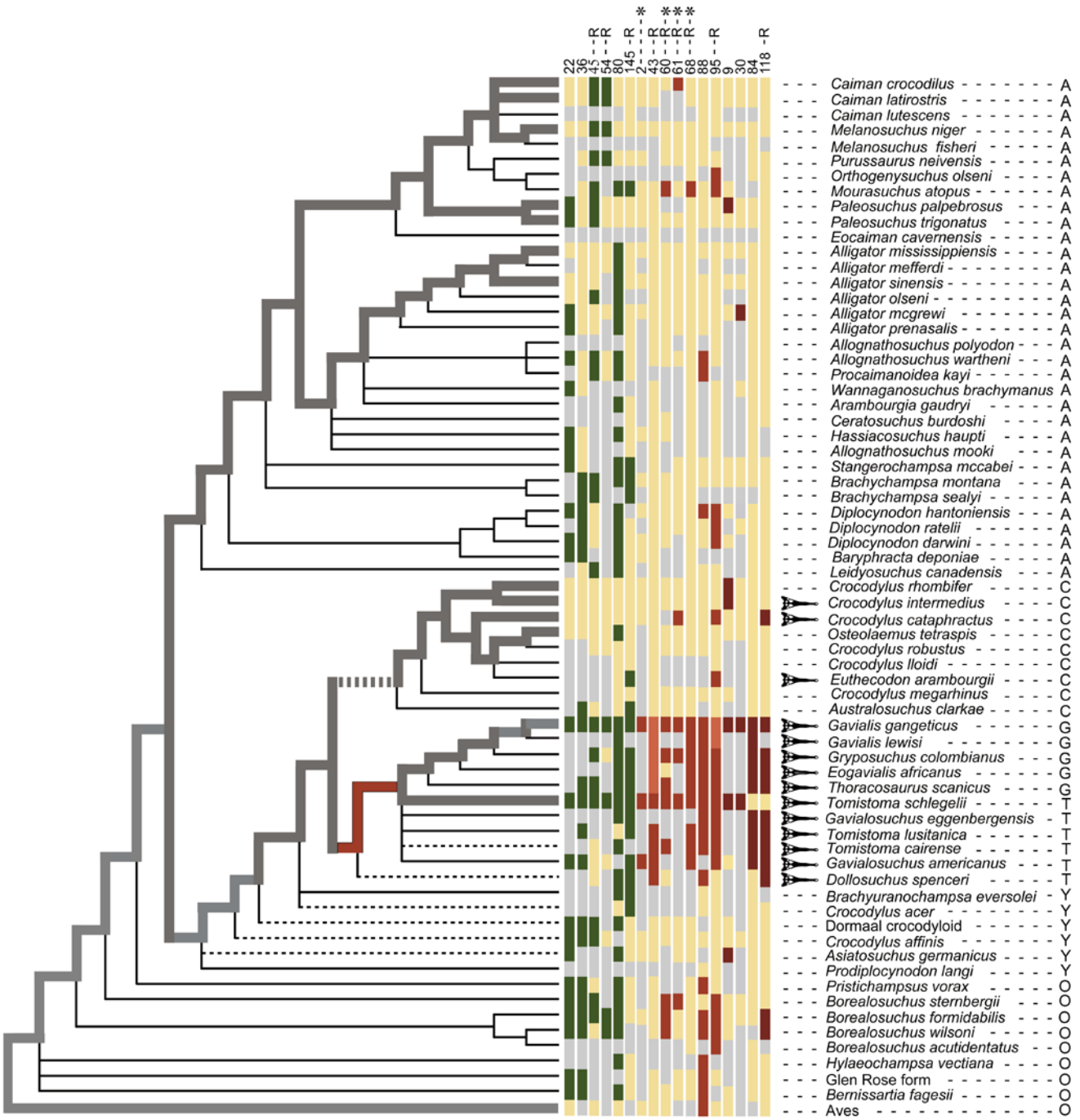

FIGURE 6. Morphological character support for Tomistominae + Gavialinae in simultaneous analysis. The strict consensus of optimal topologies for the combined matrix is shown with the phylogenetic distributions of 17 characters. Numbering of characters is from Brochu (1997). States commonly expressed in tomistomines and gavialines are colored dark green, dark red, light red, or dark rust, alternative states are tan, and missing data are light gray. Dark green = characters that provided unequivocally optimized synapomorphies for a grouping of *Tomistoma schlegelii* and *Gavialis gangeticus* in the simultaneous analysis of extant taxa but did not unequivocally support this relationship in the combined analysis of extant plus extinct taxa. Dark red = characters that provided unequivocally optimized synapomorphies for *T. schlegelii* + *G. gangeticus* in the combined evidence analysis with fossils. Light red = character states in *G. gangeticus* that were interpreted most simply as derivatives of states expressed in *T. schlegelii* for the combined analysis with fossils. Dark rust = some of the characters that provided equivocally optimized synapomorphies for *T. schlegelii* + *G. gangeticus* in the combined analysis with fossils. Asterisks mark characters that provided unequivocally optimized character support for a grouping of *T. schlegelii* and *G. gangeticus* in combined analysis whether fossils were considered or not. R = rostral/jaw character. Silhouettes to the left of species names designate taxa characterized as slender snouted by Brochu (2001). Dashed branches in the tree show the minimum number of fossil range extensions from the Eocene to the Cretaceous implied by the apical position of Gavialinae in the combined evidence topology (Brochu, 2003). Higher level groupings of taxa are abbreviated as in Figure 3 and are shown to the right of species names.

absence of extinct diversity, can be spurious (Gauthier et al., 1988).

Hidden phylogenetic signals.—Trueman (1998) showed that there was a secondary phylogenetic signal in the morphological data set of Brochu (1997) for the molecular hypothesis of crocodylian phylogeny (Fig. 1b). Specifically, reverse successive weighting of the morphological data set for extant ingroup taxa produced the molecular result, a grouping of *G. gangeticus* with *T. schlegelii*. In reverse successive weighting, characters that show no homoplasy on minimum-length trees for a data set are dismissed. The remaining characters are then reanalyzed to determine how the deletion of perfectly congruent data influences systematics results. This procedure is iterated until no cliques of congruent characters remain. For the crocodylian morphology matrix, the molecular hypothesis was supported after the first and second rounds of reverse successive weighting (Trueman, 1998). Trueman (1998:736) noted that because the secondary morphological signal replicated the molecular signal, "it is tempting to conclude that it is this signal and not the first which represents the true phylogeny."

In a subsequent study, this intriguing result was not stable to different taxon sampling schemes (Brochu, 1999). In particular, reverse successive weighting of the complete morphological data set of 45 extant plus extinct ingroup taxa did not reveal the molecular signal. Based on contrasting results for different subsets of extinct taxa, Brochu (1999:812) tentatively suggested that the secondary morphological signal for the molecular hypothesis might be "a result of convergent evolution for a suite of characters pertaining to snout morphology."

The inability to detect a secondary signal in some of these analyses could have been due to absence of the secondary signal, to deficiencies in the reverse successive weighting procedure, or to both of these factors. We therefore utilized an alternative index of secondary phylogenetic signal, PHBS (Gatesy et al., 1999), which unlike reverse successive weighting is *not* based on taxonomic congruence, character cliques, and data removal. We tested whether there was hidden character support (Barrett et al., 1991) in the morphological and molecular data sets for relationships supported by simultaneous analysis of these data sets and whether this cryptic support was influenced by the inclusion of extinct taxa (also see Harshman et al., this issue).

PHBS scores showed that there was extensive hidden support in the morphological data set at internodes that contradicted the morphological hypothesis (Fig. 3b). For the analysis of extant taxa, PHBS from the gross anatomical evidence was positive for *Caiman* monophyly (+2), *Crocodylus cataphractus* + *Osteolaemus* (+1), *G. gangeticus* + *T. schlegelii* (+8), and *G. gangeticus* + *T. schlegelii* + Crocodylinae (+11). Even stronger secondary signals were detected in the combined analysis of extant plus extinct taxa (Fig. 4). With the inclusion of fossils, PHBS at the *G. gangeticus* + *T. schlegelii* node increased by +2, and additional morphological character support also emerged at the *G. gangeticus* + *T. schlegelii* + Crocodylinae node (double decay PHBS = +16). BS for this molecular group was actually higher in the simultaneous analysis of morphology and molecules (double decay BS = +39) than in an analysis of molecular data alone (BS = +32). PHBS scores indicated that there was extensive hidden support in the morphological data set for the total evidence tree in terms of extra character steps (total double decay PHBS = +27; Fig. 4).

The unlinked DNA sequence data sets also contained hidden phylogenetic signals. Molecular double decay PHBS was positive at 6 nodes in the combined evidence topology (total of +22 character steps with fossils, +21 without fossils) and was most abundant for the grouping of *G. gangeticus* and *T. schlegelii* (double decay PHBS = +9). The nuDNA and mtDNA data sets supported *G. gangeticus* + *T. schlegelii* in separate analyses, but because homoplasy in these character partitions and in the morphological data set conflicted, molecular support for *G. gangeticus* + *T. schlegelii* increased in simultaneous analysis of all three data sets (Fig. 4). Hidden molecular support also emerged for *G. gangeticus* + *T. schlegelii* + Crocodylinae (double decay PHBS = +4) and for Alligatoroidea (+3), the two basalmost groups of Crocodylia that delimited the placement of the long outgroup root.

Evolution of the Longirostrine Narrow-Snouted Condition

Overall, double decay PHBS was positive at 8 of 11 nodes that defined groupings among extant taxa, was negative at only 1 node, and accounted for 68% of the double decay BS for relationships among the four major clades of Crocodylia (Fig. 4). Perhaps the most striking aspect of this hidden support was the combined evidence for *G. gangeticus* + *T. schlegelii*. The sum of BS scores for this relationship in separate analyses of the nuDNA, mtDNA, and morphological characters was negative (−1), but in the combined analysis of extant plus extinct taxa, double decay BS for this relationship was high (+18; Fig. 4). Within the context of all relevant characters, seven unequivocally optimized morphological synapomorphies were assigned to the internode that joined *G. gangeticus* + *T. schlegelii* (Fig. 6). Five of seven hidden synapomorphies were characteristics of the rostrum/jaws (Fig. 7), and several involve different aspects of the narrow-snouted condition. Given our minimum length trees, characters such as a "V"-shaped splenial with a deep symphysis (character 43), a surangular spur that borders the dentary toothrow (61), a linear dentary (68), and nasals excluded from the external naris (95) should not be interpreted as adaptive convergences in gavialines and tomistomines but instead constitute further support for a grouping of these taxa (Figs. 6, 7).

Some of the rostral traits have evolved independently in other narrow-snouted crocodyliformes (Langston, 1973; Clark, 1994; Brochu, 2001) and may be functionally correlated adaptations for a particular foraging mode (Langston, 1965; Hecht and Malone, 1972; Buffetaut, 1985). Although *T. schlegelii* has been observed feeding on large terrestrial mammals (Galdikas and Yeager, 1984; Galdikas, 1985), a narrow, long rostrum generally

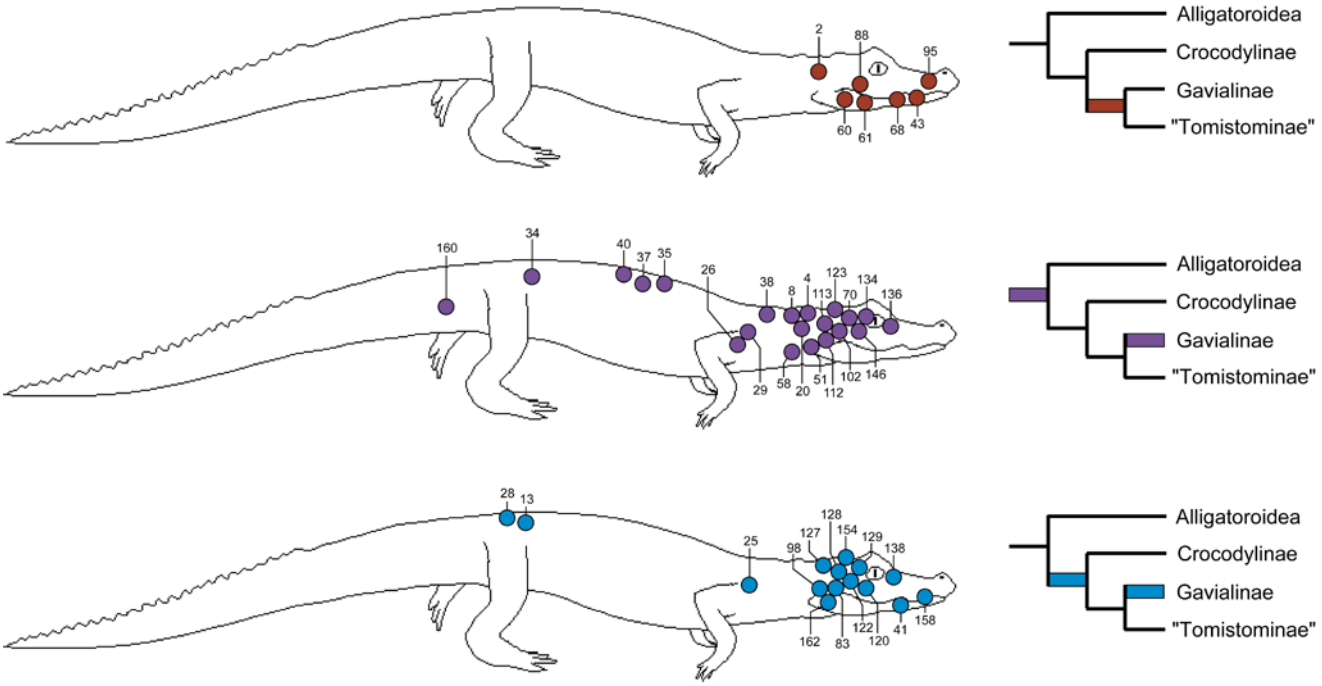

FIGURE 7. The anatomical distributions of characters that unequivocally supported a grouping of Gavialinae and Tomistominae (red circles) and traits that were consistent with widespread taxic atavism in Gavialinae (purple and blue circles). Character numbers are from Brochu (1997). Colored branches indicate the placements of character transformations on the combined evidence topology that included fossils (see Figs. 5, 6).

is thought to facilitate the capture and manipulation of agile aquatic prey (Gans, 1969; Iordansky, 1973). Taylor (1987:175–176) outlined the possible advantages of this morphology:

> The shape of the head determines the drag exerted by the surrounding water during the sideways sweep. The head should therefore be slender, and particularly shallow in depth, so that the snout presents minimum cross-sectional area to the oncoming water. . . . Increase of the length of the snout is beneficial, increasing the speed and magnitude of the movement of the terminal portion of the snout for a given angular excursion of the neck. . . . The narrow snout also minimizes the tendency for the closing jaws to expel water from the mouth and so to push prey objects away.

Recently, several authors have argued that such adaptive morphological complexes can offer corrupted phylogenetic signals and should be eliminated from phylogenetic analysis (Hedges and Maxson, 1996; Givnish and Sytsma, 1998; Luckett and Hong, 1998; Naylor and Adams, 2001; O'Keefe and Wagner, 2001). McCracken et al. (1999:707) stressed that "characters that play an important role in foraging ecology may be particularly troublesome." Our combined analysis of extant plus extinct taxa instead showed that a suite of such traits from the snout region was highly consistent with independent molecular evidence from the mt and nu genomes (Fig. 6); very few characters from other anatomical regions unequivocally supported a close relationship between Gavialinae and Tomistominae in simultaneous analysis (Fig. 7). Because widespread convergent evolution was suspected, snout characters have been excluded or downweighted in some previous analyses of crocodylian phylogeny (see discussion in Langston, 1973; Clark, 1994). For the *G. gangeticus* + *T. schlegelii* node, however, these characters provided most of the relevant morphological signal.

Dollosuchus spenceri, the basalmost tomistomine in the morphological analysis (Fig. 3b), was placed as the sister taxon to all other "tomistomines" plus Gavialinae in the combined evidence analysis (Fig. 3a). *Dollosuchus* lacks at least two of the five snout characters that supported a grouping of *G. gangeticus* with *T. schlegelii*, which in part explains the positioning of *Dollosuchus* in the minimum length topologies (Fig. 6). Because "Tomistominae," as delimited by the morphological analysis, was paraphyletic in the combined tree, parsimony reconstructions implied that gavial-specific states for some characters were derivations of ancestral "tomistomine" states (Buffetaut, 1985). For example, character 95 describes the anterior extent of the nasal bones. For "tomistomines" and primitive gavials in the analysis, the nasals do not contact the external naris, but in more derived gavials, including *G. gangeticus*, the nasals do not even touch the premaxillae (see Fig. 1; Brochu, 1997). When fossils were excluded from analysis, the different states in *G. gangeticus* and *T. schlegelii* were each equivocally optimized as uninformative autapomorphies, but in the combined evidence topology with extinct taxa considered the state in *G. gangeticus* was most simply interpreted as an accentuation of the primitive "tomistomine" condition (Fig. 6). Unfortunately, given a lack of phylogenetic resolution, hypotheses of character evolution within the Gavialinae + Tomistominae clade generally

were ambiguous. For example, a thin wedge-shaped palatine process (character 118) was equivocally optimized but characterized most "tomistomines" and gavialines (Fig. 6). Improved character and taxon sampling will be required to assign extinct species, such as *Gavialosuchus eggenbergensis*, to Gavialinae or Tomistominae and to clarify the evolutionary history of the longirostrine snout.

The phylogenetic distribution of character states did show that rostral characters grouping *G. gangeticus* with *T. schlegelii* were not strictly correlated with the slender-jawed condition or with each other. Some of these traits (60, 61, 68, and 95) also are expressed in duck-billed forms, such as *Mourasuchus atopus,* and in taxa with more generalized snouts, such as *Caiman crocodilus* (Fig. 6). Furthermore, parsimony optimizations on the combined evidence tree suggested that narrow-snouted crocodylines, such as *Crocodylus cataphractus*, *Euthecodon arambourgii*, and *Crocodylus intermedius*, convergently evolved only subsets of the characters shared by tomistomines and gavialines; *C. intermedius* lacks the entire suite of rostral traits (Fig. 6). The jaws of *C. intermedius* and *C. cataphractus*, in relative terms, are neither as long nor as narrow as the jaws of most tomistomines and gavialines, and there is a wide range of variation in rostral shape even among tomistomines and gavialines (Busbey, 1995; Brochu, 2001). Given this continuum, it was not surprising that traits associated with the long, narrow snouted condition were imperfectly correlated; no two of the seven anatomical characters that grouped *G. gangeticus* and *T. schlegelii* had identical phylogenetic distributions (Fig. 6). Some of the characters could be partially dependent, but methods designed to detect such correlations are based on null models that might or might not be valid (Maddison, 1990; Wollenberg and Atchley, 2000; O'Keefe and Wagner, 2001). Utilization of these procedures as the basis for differential character weighting schemes could offer a better understanding of the combined systematic evidence, but the additional assumptions upon which these analyses depend are not accepted by many systematists.

Evolution of Taxic Atavisms in Gavialine Crocodylians

If the combined-evidence tree of extant plus extinct taxa is taken as the current best estimate of crocodylian phylogeny, numerous morphological traits that traditionally were thought to be primitive retentions in gavialines are more simply interpreted as reversals to ancestral states, i.e., taxic atavisms (Stiassny, 1992). According to parsimony reconstructions, minimally 15 morphological characters were transformed to a derived state on the stem lineage of Crocodylia only to later revert back to the primitive condition at the base of Gavialinae or within this clade (Fig. 5; purple characters). Seven characters that grouped *G. gangeticus*, *T. schlegelii*, and Crocodylinae also reversed in gavials to ancestral states that characterized more basal nodes in the tree (Fig. 5; blue characters), and 14 other traits may constitute further evidence of retrogression in gavials but were equivocally optimized. These characters contributed the majority of the conflicting evidence in simultaneous analysis (Figs. 4, 5); of the 42 morphological characters that required more steps on the combined-evidence tree relative to the gross anatomical tree in the WSRT analysis, 32 were consistent with reversions in Gavialinae to more primitive states.

Analogous examples of taxic atavism have been hypothesized in the literature (e.g., Raikow et al., 1979; Wyss, 1988), but the pattern in Crocodylia is extreme. Characteristics of the skull table, braincase, jaws, hyoid, osteoderms, ribs, vertebrae, forelimbs, and pelvis all showed reversals in gavials to states seen in fossil and living outgroups or basal crocodylians (Fig. 7). Although these atavistic characters, by definition, changed minimally twice on the combined evidence tree, parsimony reconstructions implied that 16 of the characters changed *only* twice (Fig. 5). The conservatism of these traits, their distribution across a variety of anatomical regions, and the unified vectors of homoplastic change on the tree suggested a remarkable series of evolutionary events.

Heterochrony, in particular paedomorphosis (e.g., Kluge, 1989; Vrba et al., 1994), has been implicated as a mode of evolution that is consistent with wholesale patterns of atavistic character change, but in our analysis, the phylogenetic transformations of reversed traits did not track developmental trajectories in many cases (C. Brochu, pers. comm.; M. Norell, pers. obs.). The gavial lineage may have lost ancestral traits but may have retained conserved morphogenic systems that were then reactivated millions of years later (Stiassny, 1992; see Whiting et al., 2003, for a possible empirical example). From a developmental perspective, this hypothesis would be difficult to test, but a more complete sampling of fossil diversity might clarify the evolutionary sequence of character reversals at the base of Gavialinae. The most ancient gavialine genus in the analysis, *Thoracosaurus*, appears in the fossil record over 70 million years ago (Schwimmer, 1986) and expresses many of the atavistic character states (Fig. 5). Characters that grouped gavialines with tomistomines evolved at more basal nodes in the tree (Fig. 6). Thus, the simplest interpretation is that the shared slender-snouted condition of *G. gangeticus* and *T. schlegelii* was maintained in these lineages over the entire Cenozoic, despite the radical, overlapping evolutionary trend in many other aspects of gavialine anatomy (Fig. 7).

Simultaneous Analysis: Interpretation of Support and Future Directions

In a recent review of molecules *versus* morphology in systematics, Hillis and Wiens (2000:9–10) noted that "a few cases of conflict between molecular and morphology-based phylogenies have defied explanation. . . . Recent estimates of crocodilian phylogeny are a well-documented example of such conflict. . . . In this case, it is difficult to determine which data set is misleading and what the true phylogeny of the group may

be." In part, we agree with this statement. Morphological and molecular data sets analyzed here did conflict significantly, but these character sets were not homogeneous bodies of evidence, one right and one wrong. Data sets are composed of many individual characters, and certain characters in conflicting data sets might agree on phylogenetic groupings even though the majority of characters in the respective data sets disagree (Barrett et al., 1991). In the combined matrix analyzed here, there was extensive hidden character support in the morphological data set for controversial relationships supported by the nuDNA and mtDNA data sets (Fig. 4). By uniting double decay analysis (Wilkinson et al., 2000) with PBS and PHBS measures (Baker and DeSalle, 1997; Gatesy et al., 1999), we were able to detect this secondary signal within the context of the total evidence.

In the separate analysis of the morphological data, Gavialinae assumed a basal position in crocodylian phylogeny (Fig. 3b), but there was only meager molecular support for this hypothesis. There were 15 possible topological resolutions among the major extant lineages of Crocodylia (Alligatoroidea, Crocodylinae, Gavialinae, and Tomistominae). For the DNA sequences analyzed here, only 1 of the 15 topological possibilities fit the molecular data worse than did the traditional morphological hypothesis (Fig. 1a). In contrast, the fit of the morphological data set to the molecular hypothesis (Fig. 1b) implied fewer steps than 7 of the 15 possible trees. The imbalance of signals within separate data sets translated into extensive hidden morphological character support in simultaneous analysis (Fig. 4). There was a strong *common* phylogenetic signal for a relatively apical placement of Gavialinae in the crocodylian tree, a jump of nine internodes when compared with the morphological hypothesis (Fig. 3a).

Different investigator-defined classes of systematic data can be very internally heterogeneous in terms of phylogenetic signal. Therefore, conflict between data sets, even the significant incongruence evident in this study, does not necessarily justify indictment of the combined evidence paradigm (see Kluge, 1989, 1997; Nixon and Carpenter, 1996) and the deletion of entire classes of characters from phylogenetic analysis, as other workers have argued (Bull et al., 1993; de Queiroz, 1993; Hedges and Maxson, 1996; Givnish and Sytsma, 1998; Luckett and Hong, 1998; Naylor and Brown, 1998; McCracken et al., 1999; Naylor and Adams, 2001). When a conflicting data set is composed of mixed signals, exclusion of that entire data set from phylogenetic analysis could be analogous to throwing out the baby with the bath water; many highly consistent characters might be lost with the deletion of only a few inconsistent ones (Poe, 1996; Siddall, 1997). A melange of phylogenetic signals *within* data sets might explain why character support can increase when significantly conflicting data sets are merged in simultaneous analysis and why measures of data set incongruence, such as the ILD test, are not justified as arbiters of data set combinability (Sullivan, 1996; Farris, 1997; Siddall, 1997; Gatesy et al., 1999; Yoder et al., 2001; Baker and Gatesy, 2002).

Numerous authors have argued that congruence among separate analyses of different data sets should inspire confidence in phylogenetic results and that such analyses are a requirement for detecting incongruence among data sets (e.g., Hillis, 1987; Miyamoto and Fitch, 1995). Separate analyses of individual data sets, however, ignore hidden character support within data sets that emerges in combined analysis, and this support can be substantial (e.g., Gatesy and Arctander, 2000; Fig. 4). Separate analyses of different character sets are not necessary to detect conflicts among data sets (Baker and DeSalle, 1997) and can distort interpretations of common character support (Gatesy et al., 1999).

Instead, we suggest that the distribution of conflicts/support among data sets in a comprehensive combined analysis should be used to assess data set congruence and to question the strength of support for different clades. In contrast to a strict total evidence framework in which character partitions are not considered relevant (Kluge, 1997), our approach acknowledges that certain sets of characters *might* be dependent and that it is useful to track such *potentially* dependent characters in simultaneous phylogenetic analysis. For example, the sequence alignment of a particular nucleotide in a gene is directly affected by adjacent nucleotides in that gene (Needleman and Wunsch, 1970). Thus, strings of bases could be considered nonindependent at this critical stage of primary homology assessment. Nucleotides within the same gene also might be functionally correlated because these bases encode different interacting parts of the same gene product (Wheeler and Honeycutt, 1988; Wollenberg and Atchley, 2000). Relative to unlinked nucleotides, tightly linked sequences could be more prone to joint transport across species boundaries (Cronin, 1993), simultaneous sorting (Pamilo and Nei, 1988), correlated change by one gene conversion event (Radding, 1982), concurrent duplication (Goodman et al., 1979), and a single horizontal transfer event (Clark et al., 1994). Keeping account of potentially dependent characters in simultaneous analysis offers the researcher a better understanding of the total evidence.

Relationships among extant crocodylian species in our combined trees were robustly supported (Figs. 3a, 4), but several inconsistencies remained (see 1–5 below). We contend that such conflicts should be used *heuristically* to interpret nodal support, to better understand evolutionary patterns, to inspire experimental studies, and to suggest where future systematic work should be directed.

1) Most troubling was the concentrated, hierarchically distributed homoplasy in the combined evidence tree (Fig. 5). This remarkable atavistic pattern cannot be explained by long-branch attraction effects in the morphological data (Felsenstein, 1978) because the pattern intensified with the inclusion of primitive extinct taxa from each of the major crocodylian clades (Brochu, 1997, 2003). Ontogenetic studies of putative taxic atavisms are required to document the developmental trajectories of these traits, to formulate more detailed character descriptions, and to measure the contribution of heterochronic change, if any, to the wholesale reversals in Gavialinae.

The apical position of this taxon in the combined-evidence tree (Fig. 3a) suggests that the anatomy of the ancestral gavialine was very different from that implied by a basal positioning of Gavialinae (Fig. 3b). The extant species *G. gangeticus* and *T. schlegelii* are relicts of ancient clades, thus it will be critical to sample early representatives of Gavialinae and Tomistominae to reconstruct primitive character states for these groups (e.g., Brochu and Gingerich, 2000; Brochu, 2002). Developmental studies and the inclusion of more extinct taxa in phylogenetic analysis will test the transformational hypotheses presented here (Fig. 5).

2) For parsimony analyses of all DNA sequence data sets, outgroup taxa joined the ingroup topology at the longest branch among ingroup taxa. In all cases, this was the internode that connected Alligatoroidea to Crocodylinae + *G. gangeticus* + *T. schlegelii* (Fig. 2). Given the length of the outgroup branch, especially for the nuDNA, long-branch attraction effects could have influenced our results (Felsenstein, 1978). Maximum likelihood analyses of the mtDNA and a combined mtDNA/nuDNA data set supported the same pattern of relationships as the parsimony analyses of these data sets. Relative to the equally weighted cladistic analyses, 50% JK support was slightly reduced for the *G. gangeticus* + *T. schlegelii* clade (92% mtDNA, 99% total DNA) and the Crocodylinae + *G. gangeticus* + *T. schlegelii* clade (100% mtDNA, 90% total DNA), but gap characters were not considered evidence by the models utilized in these analyses. Maximum likelihood analysis of the nuDNA data set rooted the ingroup topology on the branch that joined *G. gangeticus* and *T. schlegelii* (JK = 59% for *G. gangeticus* + *T. schlegelii*; Fig. 2). Unrooted likelihood analyses, in which the avian outgroup sequences were deleted, produced trees that were consistent with previous molecular results (Fig. 1b); for all DNA data sets, the internode that connected *G. gangeticus* + *T. schlegelii* to the exclusion of other extant crocodylians was robustly supported by 100% JK scores.

The effects of outgroup taxon sampling on long-branch attraction (Felsenstein, 1978), long-branch misplacement (Siddall and Whiting, 1999), and rooting of the crocodylian tree currently are being investigated for a larger set of nu RAG-1 gene sequences (Pol and Gatesy, in prep.). However, a simultaneous likelihood analysis of the entire crocodylian data set, with both molecular and morphological characters, will be required to properly assess the influence of different model assumptions on phylogenetic results.

3) The combined matrix of extant taxa was exceptionally stable to the random removal of informative characters. Even JK analyses with 90% character removal yielded >70% recovery of most supported nodes, but partitioned support measures pointed to potential weaknesses in the combined evidence. In particular, the very high PBS scores for the mtDNA data set, ~88% of the total double decay BS for basal relationships, suggested that much of the character support for the combined evidence topology was due to the three mt genes (Fig. 4). Analysis of the combined matrix, with the mtDNA data excluded, favored the traditional morphological hypothesis (Fig. 1a). Because mtDNA is thought to be primarily nonrecombining and maternally inherited, a single hybridization/introgression event could explain much of the incongruence between molecular and morphological data sets in our analysis. All of the mtDNA characters are *potentially* nonindependent, so future corroboration from numerous nu loci would bolster our conclusions. Further nuDNA sequencing is underway in our and others laboratories (see Harshman et al., this issue).

4) Several authors have discussed DNA alignment ambiguity and the potential for nonindependence of adjacent indels within the context of crocodylian rDNA sequences (Gatesy et al., 1993; Wheeler et al., 1995; Poe, 1996; Lutzoni et al., 2000; White and Densmore, 2001). For our combined matrix, when gaps were treated as missing data the double decay BS was lower for *G. gangeticus* + *T. schlegelii* (decrease of seven extra steps) and for *G. gangeticus* + *T. schlegelii* + Crocodylinae (decrease of six extra steps), indicating that at least some of the support at these critical nodes was influenced by indel characters. Phylogenetic gap weight was scaled in proportion to gap length for most of our analyses. Alternative sequence alignment procedures (e.g., Kjer, 1995; Wheeler, 1996; Lutzoni et al., 2000) and gap coding methods (e.g., Simmons and Ochoterena, 2000; Geiger, 2002) could be used to test the stability of our results to different analysis assumptions.

5) The combined evidence was inconsistent with the stratigraphic distributions of many extinct taxa; *Thoracosaurus*, an early gavialine genus, implied the largest gaps in the fossil record. Minimally seven lineages that branched before *Thoracosaurus* must have been present in the Cretaceous but apparently are not found until the Eocene (Fig. 6; for quantification of stratigraphic gaps, see Brochu, 1997, 2003). If the combined evidence topology is accurate, many critical fossils await discovery. Alternatively, displacement of *Thoracosaurus* outside of Crocodylia would temper stratigraphic discrepancies. The shortest tree consistent with this basal positioning of *Thoracosaurus* was 13 extra character steps beyond minimum length and demanded the independent evolution of several characters in Gavialinae and in *Thoracosarus*. At this juncture, paleontological fieldwork in Late Cretaceous strata should be a priority.

CONCLUSIONS

Separate analyses of morphological and molecular data sets supported strongly conflicting interpretations of crocodylian phylogeny. Despite these significant discrepancies, simultaneous analysis of all character data, the combined evidence approach, produced a well-resolved, robust phylogenetic hypothesis. Calculation of PHBS scores (Gatesy et al., 1999) within a double decay framework (Wilkinson et al., 2000) showed that approximately 70% of the character support for controversial groupings was derived from secondary phylogenetic

signals that emerged with the combination of diverse data sets (Fig. 4). The hidden support was in part due to a suite of traits that described the longirostrine narrow-snouted condition in gavials and false gavials (Figs. 6, 7).

In the combined analysis of extant plus extinct taxa, 10–15% of the morphological characters showed reversals to the outgroup condition in Gavialinae, and many other characters reverted to plesiomorphic states characteristic of basal crocodylians (Fig. 5). The concentrated hierarchical homoplasy from a variety of anatomical regions (Fig. 7) accounted for most of the incongruence among data sets in simultaneous analysis and many of the hidden synapomorphies for a grouping of *G. gangeticus*, *T. schlegelii*, and crocodylines (Fig. 4). The BS for this "molecular" clade actually increased with the addition of the conflicting morphological data set to the DNA sequences. Given the current database, the lone extant gavialine, *G. gangeticus*, is best interpreted as a secondarily derived, atavistic mimic of outgroup taxa to Crocodylia and not as the primitive sister group to other extant crocodylians.

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