

# Corroboration among Data Sets in Simultaneous Analysis: Hidden Support for Phylogenetic Relationships among Higher Level Artiodactyl Taxa

John Gatesy<sup>\*,†,1</sup> Patrick O'Grady<sup>†</sup> and Richard H. Baker<sup>‡</sup>

*\*Laboratory of Molecular Systematics and Evolution and †Department of Ecology and Evolutionary Biology, University of Arizona, Biosciences West, Tucson, Arizona 85721; and ‡Department Entomology, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024*

Accepted for publication August 20, 1998

In the taxonomic congruence approach to systematics, data sets are analyzed separately, and corroboration among data sets is indicated by replicated components in topologies derived from the separate analyses. By contrast, in the total evidence and conditional combination approaches, characters from different data sets are mixed in combined phylogenetic analyses. In optimal topologies derived from such simultaneous analyses, support for a particular node may be attributed to one, some, or all of the individual data sets. Partitioned branch support (PBS) is one technique for describing the distribution of character support and conflict among data sets in simultaneous analysis. PBS is analogous to branch support (BS), but recognizes hidden support and conflicts that emerge with the combination of characters from different data sets. For both BS and PBS, support for a particular node is interpreted as the difference in cost between optimal and suboptimal topologies. A different measure, the clade stability index (CSI), assesses the robustness of a particular node through the successive removal of characters. Here, we introduce variations of the CSI, the data set removal index (DRI) and nodal

data set influence (NDI), that indicate the stability of a particular node to the removal of entire data sets. Like PBS, the DRI and NDI summarize the influence of different data sets in simultaneous analysis. However, because these new methods and PBS use different perturbations to assess stability, DRI and NDI scores do not always predict PBS scores and *vice versa*. In this report, the DRI and NDI are compared to PBS and taxonomic congruence in a cladistic analysis of 17 data sets for Artiodactyla (Mammalia). Five indices of hidden support and conflict are defined and applied to the combined artiodactyl character set. These measures identify substantial hidden support for controversial relationships within Artiodactyla. Hidden character support is ignored in the taxonomic congruence approach to systematics, but the DRI, NDI, and PBS utilize this cryptic information in estimates of support among data sets for a given node. © 1999 The

Willi Hennig Society

## INTRODUCTION

Given several data sets, systematists are faced with the task of deriving a single phylogenetic hypothesis

<sup>1</sup>To whom correspondence should be addressed at 1206-A Downey Street, Laramie, WY 82072. E-mail: hayashi@uwyo.edu.

for a particular group. Currently, there are three principle philosophies for achieving this goal within a cladistic framework: total evidence, conditional combination of data sets, and taxonomic congruence.

Total evidence and conditional combination stress the simultaneous analysis of characters from different data sets. With total evidence, all relevant character information is combined in a single phylogenetic analysis (Kluge, 1989). In conditional combination, data sets that are not "significantly" incongruent are merged and analyzed together (Bull *et al.*, 1993).

With taxonomic congruence, characters from different data sets are never allowed to interact in a simultaneous phylogenetic analysis. Instead, data sets are analyzed separately, and common components among the independent analyses are used to construct consensus trees (Nelson, 1979). Corroboration among data sets for a particular node is indicated by replication of that node in topologies derived from the separate data sets. Conflict among data sets is recorded in topological disagreements among the fundamental cladograms (Miyamoto and Fitch, 1995).

The distribution of support and conflict among data sets is also of interest to systematists, who combine data sets in simultaneous analysis (e.g., Wheeler *et al.*, 1993; Olmstead and Sweere, 1994; Whiting *et al.*, 1997; Baker *et al.*, 1998; Cannatella *et al.*, 1998; Davis *et al.*, 1998; Gatesy, 1998), but there is no consensus on how agreements and disagreements among data sets should be quantified in this framework. The incongruence length difference (ILD—Mickey and Farris, 1981; Farris *et al.*, 1994a) summarizes the *net* character conflict among data sets in simultaneous analysis, but does not measure the relative support provided by different data sets at a *particular* node. Unambiguous synapomorphies for a given clade may be derived from one, some, or all of the data sets that compose the combined character matrix. Therefore, the distribution of support among data sets for a particular node could be equated with the number of synapomorphies obtained from each of the individual data sets in simultaneous analysis. However, a simple tabulation of synapomorphies does not highlight specific conflicts among data sets.

A variation of branch support (BS—Bremer, 1988, 1994), partitioned branch support (PBS—Baker and DeSalle, 1997), does identify conflicts among data sets for particular relationships. PBS summarizes the contribution of each data set to BS scores for the total

combined character set. With this index, the net character support *and* conflict rendered by each data set at a given node can be estimated in a simultaneous analysis of all relevant data. A comparison of topologies derived from separate analyses of different data sets is not required as in taxonomic congruence (Baker and DeSalle, 1997).

The recognition of hidden support is a key difference between the simultaneous analysis and taxonomic congruence approaches (Nixon and Carpenter, 1996). When data sets are merged in simultaneous analysis, the combined character matrix sometimes supports unique relationships not favored by any of the individual data sets (Barrett *et al.*, 1991; Chippindale and Wiens, 1994; Olmstead and Sweere, 1994). In such cases, common character support for these emergent relationships is hidden by conflicting characters in the separate data sets. Taxonomic congruence does not acknowledge the importance of this hidden support. Therefore, it would be profitable to develop additional indices of support and conflict among data sets in simultaneous analysis as well as novel measures of hidden support.

The goals of this report are: (1) To define hidden character support and conflict. (2) To develop methods for quantifying hidden support and conflict in simultaneous analyses. (3) To introduce two variations of the clade stability index (CSI—Davis, 1993), the data set removal index (DRI) and nodal data set influence (NDI), that summarize the distribution of character support among data sets in simultaneous analysis. (4) To compare the DRI and NDI to PBS and taxonomic congruence in a cladistic analysis of 17 data sets for Artiodactyla (Mammalia). (5) To measure hidden support and conflict in the combined artiodactyl matrix. (6) To establish a higher level cladistic hypothesis for Artiodactyla that is based on diverse character sets.

### Definitions and Examples

The new methods in this paper are variants of existing methods for assessing nodal support (Templeton, 1983; Davis, 1993; Bremer, 1994; Baker and DeSalle, 1997). The following section contains a brief review of previous methods, descriptions of new methods, and several explanatory examples.

**Branch support (BS).** Within a cladistic framework, competing topologies are judged according to tree

length. The ability to discriminate among topologies is proportional to the differences in length of the various topologies (Goloboff, 1991). This is the logical basis for BS (Table 1—Bremer, 1988, 1994; Farris *et al.*, 1994b). For a particular data set and a particular node, BS is the minimum number of character steps for that data set on the shortest topologies that do not contain that node, minus the minimum number of character steps for that data set on the shortest topologies that do contain that node. BS can be positive, zero, or negative. If the node of interest is supported by a given data set, BS is positive. If the node is not supported by a given data set, BS is negative or zero. Our definition of BS goes beyond the original description of BS that only considered positive scores (Bremer, 1988; 1994). Nevertheless, negative and zero BS are consistent with Bremer's original concept and will be referred to as BS in the remainder of this paper.

**Partitioned branch support (PBS).** PBS extends the concept of BS to the simultaneous analysis framework (Table 1—Baker and DeSalle, 1997). For a particular combined data set, a particular node, and a particular data partition, PBS is the minimum number of character steps for that partition on the shortest topologies for the combined data set that do not contain that node, minus the minimum number of character steps for that partition on the shortest topologies for the combined data set that do contain that node. If there are multiple equally short topologies, tree lengths are averaged (Baker and DeSalle, 1997).

For any node, the sum of PBS scores for the different component data sets equals BS at that node in the simultaneous analysis of all data sets. Individual PBS scores can be positive, negative, or zero. Within a simultaneous analysis framework, a positive PBS score indicates that a given data set provides net positive support for that particular node over the alternative relationships in the shortest tree(s) without the given node, a negative PBS score shows that a data set favors the shortest tree(s) without the given node over the minimum length solution(s), and a PBS score of zero indicates the indifference of a given data set at that node (Baker and DeSalle, 1997).

Simple examples of PBS are shown in Fig. 1. The three scenarios (a–c) represent different divisions of the same total data set into three character sets (I–III). For the first partitioning of the total data set, a, support for the total data topology (clade A + B) is fairly evenly

distributed among the three data sets. In the second case, b, all of the evidence for clade A + B is squeezed into one of the three data partitions, I. The third case, c, represents an intermediate situation (Fig. 1). These simple partitioning scenarios illustrate the utility of PBS in a variety of circumstances.

PBS scores are shown for each partitioning scenario (Fig. 1). In a, the positive PBS scores for all three data sets indicate that there is net positive support for clade A + B in each component data set. In b, there is positive PBS for only one data set. There is no corroboration among data sets in this case, and the PBS scores reflect this fact. In c, there is no taxonomic congruence among different data sets (if strict consensus trees are compared), but there is corroboration between data sets I and III in simultaneous analysis. Hidden support that emerges in simultaneous analysis is revealed by the positive PBS score for partition III, despite the lack of positive BS for A + B in the separate analysis of III (Fig. 1).

Other examples of PBS are given in Figs. 2 and 3. Scenario d in Fig. 2 illustrates a case in which there is hidden conflict. Each of the two data partitions, I and II, independently support clade A + B. However, the combination of these two data sets, I + II, does not support clade A + B. BS for A + B is reduced in the combined analysis relative to the separate analyses (Fig. 2). The taxonomic congruence approach would suggest corroboration between data sets I and II in this case. In contrast, the PBS scores of zero for each data partition show that there is no corroboration between data sets in simultaneous analysis (Fig. 2).

PBS offers a simple means for assessing support rendered by different data sets within a simultaneous analysis framework. The method permits the detection of hidden conflicts and support that are not obvious from separate analyses of each data set. Furthermore, because characters are allowed to interact in simultaneous analysis, the relative weight of evidence from each data set is taken into account. A comparison of topologies derived from separate analyses of each data set (i.e., taxonomic congruence) is not required to detect congruence and incongruence among data sets at particular nodes (Baker and DeSalle, 1997).

**Character support (CS).** For a given data set, the difference between the number of steps for a particular

**TABLE 1**  
Indices Discussed in the Text and Relationships between These Indices

| Index (reference)  | Abbreviation | Definition   | Relation to other indices   |
|--|--------------|--|---|
| Branch support (Bremer, 1988; 1994)                          | BS           | For a particular data set and a particular node, BS is the minimum number of character steps for that data set on the shortest topologies that do not contain that node, minus the minimum number of character steps for that data set on the shortest topologies that contain that node.  | = Sum of CS at a node<br>= Sum of PBS at a node   |
| Partitioned branch support (Baker and DeSalle, 1997)         | PBS          | For a particular combined data set, a particular node, and a particular data partition, PBS is the minimum number of character steps for that partition on the shortest topologies for the combined data set that do not contain that node, minus the minimum number of character steps for that partition on the shortest topologies for the combined data set that do contain that node. | = Sum of CS values at a node from a particular data partition<br>= BS at a node for a particular partition + PHBS for that partition at that node                 |
| Character support (Templeton, 1983; Prager and Wilson, 1988) | CS           | For a particular data set, a particular node, and a particular character, CS is the minimum number of steps for that character on the shortest topologies for that data set that do not contain that node, minus the minimum number of steps for that character on the shortest topologies for that data set that do contain that node.  | (See BS and PBS)  |
| Hidden branch support (This paper)                           | HBS          | For a particular combined data set and a particular node, HBS is the difference between BS for that node in the combined analysis and the sum of BS values for that node from each data partition.   | = BS at a node for the combined data set – sum of BS values from each data partition<br>= Sum of all PHBS values at a node  |
| Partitioned hidden branch support (This paper)               | PHBS         | For a particular combined data set, a particular node, and a particular data partition, PHBS is the difference between PBS for that data partition at that node for the combined data set and the BS value for that node for that data partition.  | = Sum of all HCS values at node<br>= PBS for a particular partition – BS for that partition<br>= Sum of all HCS values at a node from a particular data partition |
| Hidden character support (This paper)                        | HCS          | For a particular node, and a particular character within a particular data partition within a particular combined data set, HCS is the difference between CS for that character at that node for the combined data set and CS for that character at that node for that data partition.   | (See HBS and PHBS)  |
| Hidden synapomorphy (This paper)                             | HS           | For a particular combined data set and particular clade, HS is the difference between the number of unambiguous synapomorphies for that clade in simultaneous analysis and the sum of unambiguous synapomorphies for that clade in the separate analyses of data partitions.   |   |
| Clade stability index (Davis, 1993)                          | CSI          | For a particular data set and a particular node, the CSI is the minimum number of character removals necessary to collapse that node, divided by the number of informative characters in that data set.  | = CRI / number of informative characters in the data set  |
| Character removal index (Davis, 1993)                        | CRI          | For a particular data set and a particular node, the CRI is the minimum number of character removals necessary to collapse that node.  | (See CSI)   |

TABLE 1—Continued

| Index (reference)                            | Abbreviation | Definition   | Relation to other indices   |
|--|--------------|--|---|
| Data set removal index (This paper)          | DRI          | For a particular combined data set and a particular node, the DRI is the minimum number of data set removals necessary to collapse that node.  | Like the CRI but at the level of data sets instead of characters  |
| Nodal data set influence (This paper)        | NDI          | For a particular combined data set, a particular data partition, and a particular node, NDI is the BS value at that node for the combined data set, minus the BS value at that node for the combined data set without that data partition. | = BS for total data set – BS for total data set without the partition of interest   |
| Hidden nodal data set influence (This paper) | HNDI         | For a particular combined data set, a particular data partition, and a particular node, HNDI is the NDI value for that partition at that node, minus the BS value for that partition at that node.   | = NDI for that partition – BS for that partition<br>= HBS for total data set – HBS for total data set without the partition of interest |
| Data set influence (This paper)              | DI           | For a particular combined data set and a particular data partition, DI is the sum of NDI values for that partition for each node resolved by the combined data set.  | = the sum of NDIs for a particular data partition   |

character on minimum length topologies and the number of steps for this same character on suboptimal topologies has been used to test the significance of a particular node (Templeton, 1983; Prager and Wilson, 1988). In the remainder of this paper, this difference is termed character support (CS—Table 1). CS is an extension of PBS to the level of the individual character. CS is calculated like PBS. For a particular data set, a particular node, and a particular character, CS is the minimum number of steps for that character on the shortest topologies for that data set that do not contain that node, minus the minimum number of steps for that character on the shortest topologies for that data set that do contain that node. If there are multiple equally short topologies, the numbers of character steps for the different topologies are averaged.

For any node, the sum of all CS scores equals the BS score of that node (Table 1). CS scores can be positive, negative, or zero. Within the context of a certain data set, a positive CS score indicates that a given character provides net positive support for a particular node over the alternative relationships in the shortest tree(s) without the given node, a negative CS score shows that the character favors the shortest tree(s) without the given node over the minimum length solution(s), and a zero CS score indicates the indifference of the character at that node.

**Hidden branch support (HBS).** The interaction of

different data sets in simultaneous analysis often implies hidden character support and conflicts (Barrett *et al.*, 1991; Chippindale and Wiens, 1994; Olmstead and Sweere, 1994). For a particular set of data partitions and a particular node, hidden support can be defined as increased support for the node of interest in the simultaneous analysis of all data partitions relative to the sum of support for that node in the separate analyses of each partition. In the most obvious cases of hidden support, simultaneous analysis results in relationships that are not supported by any of the separate analyses of the individual data sets (e.g., Olmstead and Sweere, 1994; Fig. 3—scenario e). For a particular set of data partitions and a particular node, hidden conflict can be defined as decreased support for the node of interest in the simultaneous analysis of all data partitions relative to the sum of support for that node in the separate analyses of the various data partitions. In the most obvious cases of hidden conflict, a node that is supported by separate analyses of each data set is not supported by simultaneous analysis (e.g., Chippindale and Wiens, 1994; Fig. 2—scenario d).

Hidden support and conflicts can be quantified with a variation of BS, hidden branch support (HBS—Table 1). For a particular combined data set and a particular node, HBS is the difference between BS for that node in the simultaneous analysis of all data partitions and the sum of BS scores for that node from each data

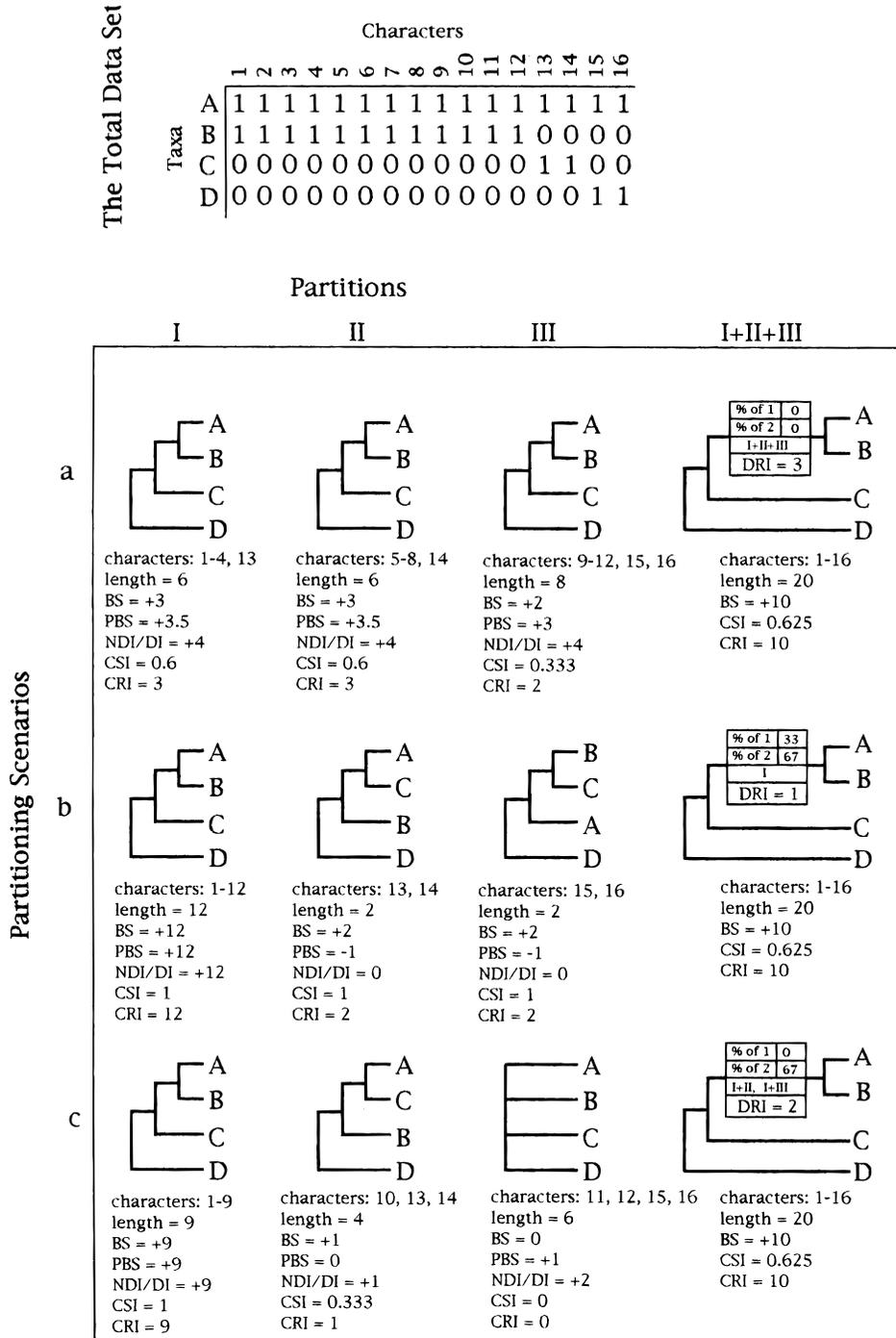
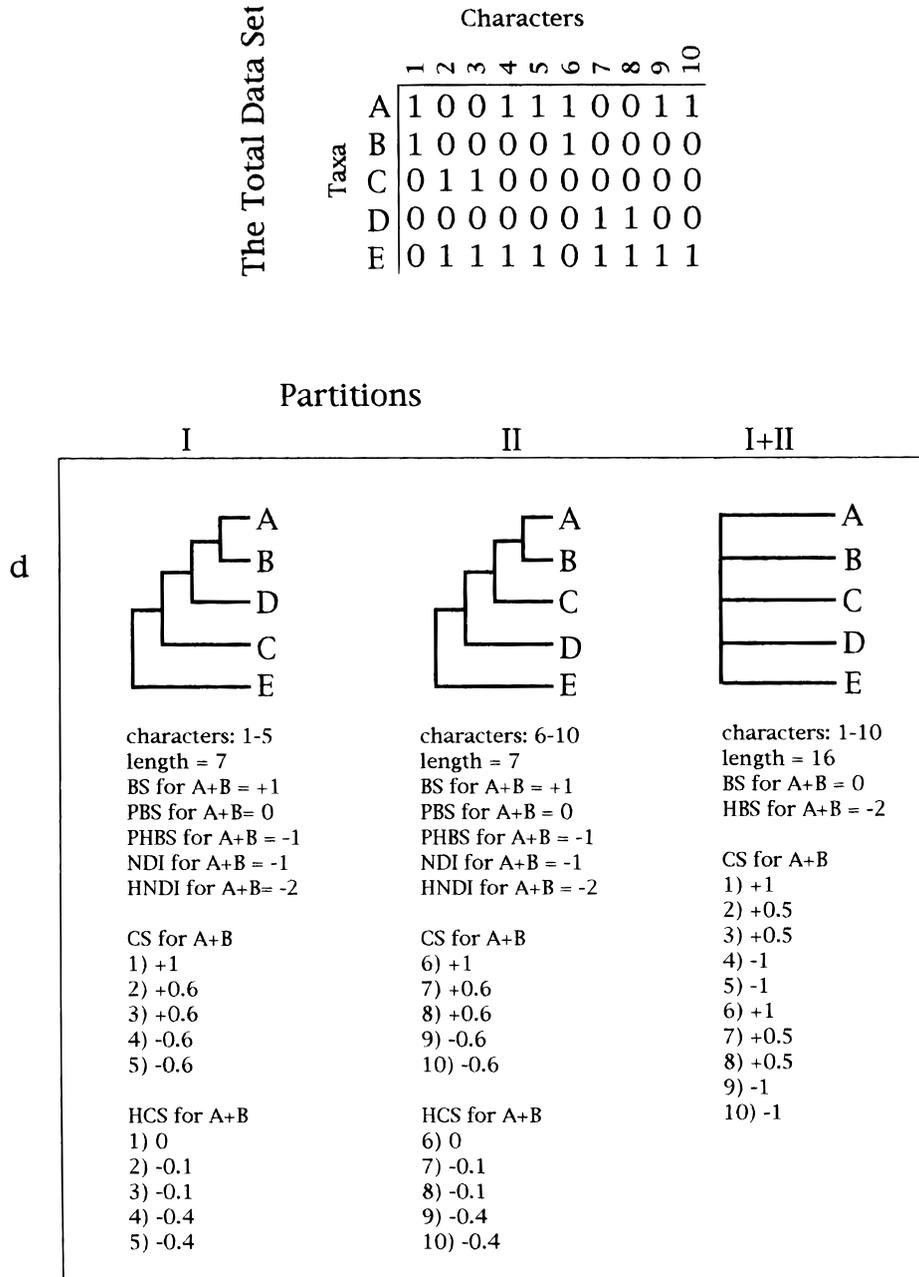


FIG. 1. Three different partitioning scenarios (a–c) for a hypothetical data set (16 characters for four taxa) that illustrate PBS, NDI, and the DRI. In each scenario, the following are shown for each of the three data partitions (I–III): strict consensus of minimum length topologies for that partition, characters in that partition, minimum tree length, BS, PBS, NDI/DI, CSI, and the CRI. The following are shown for each combined analysis (I+II+III): minimum length topology, characters in the combined data set, minimum tree length, BS, CSI, CRI, the percentage of single data set removals that collapse the total data topology (% of 1), the percentage of data set removals of size two that collapse the total data topology (% of 2), the specific data set removals that collapse the total data topology, and the DRI. All topologies are rooted with taxon D. Explanations are in the text. Abbreviations for indices are as in Table 1.



**FIG. 2.** Example that illustrates HBS, PHBS, HNDI, and HCS. A partitioning scenario (d) for a hypothetical data set (10 characters for five taxa) is characterized by hidden conflict. The following are shown for each of the two data partitions (I and II): minimum length topology for that partition, characters in that partition, minimum tree length, BS for A+B, PBS for A+B, PHBS for A+B, NDI for A+B, HNDI for A+B, CS for A+B for each character in the partition, and HCS for A+B for each character in the partition. The following are shown for the combined analysis (I+II): strict consensus of minimum length topologies, characters in the simultaneous analysis, minimum tree length, BS for A+B, HBS for A+B, and CS for A+B for each character in the simultaneous analysis. All topologies are rooted with taxon E. Explanations are in the text. Abbreviations for indices are as in Table 1.

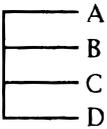
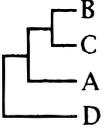
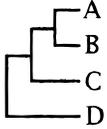
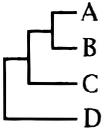
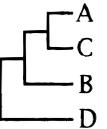
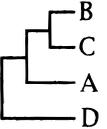
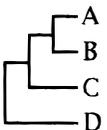
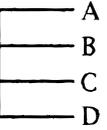
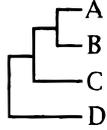
The Total Data Set

|      |   | Characters |   |   |   |   |   |   |
|------|---|------------|---|---|---|---|---|---|
|      |   | 1          | 2 | 3 | 4 | 5 | 6 | 7 |
| Taxa | A | 1          | 1 | 1 | 1 | 1 | 1 | 1 |
|      | B | 1          | 1 | 1 | 0 | 0 | 0 | 0 |
|      | C | 0          | 0 | 0 | 1 | 1 | 0 | 0 |
|      | D | 0          | 0 | 0 | 0 | 0 | 1 | 1 |

Partitions

I                      II                      III                      I+II or I+II+III

Partitioning Scenarios

|   |   |   |  |   |
|---|---|---|--|---|
| e |    |    |    |   |
|   | characters: 1, 2, 4, 5<br>length = 6<br>BS = 0<br>BS for A+B = 0<br>PBS = +1<br>PHBS = +1<br>NDI/DI = +2<br>HNDI = +2<br>1) HCS = 0<br>2) HCS = 0<br>4) HCS = +0.5<br>5) HCS = +0.5 | characters: 3, 6, 7<br>length = 4<br>BS = +1<br>BS for A+B = -1<br>PBS = 0<br>PHBS = +1<br>NDI/DI = +1<br>HNDI = +2<br>3) HCS = 0<br>6) HCS = +0.5<br>7) HCS = +0.5   | characters: 1-7<br>length = 11<br>BS = +1<br>HS = +3<br>HBS = +2<br>1) CS = +1<br>2) CS = +1<br>3) CS = +1<br>4) CS = -0.5<br>5) CS = -0.5<br>6) CS = -0.5<br>7) CS = -0.5 |   |
|   | f   |   |    |   |
|   | characters: 1-3<br>length = 3<br>BS = +3<br>BS for A+B = +3<br>PBS = +3<br>PHBS = 0<br>NDI/DI = +3<br>HNDI = 0<br>1) HCS = 0<br>2) HCS = 0<br>3) HCS = 0                            | characters: 4, 5<br>length = 2<br>BS = +2<br>BS for A+B = -2<br>PBS = -1<br>PHBS = +1<br>NDI/DI = 0<br>HNDI = +2<br>4) HCS = +0.5<br>5) HCS = +0.5                    | characters: 6, 7<br>length = 2<br>BS = +2<br>BS for A+B = -2<br>PBS = -1<br>PHBS = +1<br>NDI/DI = 0<br>HNDI = +2<br>6) HCS = +0.5<br>7) HCS = +0.5                         | characters: 1-7<br>length = 11<br>BS = +1<br>HS = 0<br>HBS = +2<br>1) CS = +1<br>2) CS = +1<br>3) CS = +1<br>4) CS = -0.5<br>5) CS = -0.5<br>6) CS = -0.5<br>7) CS = -0.5 |
| g |    |    |    |   |
|   | characters: 1-3<br>length = 3<br>BS = +3<br>BS for A+B = +3<br>PBS = +3<br>PHBS = 0<br>NDI/DI = +3<br>HNDI = 0<br>1) HCS = 0<br>2) HCS = 0<br>3) HCS = 0                            | characters: 4-7<br>length = 6<br>BS = 0<br>BS for A+B = -2<br>PBS = -2<br>PHBS = 0<br>NDI/DI = -2<br>HNDI = 0<br>4) HCS = 0<br>5) HCS = 0<br>6) HCS = 0<br>7) HCS = 0 | characters: 1-7<br>length = 11<br>BS = +1<br>HS = 0<br>HBS = 0<br>1) CS = +1<br>2) CS = +1<br>3) CS = +1<br>4) CS = -0.5<br>5) CS = -0.5<br>6) CS = -0.5<br>7) CS = -0.5   |   |

partition. For a particular combined data set and a particular node, a positive HBS score indicates that more hidden support than hidden conflict emerges at that node in simultaneous analysis. A negative HBS score indicates more hidden conflict than hidden support at that node.

**Partitioned hidden branch support (PHBS).** The distribution of HBS among different character partitions can be quantified with partitioned hidden branch support (PHBS—Table 1). For a particular combined data set, a particular node, and a particular data partition, PHBS is the difference between PBS at that node for that data partition and BS at that node for that data partition. For a particular node, the sum of PHBS scores for the various data partitions equals the HBS at that node (Table 1).

**Hidden character support (HCS).** For a particular character, the amount of CS that emerges in simultaneous analysis above or below that seen in separate analysis is the hidden character support (HCS—Table 1). For a particular node, and a particular character within a particular data partition within a particular combined data set, HCS is the difference between CS for that character at that node for the combined data set and CS for that character at that node for that data partition. For a given node and a particular data partition, the sum of HCS for characters in that partition equals the PHBS for that partition at that node. For a given node, the sum of HCS for all characters in the combined data set equals HBS for that node (Table 1).

Simple examples of HBS, PHBS, and HCS are shown in Figs. 2 and 3. In Fig. 3, a hypothetical data set of seven characters is partitioned in three different ways. These partitioning scenarios, e–g, imply various amounts of HBS, PHBS, and HCS. The positive HBS in scenario e is obvious from a comparison of topologies for the separate and combined analyses. Clade A + B is not supported by either data partition, but is favored in the simultaneous analysis of both partitions.

In each data set, there are characters that in isolation from other characters would unambiguously support A + B. However, these characters, 1–3, are masked by the conflicting characters, 4–7. When data sets I and II are combined in simultaneous analysis, characters 1–3 emerge as clear-cut evidence for A + B, but do not offer HBS. According to our definitions, HCS is zero for each of characters 1–3. HCS comes from characters 4–7 (Fig. 3). This distribution of HCS can be explained with reference to the second scenario, f.

The amount of HBS in scenario f might, at first glance, seem counterintuitive (Fig. 3). All three of the individual characters that favor the total data topology (clade A + B) over the alternatives are confined to data set I. Two characters that unambiguously favor A + C form data set II, and two characters that unambiguously favor B + C compose data set III. In this partitioning scenario, HBS is +2. There is as much HBS in this scenario as there is for scenario e. The HBS is divided equally between data sets II and III despite the lack of individual characters in these partitions that favor the total data tree. All of the characters in partition II contradict the topology favored by the simultaneous analysis *and* the topology favored by partition III. Similarly, all of the characters in partition III contradict the topology favored by the simultaneous analysis *and* the topology favored by partition II. When data sets I, II, and III are merged in simultaneous analysis, the homoplastic characters in partitions II and III offset each other. Because the homoplasy is in opposite directions, favoring A + C in partition II and B + C in partition III, the negative effects of these characters are, in part, cancelled out in the simultaneous analysis. The homoplasy is diffused and defused (Barrett *et al.*, 1991). The net result is HBS for A + B (Fig. 3).

Scenario g is identical to scenario f, but data sets II and III of f have been merged to make partition II of g (Fig. 3). In scenario g, conflicting homoplasy is

FIG. 3. Examples that illustrate HBS, PHBS, HNDI, HCS, and HS. Three different partitionings scenarios (e–g) for a hypothetical data set (seven characters for four taxa) show examples of hidden support. For each scenario, the following are shown for each of the data partitions (I–III): strict consensus of minimum length topologies for that partition, minimum tree length, BS, BS for A+B, PBS, PHBS, NDI/DI, HNDI, and HCS for each character in the partition. The following are shown for each combined analysis (I+II or I+II+III): minimum length topology, characters in the combined data set, minimum tree length, BS, HS, HBS, and CS for each character in the simultaneous analysis. All topologies are rooted with taxon D. Explanations are in the text. Abbreviations for indices are as in Table 1.

accounted for *within* data set II. No conflicts are dampened by the simultaneous analysis in g. HBS is zero in this case as are all PHBS and HCS scores.

Figure 2, shows an example of hidden conflict. In scenario d, clade A + B is favored by separate analyses of data sets I and II. However, the analysis of the combined character set, I + II, does not support A + B. In this case, HBS for A + B is -2. The interaction of characters in the simultaneous analysis reduces support for A + B relative to the sum of support in the separate analyses of data sets I and II.

**Hidden synapomorphy (HS).** In some cases, a positive HBS score simply records the dispersion of homoplasy in simultaneous analysis. There may be no hidden synapomorphies for the clade of interest (e.g., Fig. 3—scenario f). This may be troubling to those who exclusively equate character support for a node with synapomorphy. However, by defining hidden support in terms of synapomorphy as opposed to BS, synapomorphies for a particular clade that are hidden by conflicting characters in separate analyses can be distinguished. Also, the absence of such hidden synapomorphies can be recorded.

For a particular combined data set and a given clade supported by that combined data set, hidden synapomorphy (HS—Table 1) can be defined as the number of unambiguous synapomorphies for that clade in the simultaneous analysis of the combined data set, minus the sum of unambiguous synapomorphies for that clade in the separate analyses of individual data partitions. The contribution of a particular data partition to HS for a given clade is the difference between the number of unambiguous synapomorphies for that clade from that partition in simultaneous analysis and the number of unambiguous synapomorphies for that clade from that partition in separate analysis.

By using HS in combination with HBS, HBS that is due solely to the dispersion of homoplasy in simultaneous analysis can be discerned. For example, there are no hidden synapomorphies for clade A + B in scenario f of Fig. 3 despite an HBS score of +2. HBS in this case results from the combination of conflicting homoplasy in data sets II and III; there is no HS. In contrast, there is both HBS and HS in scenario e of Fig. 3. Because A + B is not supported in the separate analyses of data sets I and II from scenario e, there are no unambiguous synapomorphies for A + B in these separate analyses.

However, characters 1–3 offer unambiguous synapomorphies for A + B in the combined analysis, so in this case, HS is +3. Data set I contributes HS of +2, and data set II contributes HS of +1.

**The clade stability index (CSI) and the character removal index (CRI).** For BS, support is interpreted as the difference in cost between optimal and suboptimal topologies. A different approach, the CSI (Table 1—Davis, 1993), uses successive character removal to test the stability of a given clade. The CSI for a particular node is the minimum number of character removals necessary to collapse that node divided by the total number of informative characters in the data set. A clade that is not in the strict consensus of most parsimonious trees for a data set has a CSI of 0. A clade that does not collapse until all informative characters are removed has a CSI of 1.

To calculate the CSI for a particular node, all possible combinations of character removals are tested until that node collapses. So, for a data set with five informative characters, maximally five phylogenetic searches are required to determine whether the CSI is 0.2 (i.e., whether the clade collapses with the removal of one of the five informative characters). For this same data set, there are 10 possible combinations of two characters, and so on. With large data sets and well supported nodes, the computation of CSI can be time-consuming, but short cuts are possible in some situations (Davis, 1993).

The raw number of character removals required to collapse a node has also been suggested as an index of clade stability (Davis, 1993). In the remainder of this paper, we refer to this measure as the character removal index (CRI—Table 1).

**The data set removal index (DRI).** In his concluding remarks, Davis (1993) suggested that, "The CSI also should prove useful in the analysis of relative degrees of stability of clades *among* data sets." An analogous measure at this level would require the sequential removal of data sets as opposed to individual characters. The stability of a particular node to the removal of individual data sets and successively larger combinations of data sets is here termed the data set removal index (DRI—Table 1). For a particular combined data set and a particular node, the DRI is the minimum number of data set removals necessary to collapse that node. A DRI of 1 indicates that only one component data set must be removed from the combined data set

in order to collapse that node. A DRI of 2 indicates that the removal of any single data set does not collapse the node of interest, but the node collapses with the removal of at least one combination of two data sets. For a combined data set with five subsets of characters, a DRI of 5 indicates that all five data sets must be eliminated to lose the node of interest (i.e., each individual data set and each combination of data sets support the node). A group not found in the simultaneous analysis of all data sets has a DRI of 0.

Simple examples are shown in Fig. 1. DRIs are indicated for each of the three divisions (a–c) of the same total data set. In a, the DRI of 3 shows that each component data set and each combination of data sets support the topology favored by the total combined data set. In b, the DRI is 1. The removal of a single data set, I, collapses clade A + B. In c, there is no taxonomic congruence among strict consensus trees derived from different data sets. However, the DRI is 2 because the removal of any one data set does not perturb clade A + B. Hidden support for A + B in data partitions II and III is reflected in the DRI (see Olmstead and Sweere, 1994, for an analogous empirical case).

The CSI identifies particular characters or particular combinations of characters that are critical for the resolution of a node (Davis, 1993). Similarly, the DRI identifies particular data sets or particular combinations of data sets that are critical for the resolution of a node. Like PBS, the DRI accounts for hidden support, hidden conflicts, and the amount of support provided by each data set in simultaneous analysis. However, in contrast to PBS, the DRI subsumes taxonomic congruence. For example, in DRI calculations for a combined data set composed of three data partitions, the data set removals of size two replicate the analyses of individual data sets in the taxonomic congruence approach. The interaction among data sets can be assessed with the DRI, but unlike PBS, portions of the total data set are removed in the various perturbations. Therefore, DRI and PBS scores do not necessarily predict each other. This same relationship exists between the CSI and BS (Davis, 1993).

**Nodal data set influence (NDI).** PBS summarizes the relative contribution of different data sets to the support of particular nodes in simultaneous analysis. Nodal data set influence (NDI—Table 1) is an analogous measure that is quantified through data set removal. NDI assesses the influence of specific data set

removals on levels of BS at a particular node. For a particular combined data set, a particular data partition, and a particular node, NDI is the BS score at that node for the combined data set, minus the BS score at that node for the combined data set without that data partition, or alternatively, PBS for that partition at that node plus the amount of HBS that does not emerge in simultaneous analysis at that node because of the removal of that partition. Given that PBS equals BS plus PHBS for a particular partition (Table 1), NDI is the sum of BS for a particular data partition, PHBS for that partition, and HBS that the partition brings out of other data sets in simultaneous analysis. Simple examples of NDI are shown in Figs. 1–3.

**Hidden nodal data set influence (HNDI).** The effect of a data set removal on the level of hidden support and conflict for a particular node can be quantified with a variation of NDI, hidden nodal data set influence (HNDI—Table 1). For a particular combined data set, a particular data partition, and a particular node, HNDI is the NDI at that node for that partition, minus the BS at that node for that partition, or alternatively, HBS at that node for the total data set, minus the HBS at that node for the total data set without that partition. Note that for a particular node and a particular data set removal, HNDI measures both the HBS in the data set that is removed, *plus* the HBS in the remaining character partitions that fails to emerge in simultaneous analysis because of the data set removal. In contrast, PHBS only measures the hidden support within a particular data partition that comes with simultaneous analysis. The effect of a particular data set removal on the level of HBS in other data partitions can be quantified by subtracting PHBS for the deleted partition from HNDI for that partition, or alternatively, by subtracting PBS for that partition from NDI for that partition.

Simple examples of HNDI are shown in Figs. 2 and 3. In scenario e of Fig. 3, NDI for data set II is +1, and BS in data set II for clade A + B is –1. HNDI for data set II is the difference between these two measures, +2 (Fig. 3). Alternatively, the HNDI for data set II equals the PHBS from data set II (+1) plus the HBS in data set I that does not emerge in simultaneous analysis because of the removal of data set II. Because I and II are the only data sets in scenario e, the removal of data set II eliminates all HBS (+1) in data set I. By definition, if there is only one data set, there can be no HBS.

Therefore, the HNDI in partition II equals the PHBS in partition II plus the PHBS in partition I (Fig. 3). In scenario g of Fig. 3, there is no HNDI. Removal of either data set, I or II, has no effect on the amount of HBS. Figure 2 illustrates a simple case in which there are negative HNDI scores.

**Data set influence (DI).** DRIs show which data sets and combinations of data sets exert the most influence in simultaneous phylogenetic analysis. However, it may also be important to recognize which data sets have the least influence in simultaneous analysis. Baker and DeSalle (1997) and Baker *et al.* (1998) used the sum of all PBS scores for a particular data set to summarize the relative contribution of that data set to the simultaneous analysis. Data set influence (DI—Table 1) is an alternative measure of the relative importance of data sets in simultaneous analysis. DI measures the effect of a particular data set removal on levels of BS over the entire cladogram. For a particular combined data set and a particular data partition, DI is the sum of NDI scores for that partition at each node supported by the total combined data set. The DI differs from the sum of PBS in recognizing HBS that a data set brings out of other data sets in simultaneous analysis. DI can be divided by the minimum possible number of steps for informative characters in a data set to make DIs from data sets of different sizes more comparable (scaled DI).

### **Artiodactyl Relationships: Background**

In the following section, BS, PBS, HBS, PHBS, HS, DRI, NDI, HNDI, and DI are utilized in a cladistic analysis of Artiodactyla, even-toed hoofed mammals. Because there are multiple sources of character information for Artiodactyla, this group is ideal for demonstrating the methods presented above. Extensive character conflicts among data sets have been recorded within Artiodactyla (Gatesy, 1998). We contend that it is valid to combine the data sets evaluated here and explore the importance of particular data sets and combinations of data sets in simultaneous phylogenetic analysis.

Higher level artiodactyl relationships have been vigorously debated over the past 15 years. The primary controversy centers on whether Cetacea (whales, dolphins, and porpoises) should be included within Artiodactyla (Graur and Higgins, 1994). However, even disregarding the placement of Cetacea, morphological

estimates of artiodactyl phylogeny contrast sharply with molecular topologies (Gentry and Hooker, 1988; Thewissen, 1994; Gatesy *et al.*, 1996; Gatesy, 1998). The affinities of Hippopotamidae (hippos) are particularly contentious (e.g., Pickford, 1983, Sarich, 1993; Irwin and Arnason, 1994). Nuclear DNA sequences (Gatesy *et al.*, 1996; Gatesy, 1997, 1998) and retropositional insertions (Shimamura *et al.*, 1997) suggest a clade composed of Hippopotamidae, Pecora (antelopes, giraffes, deer, and kin), Tragulidae (chevrotains), and Cetacea with other extant artiodactyl taxa more distantly related. In contrast, morphological characters support a monophyletic Artiodactyla in which Hippopotamidae, Suidae (pigs), and Tayassuidae (peccaries) cluster. In this scheme, the other major clade of extant artiodactyls, Selenodontia, is made up of Camelidae (camels), Tragulidae, and Pecora (Gentry and Hooker, 1988).

## **MATERIALS AND METHODS**

### **Data**

Seventeen data sets were compiled from the literature. Each data set includes representatives from the four basic subdivisions of extant artiodactyls (Ruminantia = Pecora + Tragulidae, Suina = Suidae + Tayassuidae, Camelidae, and Hippopotamidae), at least one cetacean exemplar (an exception is the morphological data set that is coded just for Artiodactyla), and an outgroup taxon. Data sets include: nuclear (nu) amino acid sequences ( $\alpha$ -hemoglobin,  $\beta$ -hemoglobin, pancreatic ribonuclease,  $\alpha$ -crystallin A, and cytochrome *c*), mitochondrial (mt) DNA sequences (cytochrome *b*, 12S ribosomal [r] DNA, and 16S rDNA), nu DNA sequences ( $\beta$ -casein exon 7,  $\beta$ -casein intron 7,  $\kappa$ -casein exon 4,  $\gamma$ -fibrinogen exons 2–4,  $\gamma$ -fibrinogen introns 2–3, protamine P1 exons 1–2 + 5' noncoding region + 3' noncoding region, and protamine P1 intron 1), skeletal/dental characters (Gentry and Hooker, 1988), and SINE retropositional insertions (Shimamura *et al.*, 1997). Amino acid sequences were downloaded from NCBI, and DNA sequences are from NCBI and Gatesy (1998). Species sampled for each data set are shown in Appendix 1.

There are many opinions on what constitutes a systematic data set (see Kluge, 1989, and Miyamoto and

Fitch, 1995, for two extremes). In taxonomic congruence, a consensus of fundamental cladograms determines the final scheme of relationships. Therefore, the delineation of data sets is critical. With simultaneous analysis, data sets are pooled, so the definition of data sets has no impact on final systematic results. In this combined framework, character sets should be defined so as to gain insights into the distribution of nodal support and conflict among biologically important data partitions (e.g., DeSalle and Brower, 1997). For example, a separation of molecular data from morphological data would highlight discrepancies between characters that presumably have very different evolutionary constraints. Mt and nu partitions would illuminate congruence between these genomes, and separation of individual genetic loci might suggest previously undetected gene duplications (i.e., paralogy). In addition to these partitioning scenarios, there are many other potentially useful ways that a combined data set could be divided.

In this study, distinctions were made between the transposon data, the morphological characters, and specific genes or gene products (see above). There has been some concern in the literature that protein-coding DNA sequences may be prone to convergence in aquatic taxa such as Hippopotamidae and Cetacea (Gatesy *et al.*, 1996; Montgelard *et al.*, 1997; Theodor, 1997). For example, given that *Hippopotamus* and cetaceans nurse their offspring underwater, it is conceivable that sequence similarities in the milk proteins of these taxa are due to functional convergence. Introns that do not code for functional proteins should be less prone to this type of selective convergence. Therefore, in this paper, introns and exons from the same gene were considered different data sets in order to partition support and conflict among these different genic regions.

### Sequence Alignment

**Amino acid sequences.** The alignments were trivial. No internal gaps were introduced into alignments for  $\alpha$ -hemoglobin,  $\beta$ -hemoglobin,  $\alpha$ -crystallin A, cytochrome *c*, and pancreatic ribonuclease.

**DNA sequences.** The alignments for mt cytochrome *b*,  $\gamma$ -fibrinogen,  $\kappa$ -casein,  $\beta$ -casein, and protamine P1 are variants of computer-generated MALIGN (Wheeler and Gladstein, 1994) output for a variety of parameter

settings. Some alignment gaps were consolidated by eye using SeqApp (Gilbert, 1992); these adjustments greatly reduced the number of gaps and the cost of each multiple alignment (see Gatesy, 1997). Alignments for mt cytochrome *b*,  $\gamma$ -fibrinogen exons 2–4,  $\gamma$ -fibrinogen introns 2 and 3,  $\beta$ -casein exon 4,  $\beta$ -casein exon 7, and  $\beta$ -casein intron 7 are identical to those shown in Gatesy (1998). The alignment for protamine P1 (exons 1 and 2) is slightly different from the alignment in Gatesy (1998). The removal of several distant outgroup taxa warranted the rearrangement of some gaps. The alignment for protamine P1 intron 1 is new to this study.

The 12S and 16S mt rDNA genes are more prone to insertion and deletion events than the other loci. These genes were each aligned algorithmically with MALIGN using the following parameters: leading = 3, trailing = 3, internal = 4, changecost = 2, contig, score 3, quick, alignaddswap, alignswap, lowmem, iter (Wheeler and Gladstein, 1994). A discussion of alignment ambiguity in the artiodactyl mt rDNAs and nu introns is in preparation (Gatesy and O'Grady, in prep). All data sets are available at the web site for the AMNH molecular lab: [research.amnh.org/molecular/sequence.html](http://research.amnh.org/molecular/sequence.html).

### Phylogenetic Analysis

**Amino acid sequences.** Transformations between any two amino acids were assigned unit cost with the "datatype=protein" command in PAUP 3.1.1 and PAUP\* 4.0d59 – 4.061a (Swofford, 1993; in prep). Searches were branch and bound or heuristic with minimally 100 random taxon addition replicates and TBR branch swapping. All characters were unordered, gaps were treated as missing data, and branches with maximum length of zero were collapsed. All character transformations were equally weighted.

**DNA sequences.** For all DNA sequence data, nucleotide base ambiguities were coded as N (any base). Cladistic analyses of each alignment were done using PAUP 3.1.1 or PAUP\* (Swofford, 1993; in prep.). Searches were either branch and bound or heuristic as described above.

**Retroposons.** The presence and absence of SINE retroposons at specific loci were coded as in Shimamura *et al.* (1997). Gains and losses of retroposons were given equal weight in phylogenetic analysis. The search was branch and bound as described above.

**Morphological data.** The relatively primitive, extinct Leptomerycidae is more closely related to Pecora than to Tragulidae and other extant artiodactyls (Webb and Taylor, 1980; Gentry and Hooker, 1988). Because extant pecoran families were not scored in Gentry and Hooker's (1988) higher level artiodactyl matrix, dental and skeletal characters for Leptomerycidae were assigned to each of the pecoran artiodactyl families in the combined data set (Bovidae, Cervidae, and Giraffidae). Characters were unordered and the search was branch and bound as described above.

**Combined analyses.** To combine amino acid sequences, DNA sequences, retroposons, and morphological characters from the 17 separate data sets in one simultaneous analysis, the "datatype=protein" command of PAUP 3.1.1 and PAUP\* (Swofford, 1993; in prep) was used. This option includes character states for all 20 amino acids as well as some ambiguity codes for amino acids. Extra character states (0, 1, 2, and 3) were added with the "symbols" command to accommodate the morphological data and the retroposon characters. The DNA data were incorporated into the combined data set by converting all IUPAC ambiguities in the DNA data to X, the ambiguity code for any amino acid. All character transformations were given equal weight, and the parsimony search was heuristic as described above.

### Outgroups

Outgroup relationships of Artiodactyla are not generally agreed upon. Some morphological data sets suggest that Cetacea is only distantly related to Artiodactyla. In this scheme, Cetacea is aligned with Perissodactyla (odd-toed hoofed mammals) and/or Paenungulata (elephants, hyaxes, and manatees) to the exclusion of Artiodactyla (Prothero *et al.*, 1988; Novacek, 1989; Thewissen, 1994). However, the majority of molecular evidence as well as several complex morphological characters support a close relationship between artiodactyls and cetaceans (reviewed in Novacek, 1992; Thewissen, 1994; Gatesy, 1998). Perissodactyla appears to be the extant sister group to artiodactyls + cetaceans (e.g., Shoshani, 1986; Stanhope *et al.*, 1996; Gatesy, 1998), but again this relationship remains controversial (e.g., Xu *et al.*, 1996).

In this study, all molecular trees for artiodactyls + cetaceans were rooted with perissodactyl exemplars.

This rooting assumes that cetaceans and artiodactyls are more closely related to each other than either is to Perissodactyla. The hypothesis is strongly supported by phylogenetic analyses of complete mt genomes (Allard and Carpenter, 1996; Xu *et al.*, 1996; Zardoya and Meyer, 1996) and five nu loci (Stanhope *et al.*, 1996; Gatesy, 1998). The morphological topology for Artiodactyla was rooted with the hypothetical ancestor characterized by Gentry and Hooker (1988). In simultaneous analyses of molecular and morphological data sets, this hypothetical ancestor was equated with Perissodactyla.

### DRI

DRI were calculated as follows. First, single data sets were sequentially removed from the combined artiodactyl data set. This resulted in 17 combined data sets that were each composed of 16 data sets. Each combination of 16 data sets was analyzed with PAUP\* (Swofford, in prep). Searches were heuristic and included 100 random taxon addition replicates and TBR branch swapping. Nodes that collapsed with the removal of a single data set were noted, as were the particular data set removals that collapsed the node. Next, all possible combinations of two data sets were sequentially removed from the total data set. Each resulting combination of 15 data sets was then analyzed as above. Finally, data set removals of sizes 3, 15, and 16 were each analyzed as above. For each node in the total data tree, the DRI, the specific data set removals that collapse the node and the percentage of data set removals of a given class (e.g., one data set removals, two data set removals, etc.) that collapse the node were recorded.

### BS, PBS, HBS, PHBS, NDI, HNDI, and DI

BS, PBS, HBS, PHBS, NDI, HNDI, and DI were calculated with PAUP 3.1.1 and PAUP\* (Swofford, 1993; in prep.). For each clade of interest, the "constraints" command of PAUP was used to force the monophyly or nonmonophyly of that group. Minimum length constrained topologies were derived from branch and bound searches or heuristic searches with 50 random taxon addition replicates and TBR branch swapping. Tree lengths from constrained searches were compared to tree lengths for unconstrained searches to determine

BS, PBS, HBS, PHBS, NDI, HNDI, and DI. All statistics were calculated as described under Definitions and Examples above. In calculations of the scaled DI, the minimum number of character steps for a data set was determined with MacClade 3.03 (Maddison and Maddison, 1992). The following values were measured: positive BS for each node supported by the combined data set, positive BS for each node supported by each individual data set, positive and negative BS for each individual data set for each node in the total data tree, PBS for each data set for each node in the total data tree, HBS for each node in the total data tree, PHBS for each data set for each node in the total data tree, DI for each individual data set, scaled DI for each data set, NDI scores for particular data sets or combinations of data sets that collapse nodes in the DRI analyses described above, NDIs for each data set at each node in the total data tree, and HNDIs for each data set at each node in the total data tree.

## HS

PAUP\* was used to identify HS in the artiodactyl matrix. The "list of apomorphies" option of PAUP\* was used to diagnose selected clades supported by the simultaneous analysis of 17 data sets. Unambiguous synapomorphies for these clades were tabulated for the combined data set and for each of the 17 individual data sets. Differences in the number of unambiguous synapomorphies in simultaneous analysis versus separate analysis for each data set determined the extent of HS.

## Consensus Trees–Taxonomic Congruence

Trees derived from each of the 17 artiodactyl data sets were compared to assess topological similarity. Because of an uneven sampling of taxa among the 17 data sets (Appendix 1), several approaches were taken. First, topological relationships of the five taxa that are common to all 17 data sets (Bovidae, Camelidae, Hippopotamidae, Suidae, and outgroup) were compared. Each reduced data set was subjected to branch and bound parsimony searches with PAUP 3.1.1 (Swofford, 1993). Clades supported by two or more of the 17 data sets were noted, and a strict consensus tree (Schuh and Polhemus, 1980), a semistrict consensus tree (Bremer, 1990), and a 50% majority rule consensus tree (Margush

and McMorris, 1981) were constructed from optimal trees or strict consensus trees for each data set. Second, topological relationships of the six taxa that are common to 16 of the 17 data sets (Bovidae, Camelidae, Hippopotamidae, Suidae, Mysticeti, and Perissodactyla) were compared. Clades supported by 2 or more of the 16 data sets were noted, and consensus trees were constructed. Third, the 8 data sets that contain representatives of all 13 taxa sampled in this study were compared as above. Clades supported by 2 or more of the 8 data sets were noted, and consensus trees were constructed.

## Incongruence Length Difference (ILD) Tests

The ILD test (Farris *et al.*, 1994a) was used to assess the null hypothesis of congruence between data sets. This procedure employs the ILD, the number of extra character steps gained by combining data sets in simultaneous analysis (Mickey and Farris, 1981), as a test statistic. ILDs derived from random partitions of the combined data set determine the extremity of a particular empirical ILD (Farris *et al.*, 1994a). In all ILD tests described below, uninformative characters were excluded, and searches were heuristic with simple taxon addition and TBR branch swapping. To establish a null distribution for each test, 999 random data partitions were generated, and ILDs were calculated for each replicate with PAUP\* (Swofford, in prep.). *P* of 0.05 was taken as the threshold for significance.

Four groups of ILD tests were done. Group 1 included taxa with extensive missing data. Groups 2–4 had little missing data.

(1) Each individual data set was compared to the sum of the characters from the other 16 data sets. In each test, taxa were limited to those in the individual data set. For example, in the comparison of cytochrome *c* to the remaining characters in the combined data set, the ILD test was applied to the six taxa in the cytochrome *c* data set.

(2) ILD tests were restricted to the five taxa common to all 17 data sets (Bovidae, Hippopotamidae, Suidae, Camelidae, and outgroup). Each individual data set was compared to the sum of the characters from the other 16 data sets.

(3) ILD tests were restricted to the six taxa common to 16 of the 17 data sets (Bovidae, Hippopotamidae, Suidae, Camelidae, Mysticeti, and Perissodactyla).

Each individual data set was compared to the sum of the characters from the other 15 data sets.

(4) ILD tests were restricted to the eight data sets sampled for all 13 taxa. Each individual data set was compared to the sum of characters from the other seven data sets.

### *Additional Phylogenetic Searches*

Additional phylogenetic searches were executed for three large data sets (mt cytochrome *b*: 99 taxa,  $\beta$ -casein exon 7:68 taxa, and a combined matrix of seven genes: 76 taxa). Sequences for these analyses were from GenBank and Gatesy (1998). Alignments were from Gatesy (1998). Phylogenetic analyses with PAUP were as for the other DNA data sets above, but nucleotide ambiguities were coded according to IUPAC rules. PAUP results for each matrix were checked with NONA 1.16 (Goloboff, 1993a). Search options in NONA were hold\*, hold/1000, pack, amb-, and mult\*100. In contrast to PAUP, the "amb-" option in NONA collapses nodes that are ambiguously supported. Differences in numbers of optimal trees found with the different programs were noted, and strict consensus trees were compared. Topologies were rooted with Xenarthra, the putative sister group of other extant eutherians (Miyamoto and Goodman, 1986), or Monotremata, the proposed sister group of Theria (Novacek, 1989).

## RESULTS/DISCUSSION

### *Individual Data Sets: Taxonomic Congruence*

The individual data sets support a variety of topologies (Fig. 4). Some of the strict consensus trees derived from the individual analyses are poorly resolved ( $\alpha$ -crystallin A and pancreatic ribonuclease), others are

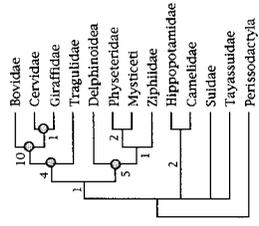
well resolved but wildly contradictory (e.g., protamine P1 intron 1 versus  $\alpha$ -hemoglobin), and several are well resolved and highly congruent ( $\beta$ -casein exon 7,  $\beta$ -casein intron 7,  $\kappa$ -casein,  $\gamma$ -fibrinogen exons,  $\gamma$ -fibrinogen introns, and SINE retroposons). BS for individual nodes supported by the separate analyses ranges from +1 to +23 (Fig. 4).

Figure 5 shows results for the taxonomic congruence approach taken in this report. For the five taxa common to all 17 data sets, strict, semistrict, and 50% majority rule consensus trees derived from the 17 separate analyses are totally unresolved (Fig. 5A). Seven components are replicated two or more times among the 17 separate analyses (Fig. 5B and C). Of these seven components, two are consistent with the single topology derived from simultaneous analysis of all 17 data sets (Fig. 5B). Only one of these seven components, the Hippopotamidae + Bovidae clade, is replicated more than three times (Fig. 5B).

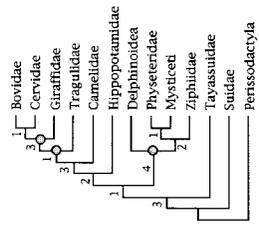
For the six taxa common to 16 of the 17 data sets, the strict, semistrict, and 50% majority rule consensus trees derived from the 16 individual analyses are totally unresolved (Fig. 5D). Eight components are replicated two or more times (Figs. 5E and 5F). Of these eight components, three are consistent with the single topology derived from simultaneous analysis of all 16 data sets. These three are the only nodes replicated more than twice (Fig. 5E).

For the eight data sets with "complete" taxonomic sampling, the strict and semistrict consensus trees are poorly resolved, and the 50% majority rule consensus tree has five components (Fig. 5G). Fourteen components are replicated two or more times in the eight separate analyses (Figs. 5H and 5I). Of these 14 components, 10 are consistent with the single topology derived from simultaneous analysis of all eight data sets (Fig. 5H).

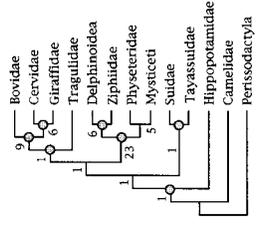
FIG. 4. Strict consensus trees of minimum length topologies for each of the 17 artiodactyl data sets and the simultaneous analysis of all 17 data sets. For each topology, the following information is given: number of informative characters (inf. chars.), number of equally parsimonious trees, tree length, the number of extra steps required to fit the data set onto the total data topology (in parentheses following the tree length), consistency index disregarding uninformative characters (CI—Kluge and Farris, 1969), and retention index (RI—Farris, 1989). Positive BS is shown at internodes, and nodes consistent with the total data tree are marked by gray dots. Species for each higher level taxonomic group are listed in Appendix 1.



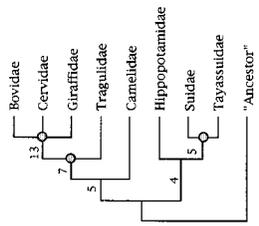
**16S rDNA**  
103 inf. chars.  
1 tree, 483 (13) steps  
CI=0.4844, RI=0.4695



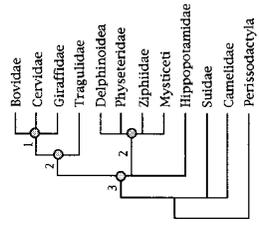
**12S rDNA**  
87 inf. chars.  
1 tree, 335 (22) steps  
CI=0.4944, RI=0.4380



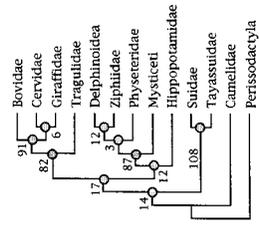
**CYTOCHROME B**  
394 inf. chars.  
1 tree, 1584 (112) steps  
CI=0.4507, RI=0.3184



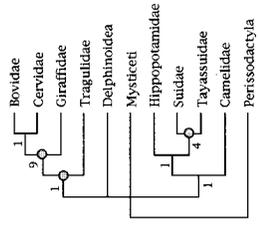
**SKELETAL/DENTAL**  
62 inf. chars.  
1 tree, 137 (10) steps  
CI=0.7333, RI=0.7956



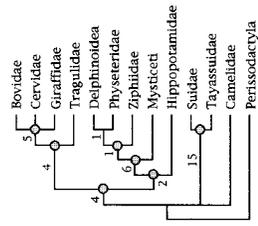
**SINE RETROPOSONS**  
8 inf. chars.  
1 tree, 9 (0) steps  
CI=1.0000, RI=1.0000



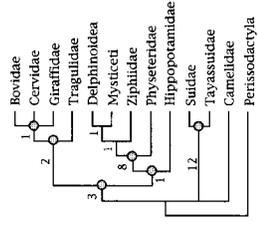
**ALL DATA**  
1291 inf. chars.  
1 tree, 4822 steps  
CI=0.5417, RI=0.4980



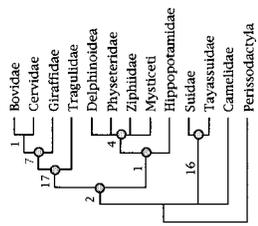
**PROTAMINE P1 INTRON 1**  
37 inf. chars.  
5 trees, 170 (8) steps  
CI=0.6386, RI=0.6591



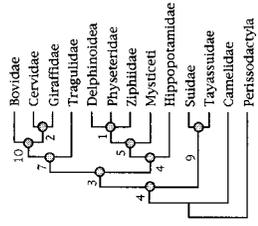
**Y-FIBRINOGEN INTRONS 2-3**  
69 inf. chars.  
4 trees, 247 (2) steps  
CI=0.6901, RI=0.7143



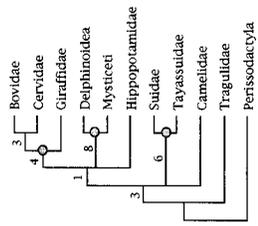
**Y-FIBRINOGEN EXONS 2-4**  
56 inf. chars.  
4 trees, 161 (2) steps  
CI=0.6887, RI=0.7273



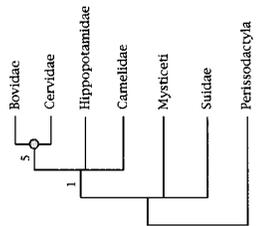
**K-CASEIN EXON 4**  
97 inf. chars.  
28 trees, 362 (1) steps  
CI=0.6845, RI=0.6991



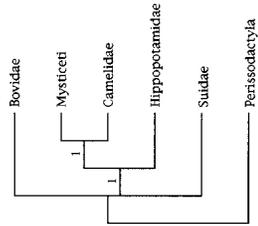
**B-CASEIN EXON 7**  
91 inf. chars.  
2 trees, 360 (0) steps  
CI=0.6911, RI=0.7163



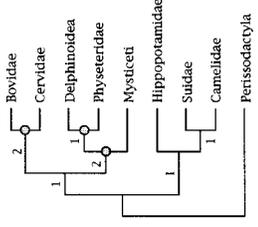
**PROTAMINE P1 EXONS 1 and 2**  
62 inf. chars.  
4 trees, 231 (8) steps  
CI=0.6014, RI=0.5714



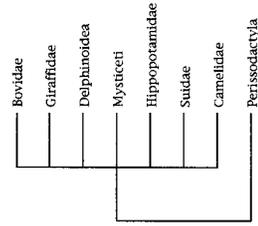
**PANCREATIC RIBONUCLEASE**  
23 inf. chars.  
2 trees, 102 (5) steps  
CI=0.7400, RI=0.8306



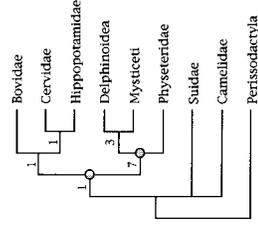
**CYTOCHROME C**  
2 inf. chars.  
1 tree, 7 (2) steps  
CI=1.0000, RI=1.0000



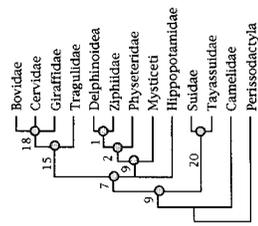
**B-HEMOGLOBIN**  
24 inf. chars.  
1 tree, 99 (5) steps  
CI=0.7167, RI=0.6047



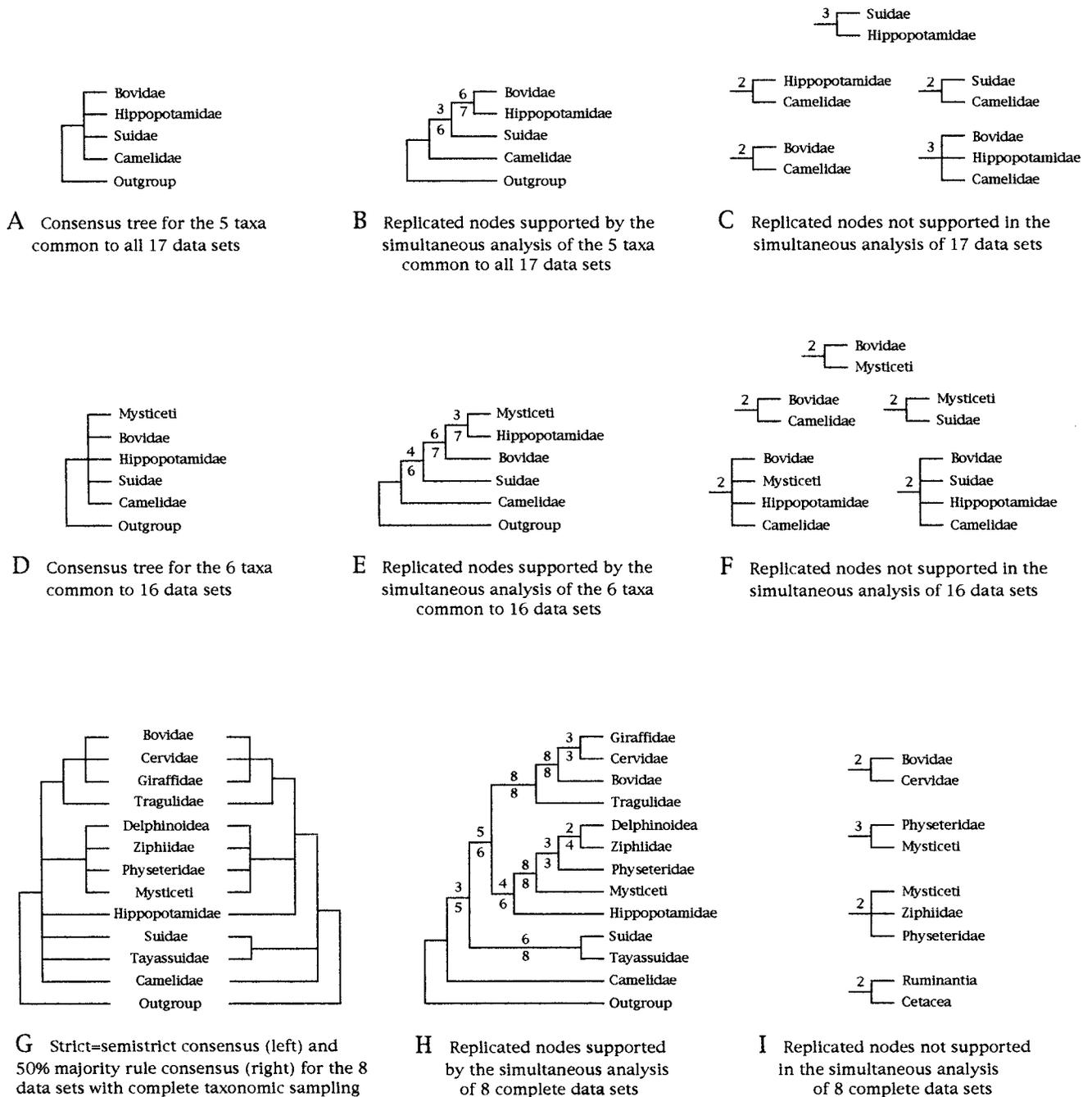
**A-CRYSTALLIN A**  
5 inf. chars.  
2 trees, 12 (2) steps  
CI=0.6250, RI=0.5714



**B-HEMOGLOBIN**  
26 inf. chars.  
2 trees, 97 (5) steps  
CI=0.7419, RI=0.6735



**B-CASEIN INTRON 7**  
145 inf. chars.  
6 trees, 477 (0) steps  
CI=0.7034, RI=0.7555



**FIG. 5.** Taxonomic congruence for the 17 artiodactyl data sets. (A) Strict = semistrict = 50% majority rule consensus for the five taxa common to all 17 data sets. (B) The minimum length topology for simultaneous analysis of the 17 artiodactyl data sets for the five taxa common to all 17 data sets. (C) Nodes replicated in the separate analyses of the 17 individual data sets that conflict with the simultaneous analysis of all 17 data sets. (D) Strict = semistrict = 50% majority rule consensus for the six taxa common to 16 artiodactyl data sets. (E) The minimum length topology for simultaneous analysis of the 16 artiodactyl data sets that were sampled for the six taxa shown. (F) Nodes replicated in the separate analyses of the 16 individual data sets that conflict with the simultaneous analysis of all 16 data sets. (G) Consensus trees derived from separate analyses of the eight artiodactyl data sets with complete taxonomic sampling. (H) The minimum length topology for simultaneous analysis of the eight data sets with complete taxonomic sampling. (I) Nodes replicated in the separate analyses of the eight taxonomically complete data sets that are inconsistent with the simultaneous analysis of all eight data sets. For B, E, and H, the number of times each node was replicated in the separate analyses is shown above internodes, and the number of positive PBS scores in simultaneous analysis is shown below internodes. For C, F, and I, the number of times each node was replicated in the separate analyses is shown above internodes.

## Simultaneous Analysis

Simultaneous analysis of the 17 artiodactyl data sets results in one fully resolved topology (Fig. 4). For the 10 nodes favored by this analysis, BS scores range from +3 to +108, and 8 of these 10 nodes have BS scores greater than +10. Four nodes, Pecora (Cervidae + Bovidae + Giraffidae), Ruminantia (Pecora + Tragulidae), Cetacea (Physeteridae + Ziphiidae + Mysticeti + Delphinoidea), and Suina (Suidae + Tayassuidae), are characterized by BS scores greater than +80 (Fig. 4).

Extra steps are required to fit 14 of the 17 individual data sets to the topology supported by simultaneous analysis of all 17 data sets.  $\beta$ -Casein exon 7,  $\beta$ -casein intron 7, and the SINE retroposons are exceptions and require no additional steps on the total data tree (Fig. 4).

## PBS

PBS scores for each node supported by the simultaneous analysis are shown in Fig. 6, and the sums of PBS scores for each data set are shown in Fig. 7A. The nodes with the three highest BS scores, Pecora, Cetacea, and Suina, have no negative PBS scores. Weakly supported clades such as Cervidae + Giraffidae and Odontoceti (Ziphiidae + Physeteridae + Delphinoidea) show a more even mixture of positive and negative PBS scores. Ruminantia has the most positive PBS scores in the analysis (14), and Cervidae + Giraffidae has the least (three). Cervidae + Giraffidae is the only node supported by the simultaneous analysis that has more negative (four) than positive (three) PBS scores. This is due to the extent of positive support for this relationship from mt cytochrome *b* (+8) and the relative indifference of the 16 other data sets at this node (Fig. 6).

As in many previous studies, the simultaneous analysis does not support the monophyly of Artiodactyla (Graur and Higgins, 1994). Indeed, Cetacea is nested three nodes within Artiodactyla. The node that joins Cetacea with Ruminantia, Hippopotamidae, and Suina to the exclusion of Camelidae has a BS score of +14, but 5 data sets contradict this controversial group (Fig. 6). 12S mt rDNA has a PBS score of -8 at this node; this is the smallest PBS score at any node in the simultaneous analysis. The Cetacea + Hippopotamidae clade has a slightly lower BS score of +12, but has net positive support from 7 data sets (Fig. 6). Nine of the 17 data sets contribute positive PBS to the Cetacea + Hippopotamidae + Ruminantia node (BS = +17). The only

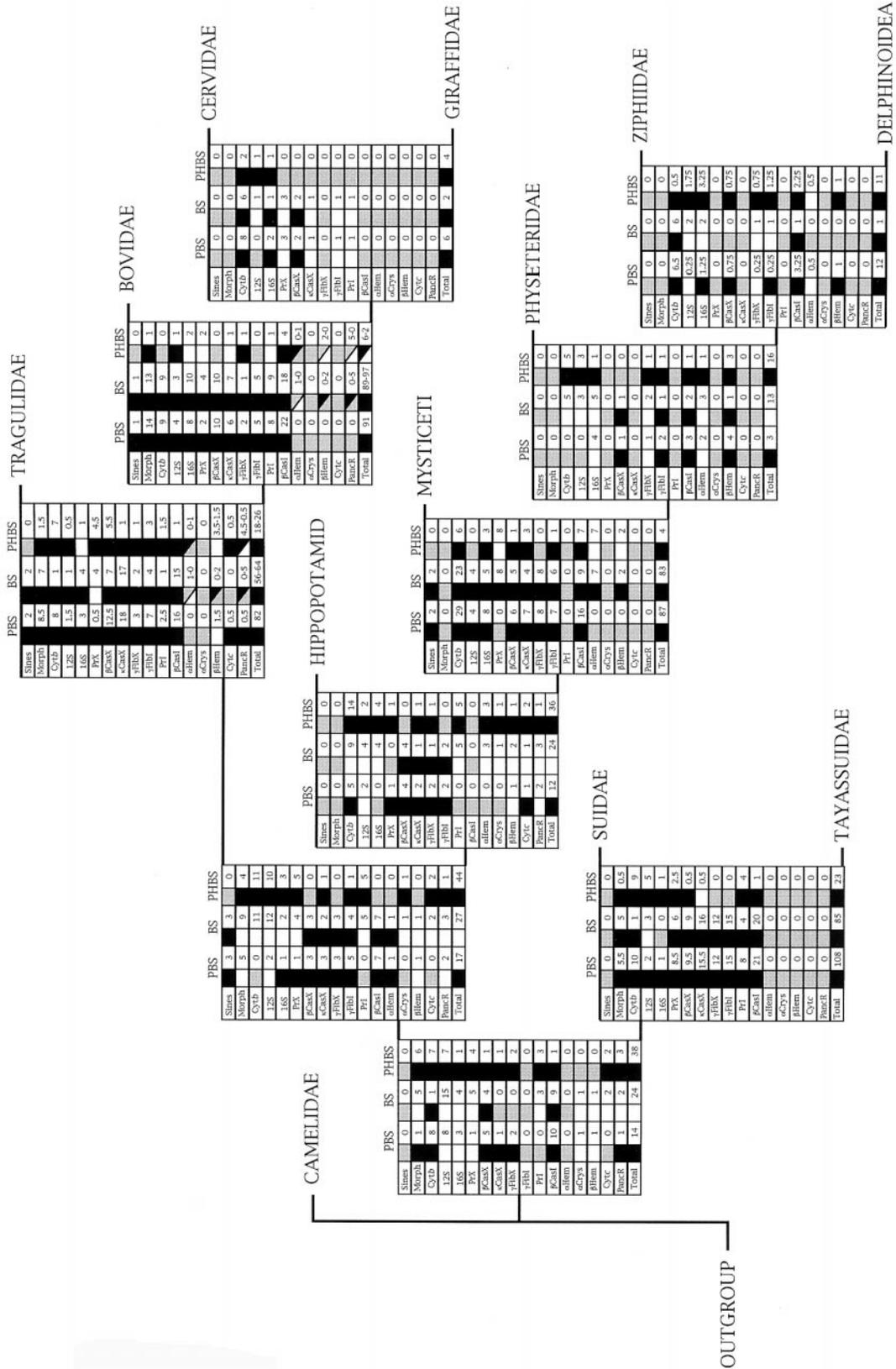
substantive conflict comes from the skeletal and dental evidence (PBS = -5).

Relationships within Cetacea are not the focus of this study. However, two subclades of cetaceans are supported in the combined analysis (Fig. 4). A monophyletic Odontoceti (Physeteridae + Delphinoidea + Ziphiidae) contradicts both separate and combined analyses of mt cytochrome *b*, 12S mt rDNA, and 16S mt rDNA that unanimously group Physeteridae (sperm whales) and Mysticeti (baleen whales) to the exclusion of Delphinoidea (dolphins, porpoises, and kin—Milinkovitch *et al.*, 1993, 1994, 1995, 1996). In the combined analysis presented here, PBS at the odontocete node is negative for only one of the mt genes, 16S rDNA (PBS = -4); the other mt genes have PBS scores of zero at this node (Fig. 6). Positive PBS for Odontoceti comes from four data sets, but negative PBS comes from three partitions. A subclade of Odontoceti, Delphinoidea + Ziphiidae (beaked whales), is corroborated by six data sets. These partitions include two mt genes, three nu DNA data sets, and one set of nu amino acid sequences (Fig. 6).

## HBS

HBS at each node and PHBS at each node for each data set are shown in Fig. 6. Nine of 10 nodes in the total data tree are characterized by net positive HBS. The 3 nodes that support the derivation of Cetacea from within Artiodactyla have by far the largest amount of HBS. The Hippopotamidae + Cetacea clade has an HBS score of +36, and 12 data sets have positive PHBS at this node. For the Hippopotamidae + Cetacea + Ruminantia clade these values are +44/11, and for the Hippopotamidae + Cetacea + Ruminantia + Suina clade these values are +38/12. There are no negative PHBS scores at any of these nodes (Fig. 6). HBS totals to +118 for these three clades, and the sum of BS at these nodes is just +43. The only other node that has more HBS than BS is Odontoceti (HBS = +16, BS = +3). The node for Pecora has the least HBS and is characterized by six negative PHBS scores (Fig. 6).

There is net positive HBS in 14 of the 17 data sets (Fig. 7C). The HBS for some data sets is remarkably high. For example, mt cytochrome *b* has more HBS on the total data tree (+61.5) than BS on its own shortest tree (+54). 12S mt rDNA, cytochrome *c*,  $\alpha$ -crystallin



A, and protamine P1 intron 1 show similar patterns.  $\alpha$ -Hemoglobin and  $\beta$ -hemoglobin are the only data sets with net negative HBS (Fig. 7C).

## HS

HS in the combined analysis was determined for three critical clades: Hippopotamidae + Cetacea, Hippopotamidae + Cetacea + Ruminantia, and Hippopotamidae + Cetacea + Ruminantia + Suina. There is an abundance of HS for each of these clades (Table 2).

Forty-one unambiguous synapomorphies support Hippopotamidae + Cetacea in the simultaneous analysis; 26 of these synapomorphies are derived from data sets that do not support this clade in separate analyses (HS = +26). There are 12 data sets with positive PHBS at this node (Fig. 6). Of these 12 data sets, there is positive HS in 7. Five data sets with positive PHBS have zero HS (Table 2), so PHBS in these cases is due solely to the dispersion of homoplasy in simultaneous analysis. Conversely, the  $\beta$ -casein intron 7 data set has HS of +2 and zero PHBS. Character conflicts offset the HS provided by the  $\beta$ -casein intron 7 data set.

Eleven data sets have positive PHBS for Hippopotamidae + Cetacea + Ruminantia (Figure 6). Of these 11, 6 have positive HS, and total HS is +8. The  $\alpha$ -hemoglobin data set provides more unambiguous synapomorphies for this clade in separate than in simultaneous analysis (Table 2) and illustrates a case of hidden conflict in terms of synapomorphy (HS = -1).

For Hippopotamidae + Cetacea + Ruminantia + Suina, total HS is +22. Twelve data sets have positive PHBS scores at this node (Fig. 6). There is positive HS for 7 of these 12 partitions, and three partitions with zero PHBS have positive HS. (Table 2). In terms of synapomorphy, hidden support is offset by hidden

conflict for the mt cytochrome *b* data set (HS = 0). Eight unambiguous synapomorphies for Hippopotamidae + Cetacea + Ruminantia + Suina emerge in simultaneous analysis, but eight unambiguous synapomorphies that support this clade in separate analysis do not unambiguously support the clade in simultaneous analysis.

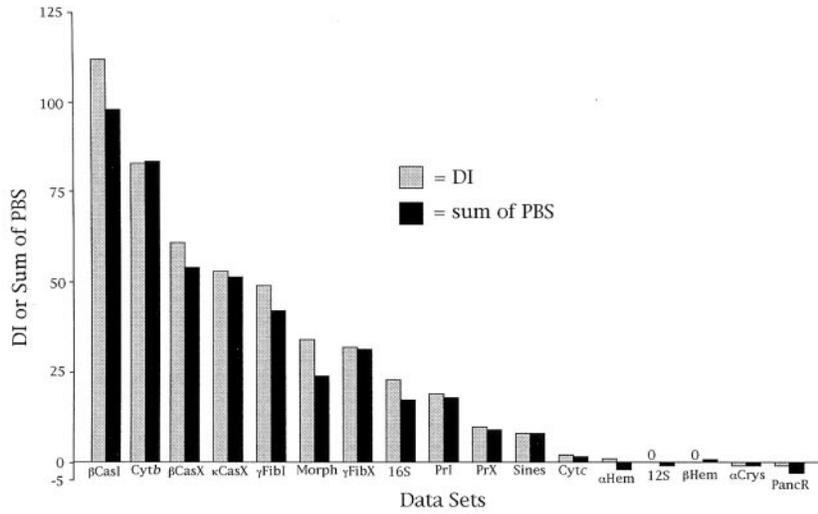
## DRIs

DRIs for the simultaneous analysis of 17 artiodactyl data sets are shown in Fig. 8. The nodes with the four highest BS scores also have the highest DRIs. Pecora, Ruminantia, Cetacea, and Suina each have DRIs greater than 3, so at least four data sets must be removed to lose these four nodes. Similarly, the least stable nodes according to the DRIs are the least stable nodes with reference to BS scores. Cervidae + Giraffidae (BS = +6) and Odontoceti (BS = +3) each have DRIs of 1. The removal of either of two data sets collapses the odontocete node.  $\beta$ -Hemoglobin is not sampled for all odontocete taxa in our analysis and represents only 2% of the informative characters. However, the dismissal of this seemingly insignificant, incompletely sampled data set collapses Odontoceti (Fig. 8).

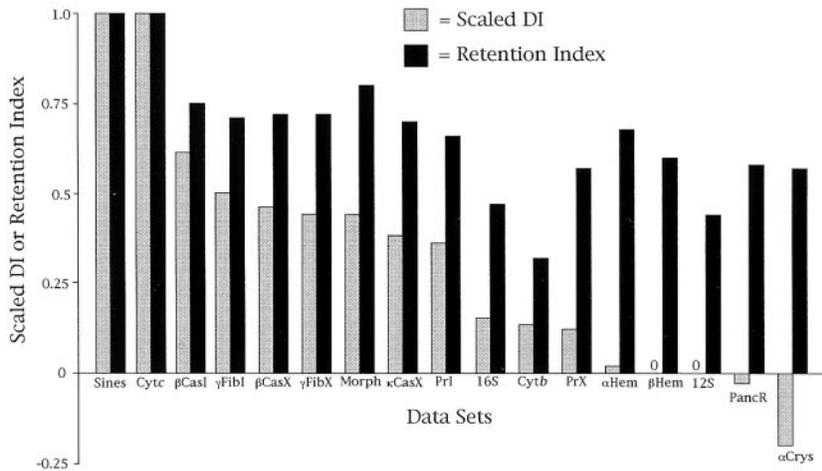
Cetacea + Hippopotamidae, Cetacea + Hippopotamidae + Ruminantia, and Cetacea + Hippopotamidae + Ruminantia + Suina are also relatively unstable to data set removal. The deletion of two crucial data sets collapses all three of these nodes (DRI = 2). These key data sets are from the same nu locus,  $\beta$ -casein (Fig. 8). The importance of  $\beta$ -casein intron 7 for the resolution of the Hippopotamidae + Cetacea node is not obvious from either separate analyses of individual data sets or PBS scores.  $\beta$ -Casein intron 7 does not support Hippopotamidae + Cetacea on its own (BS =

**FIG. 6.** BS, PBS, HBS, and PHBS in the simultaneous analysis of 17 artiodactyl data sets. At each node, the following information is given: PBS for each data set, the sum of all PBS scores (= BS), positive, zero, or negative BS for each partition, the sum of these BS scores, PHBS for each data set, and the sum of all PHBS scores (= HBS). Positive values are marked by black boxes to the left, negative values are marked by white boxes to the left, and values of zero are marked by gray boxes to the left. Because of missing taxa for several data sets, some BS scores are ambiguous. That is, BS cannot be unambiguously assigned to one node. In these cases, the range of BS scores is given (e.g., "1-0" = BS from negative one to zero). Data sets are abbreviated as: Sines = SINE retroposons, Morph = skeletal/dental characters, Cytb = mt cytochrome *b*, 12S = 12S mt rDNA, 16S = 16S mt rDNA, PrX = protamine P1 exons 1-2 + 5' and 3' noncoding regions,  $\beta$ CasX =  $\beta$ -casein exon 7,  $\kappa$ CasX =  $\kappa$ -casein exon 4,  $\gamma$ FibX =  $\gamma$ -fibrinogen exons 2-4,  $\gamma$ FibI =  $\gamma$ -fibrinogen introns 3-4, PrI = protamine P1 intron 1,  $\beta$ CasI =  $\beta$ -casein intron 7,  $\alpha$ Hem =  $\alpha$ -hemoglobin,  $\alpha$ Crys =  $\alpha$ -crystallin A,  $\beta$ Hem =  $\beta$ -hemoglobin, Cytc = cytochrome *c*, PancR = pancreatic ribonuclease. Abbreviations for indices are as in Table 1.

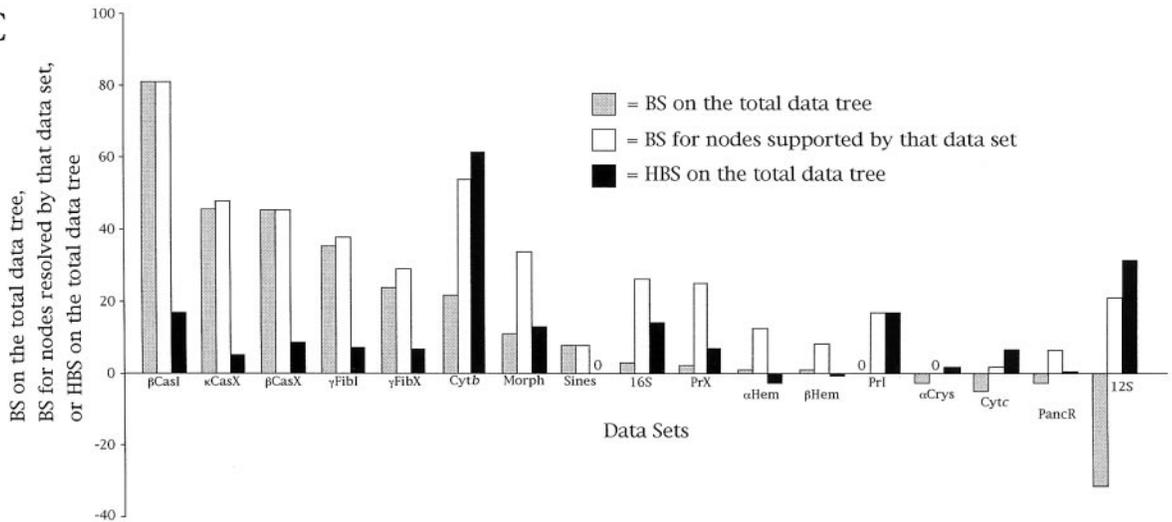
A



B



C



0, Fig. 4). Furthermore, PBS for  $\beta$ -casein intron 7 is zero at the Hippopotamidae + Cetacea node. Six other data sets have higher PBS scores than  $\beta$ -casein intron 7 (Fig. 6), but the Hippopotamidae + Cetacea node does not collapse with the removal of  $\beta$ -casein exon 7 plus any one of these six data sets. At this node, only the DRI roots out the importance of  $\beta$ -casein exon 7 plus  $\beta$ -casein intron 7 in simultaneous analysis (Fig. 8).

The Delphinoidea + Ziphiidae node offers a similar example. The removal of mt cytochrome *b* +  $\beta$ -hemoglobin collapses Delphinoidea + Ziphiidae (DRI = 2, Figure 8). However,  $\beta$ -hemoglobin is not even sampled for Ziphiidae (Appendix 1), so the separate analysis of  $\beta$ -hemoglobin obviously does not support Delphinoidea + Ziphiidae (Fig. 4). PBS for  $\beta$ -hemoglobin is only +1.0 at this node (Fig. 6). Removal of the two data sets with the highest PBS scores, mt cytochrome *b* (+6.5) and  $\beta$ -casein intron 7 (+3.25), does not collapse Delphinoidea + Ziphiidae. As in the case above, the DRI analysis shows which combinations of data sets are critical for the resolution of a particular node. These critical combinations are not necessarily predictable based on separate analyses of data sets or PBS scores.

The data set removals of size 16 basically replicate the taxonomic congruence analysis (Fig. 5), but data set removals of size 15 record important interactions among data sets that are not recognized in the taxonomic congruence approach. In separate analyses,  $\beta$ -casein intron 7, mt cytochrome *b*, cytochrome *c*, and protamine P1 exons do not support Hippopotamidae + Cetacea (Fig. 4). However, various combinations of these data sets ( $\beta$ -casein intron 7 + mt cytochrome *b*,  $\beta$ -casein intron 7 + protamine P1 exons, and  $\beta$ -casein intron 7 + cytochrome *c*) do support this clade. The DRI analysis reveals hidden support for relationships supported by the total combined data set. Additionally, with the DRI, the relative strength of support from different data sets is taken into account. For example,  $\beta$ -casein intron 7 strongly supports Hippopotamidae

+ Cetacea + Ruminantia (BS = +7, Fig. 4). In contrast, separate analyses of 10 different data sets do not support this clade (Fig. 4). When  $\beta$ -casein intron 7 is paired with any one of these 10 data sets, Hippopotamidae + Cetacea + Ruminantia is supported.

### NDIs and HNDIs

NDIs and HNDIs for each data set at each node are shown in Fig. 9. For the most part, these values are similar to corresponding PBS and PHBS scores, but most HNDIs are higher than PHBS scores for Ziphiidae + Delphinoidea and Ruminantia. NDIs for combinations of data set removals that collapse particular nodes in the DRI analysis are shown in Fig. 8.

### DIs

DIs for each of the 17 artiodactyl data sets are shown in Fig. 7. According to these scores, the data sets have the following order of influence in simultaneous analysis:  $\beta$ -casein intron 7 (+112) > mt cytochrome *b* (+83) >  $\beta$ -casein exon 7 (+61) >  $\kappa$ -casein exon 4 (+53) >  $\gamma$ -fibrinogen introns 3–4 (+49) > skeletal/dental characters (+34) >  $\gamma$ -fibrinogen exons 2–4 (+32) > 16S mt rDNA (+23) > protamine P1 intron 1 (+19) > protamine P1 exons 1–2 + 5' and 3' noncoding regions (+10) > SINEs (+8) > cytochrome *c* (+2) >  $\alpha$ -hemoglobin (+1) > 12S mt rDNA (0) =  $\beta$ -hemoglobin (0) >  $\alpha$ -crystallin A (–1) = pancreatic ribonuclease (–1). If the DI is scaled to the minimum number of character steps for informative characters in a particular data set, the order of influence changes to: SINEs (+1.00) = cytochrome *c* (+1.00) >  $\beta$ -casein intron 7 (+0.61) >  $\gamma$ -fibrinogen introns 3–4 (+0.50) >  $\beta$ -casein exon 7 (+0.46) >  $\gamma$ -fibrinogen exons 2–4 (+0.44) = skeletal/dental characters (+0.44) >  $\kappa$ -casein exon 4 (+0.38) > protamine P1 intron 1 (+0.36) > 16S mt rDNA (+0.15) > mt cytochrome *b* (+0.13) > protamine P1 exons 1–2

FIG. 7. Measures of support and influence for the 17 artiodactyl data sets. For each data set, the following are plotted: (A) DI (gray bars) and the sum of PBS for all nodes in the total data tree (black bars). (B) DI divided by the minimum possible number of steps for informative characters in the data set (= scaled DI, gray bars) and the retention index (RI—Farris, 1989) of minimum length cladograms for that partition (black bars). (C) The sum of positive, zero and negative BS for all nodes in the total data tree (gray bars), the sum of positive BS scores for all nodes supported by the individual data set (white bars), and the sum of HBS for all nodes in the total data tree (black bars). Abbreviations for data sets are as in Fig. 6. Abbreviations for indices are as in Table 1.

TABLE 2

The Distribution of HS among 17 Data Partitions for Three Nodes: Cetacea + Hippopotamidae, Cetacea + Hippopotamidae + Ruminantia, and Cetacea + Hippopotamidae + Ruminantia + Suina

|                                       | Unambiguous Synapomorphies in Separate Analysis | Unambiguous Synapomorphies in Simultaneous Analysis | Hidden Synapomorphy (HS) | Partitioned Hidden Branch Support (PHBS) |
|---------------------------------------|---|---|--------------------------|--|
| Hippopotamidae + Cetacea              | Sines   | 0   | 0                        | 0  |
|                                       | Morph   | 0   | 0                        | 0  |
|                                       | Cyt <i>b</i>                                    | 0   | 14                       | 14                                       |
|                                       | 12S   | 0   | 1                        | 1  |
|                                       | 16S   | 0   | 3                        | 3  |
|                                       | PrX   | 0   | 3                        | 3  |
|                                       | $\beta$ CasX                                    | 7   | 7                        | 0  |
|                                       | $\kappa$ CasX                                   | 3   | 3                        | 0  |
|                                       | $\gamma$ FibX                                   | 3   | 3                        | 0  |
|                                       | $\gamma$ FibI                                   | 2   | 2                        | 0  |
|                                       | PrI   | 0   | 0                        | 0  |
|                                       | $\beta$ CasI                                    | 0   | 2                        | 2  |
|                                       | $\alpha$ Hem                                    | 0   | 1                        | 1  |
|                                       | $\alpha$ Crys                                   | 0   | 0                        | 0  |
|                                       | $\beta$ Hem                                     | 0   | 0                        | 0  |
|                                       | Cyt <i>c</i>                                    | 0   | 1                        | 1  |
|                                       | PancR   | 0   | 1                        | 1  |
| Total                                 | 15  | 41  | 26                       | 36                                       |
| Hippopotamidae + Cetacea + Ruminantia | Sines   | 3   | 3                        | 0  |
|                                       | Morph   | 0   | 3                        | 3  |
|                                       | Cyt <i>b</i>                                    | 0   | 2                        | 2  |
|                                       | 12S   | 0   | 1                        | 1  |
|                                       | 16S   | 0   | 1                        | 1  |
|                                       | PrX   | 0   | 1                        | 1  |
|                                       | $\beta$ CasX                                    | 5   | 5                        | 0  |
|                                       | $\kappa$ CasX                                   | 2   | 3                        | 1  |
|                                       | $\gamma$ FibX                                   | 4   | 4                        | 0  |
|                                       | $\gamma$ FibI                                   | 5   | 5                        | 0  |
|                                       | PrI   | 0   | 0                        | 0  |
|                                       | $\beta$ CasI                                    | 9   | 9                        | 0  |
|                                       | $\alpha$ Hem                                    | 2   | 1                        | -1                                       |
|                                       | $\alpha$ Crys                                   | 0   | 0                        | 0  |
|                                       | $\beta$ Hem                                     | 0   | 0                        | 0  |
|                                       | Cyt <i>c</i>                                    | 0   | 0                        | 0  |
|                                       | PancR   | 0   | 0                        | 0  |
| Total                                 | 30  | 38  | 8                        | 44                                       |

+ 5' and 3' noncoding regions (+0.12) >  $\alpha$ -hemoglobin (+0.02) >  $\beta$ -hemoglobin (0) = 12S mt rDNA (0) > pancreatic ribonuclease (-0.03) >  $\alpha$ -crystallin A (-0.20) (Figs. 7A and 7B).

With taxonomic congruence, optimal trees from different data sets are treated as equals in the construction

TABLE 2—Continued

|   | Unambiguous Synapomorphies in Separate Analysis | Unambiguous Synapomorphies in Simultaneous Analysis | Hidden Synapomorphy (HS) | Partitioned Hidden Branch Support (PHBS) |    |
|---|---|---|--------------------------|--|----|
| Hippopotamidae + Cetacea + Ruminantia + Suina | Sines   | 0   | 0                        | 0  |    |
|   | Morph   | 0   | 1                        | 1  |    |
|   | Cyt <i>b</i>                                    | 22  | 22                       | 0  |    |
|   | 12S   | 0   | 2                        | 2  |    |
|   | 16S   | 0   | 3                        | 3  |    |
|   | PrX   | 0   | 1                        | 1  |    |
|   | $\beta$ CasX                                    | 7   | 7                        | 0  |    |
|   | $\kappa$ CasX                                   | 0   | 4                        | 4  |    |
|   | $\gamma$ FibX                                   | 0   | 3                        | 3  |    |
|   | $\gamma$ FibI                                   | 0   | 3                        | 3  |    |
|   | PrI   | 0   | 0                        | 0  |    |
|   | $\beta$ CasI                                    | 12  | 12                       | 0  |    |
|   | $\alpha$ Hem                                    | 0   | 2                        | 2  |    |
|   | $\alpha$ Crys                                   | 0   | 0                        | 0  |    |
|   | $\beta$ Hem                                     | 0   | 1                        | 1  |    |
|   | Cyt <i>c</i>                                    | 0   | 0                        | 0  |    |
|   | PancR   | 0   | 2                        | 2  |    |
|   | Total   | 41  | 63                       | 22                                       | 38 |

Note. Data sets are abbreviated as in Fig. 6.

of consensus trees. This implicitly assumes that different data sets offer equal evidence for phylogenetic relationships (Miyamoto, 1985; Kluge, 1989; Eernisse and Kluge, 1993; Kluge and Wolf, 1993). For the 17 data sets examined here, this is clearly not the case. The DI scores for the different data sets span two orders of magnitude (Fig. 7A).

The amino acid sequences generally have little influence in the combined analysis. PBS for the sum of all amino acid sequences is -3, and DIs for the amino acid data sets range from +2 to -1 (Fig. 7A). The low DIs for these data sets are the result of character conflicts, few informative characters, and incomplete taxonomic sampling. The 12S mt rDNA data set has complete taxonomic sampling but is also relatively inconsequential in the combined analysis (DI = 0). Not surprisingly, 12S mt rDNA is highly incongruent with the other data sets according to some ILD tests (see below).

According to DI,  $\beta$ -casein,  $\kappa$ -casein,  $\gamma$ -fibrinogen, mt cytochrome *b*, and morphology have the most influence in the combined analysis. The mt cytochrome *b*

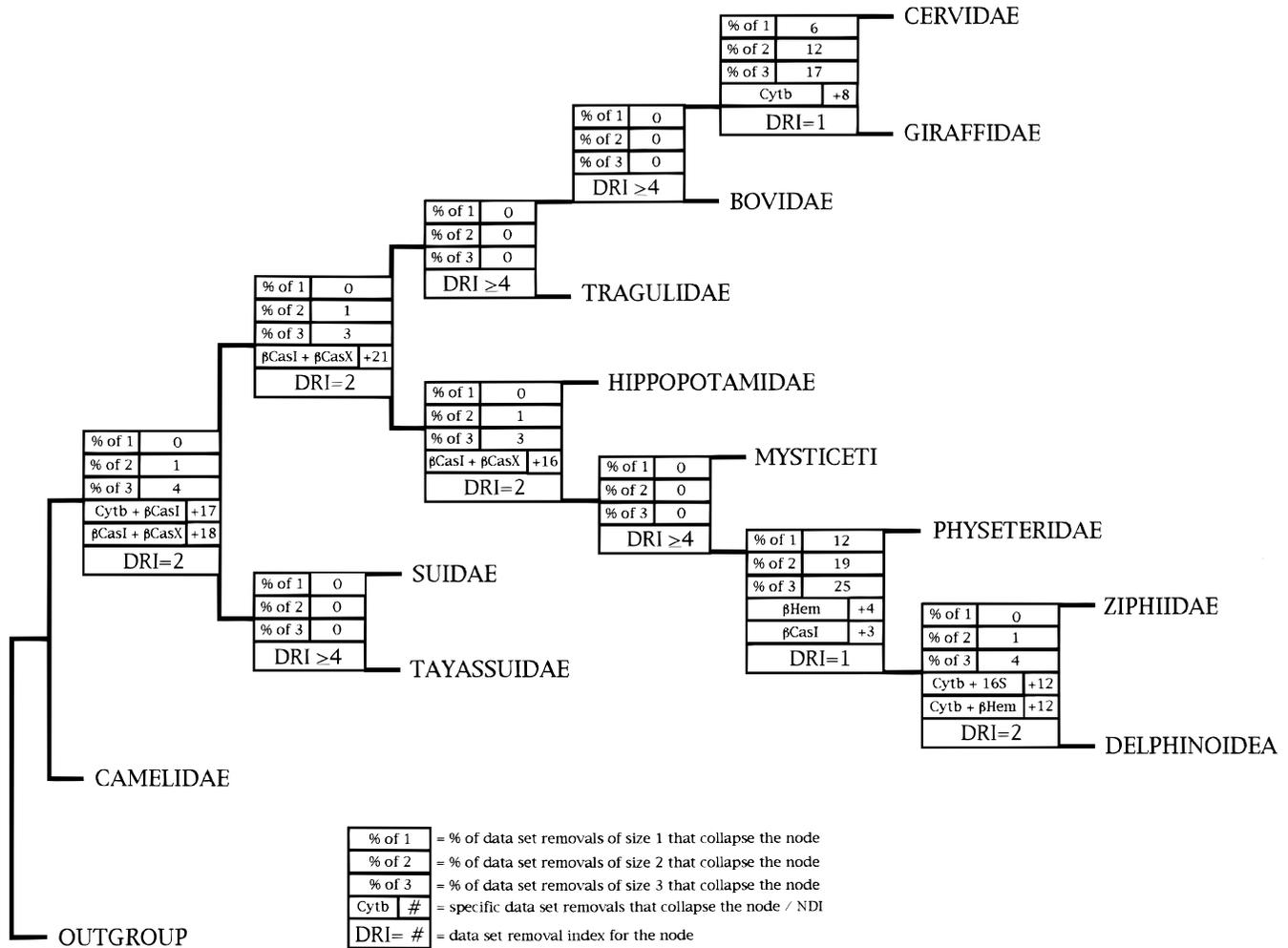


FIG. 8. DRIs for the simultaneous analysis of 17 artiodactyl data sets. The following information is given at each node: the percentage of data set removals of sizes 1, 2, and 3 that collapse the node (% of X), specific data sets or combinations of data sets that, when removed, collapse the node, NDIs for these data sets or combinations of data sets, and the DRI. Abbreviations for data sets are as in Fig. 6. Abbreviations for indices are as in Table 1.

data set has a high DI (+83), but this influence appears to be the result of the size of the mt cytochrome *b* data set and not necessarily the quality of the data set (scaled DI = +0.13, Fig. 7B). The protamine data sets are by far the weakest nu DNA partitions. The two protamine data sets have low consistency indices, retention indices, PBS scores, DIs, and scaled DIs (Figs. 4 and 7).

Shimamura *et al.* (1997) suggested that insertions of SINE retrotransposons at specific loci should be free of convergence and reversal. Apparently, there is no clear bias as to where SINEs insert themselves and no known mechanism for the precise excision of these elements

once inserted (Shimamura *et al.*, 1997). Within the context of the other 16 data sets, the scaled DI for the SINE retrotransposons is +1.00 (Fig. 7B). As predicted by Shimamura *et al.* (1997), there is no homoplasy in the SINE data set (Fig. 4); each insertion of a transposon is a unique and unreversed evolutionary event. However, the SINE data set is so small, eight informative characters, that in the equal weighting scheme employed here, the SINEs have little overall influence, DI = +8 (Fig. 7A).

For a particular data set, DI minus the sum of PBS scores estimates the amount of HBS a data set brings

out of other data sets in simultaneous analysis.  $\beta$ -Casein intron 7 brings out the most HBS in the other 16 data sets. DI minus the sum of PBS scores is +13.75 for  $\beta$ -casein intron 7 (Fig. 7A).

### Incongruence Length Difference Tests

Four groups of ILD tests were done (see Materials and Methods). The first group of ILD tests included taxa with extensive missing data. These tests suggested significant character conflicts in five cases (mt cytochrome *b*,  $P = 0.014$ ; 12S mt rDNA,  $P = 0.001$ ; 16S mt rDNA,  $P = 0.015$ ; skeletal/dental,  $P = 0.005$ ,  $\beta$ -hemoglobin,  $P = 0.017$ ). In ILD tests for the five taxa common to all 17 data sets (group 2), only two comparisons rejected the null hypothesis of congruence between partitions (skeletal/dental,  $P = 0.004$  and mt 12S rDNA,  $P = 0.004$ ). For ILD tests that included the six taxa common to 16 of the 17 data sets (group 3), four comparisons rejected the null hypothesis (Protamine P1 intron 1,  $P = 0.008$ ; pancreatic ribonuclease,  $P = 0.042$ ; 12S mt rDNA,  $P = 0.009$ ; mt cytochrome *b*,  $P = 0.046$ ). For the eight data sets sampled for all 13 taxa in this study (group 4), each of the three mt data sets was significantly incongruent with the remainder of the data (mt cytochrome *b*,  $P = 0.012$ ; 12S mt rDNA,  $P = 0.001$ ; 16S mt rDNA,  $P = 0.009$ ). 12S mt rDNA was significantly incongruent with the remainder of the character evidence in all four groups of ILD tests. However, if  $P$  values are multiplied by the number of ILD tests in each group,  $P$  is less than 0.05 in only a few cases: group one, 12S mt rDNA ( $P = 0.017$ ) and group four, 12S mt rDNA ( $P = 0.008$ ).

### Interpretation of Support in the Simultaneous Analysis

A summary of nodal support in the simultaneous analysis is shown in Fig. 10. In most cases, measures of support among characters and among data sets agree.

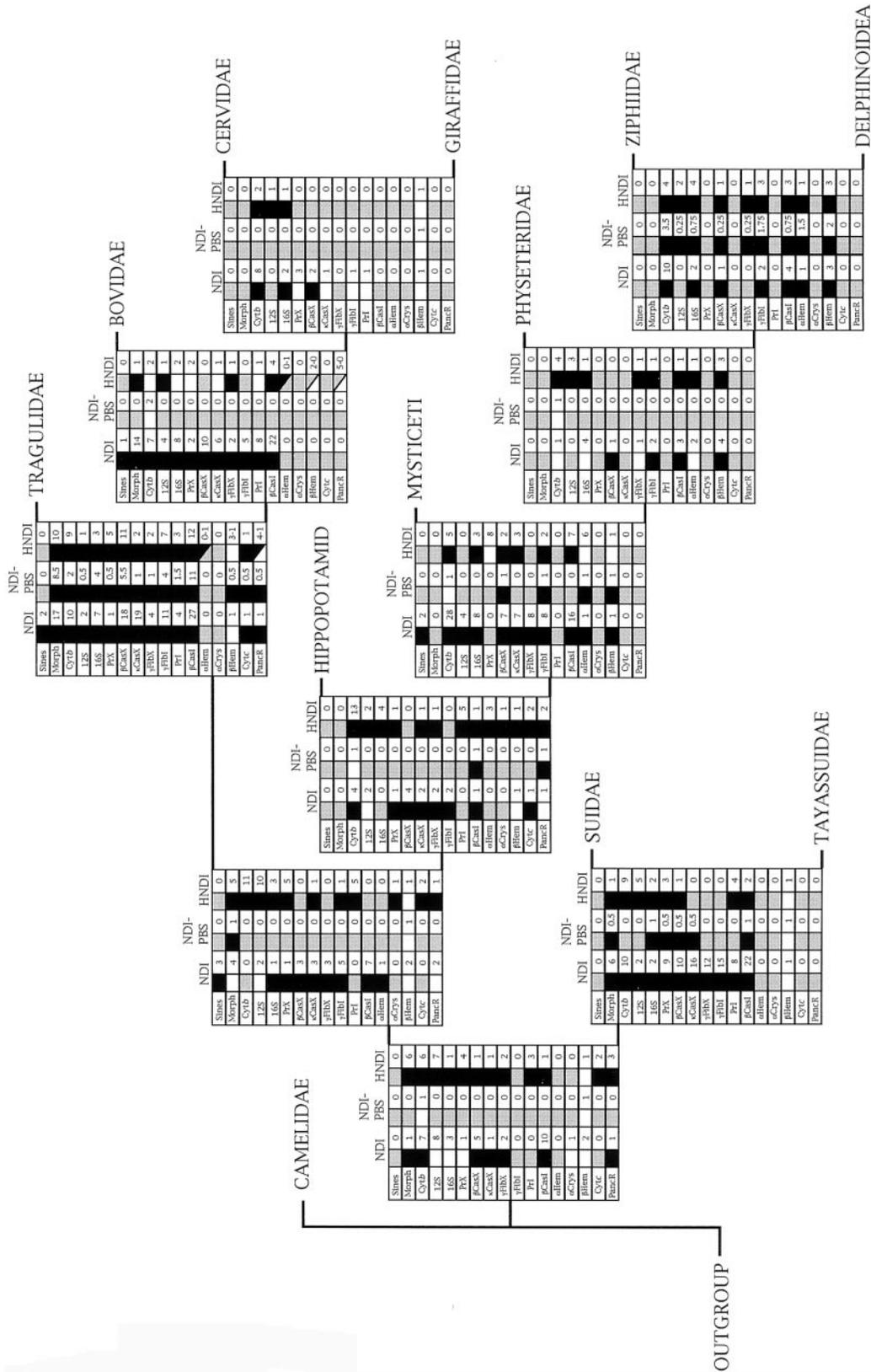
Four nodes are solidly supported. Suina (Tayassuidae + Suidae), Pecora (Bovidae + Giraffidae + Cervidae), Cetacea (Physeteridae + Delphinoidea + Mysticeti + Ziphiidae), and Ruminantia (Tragulidae + Pecora) have DRIs of at least 4, BS greater than +80, minimally 11 positive NDI scores, and minimally 9 positive PBS scores (Fig. 10, nodes J, B, F, and C respectively). Each of these nodes is supported by both mt and nu DNA data sets (Figs. 6 and 9). Cetacea and Pecora have no negative PBS or NDI scores.

In contrast, Odontoceti (Physeteridae + Ziphiidae + Delphinoidea) and Cervidae + Giraffidae are weakly supported (Fig. 10, nodes E and A). These clades are not stable to data set removal (DRI = 1), have low BS, and show a nearly even mixture of positive and negative PBS/NDI scores. Most of the support for Cervidae + Giraffidae is concentrated within the mt cytochrome *b* data set (PBS = +8, NDI = +8, DRI = 1 with mt cytochrome *b* the critical data set removal). Odontoceti is characterized by a lower BS score than Cervidae + Giraffidae, but Odontoceti has better support among data sets (Figs. 6, 9, and 10).

The Ziphiidae + Delphinoidea clade is more stable than either Odontoceti or Cervidae + Giraffidae (Fig. 10, node D). BS is +12, and the clade is characterized by six positive PBS scores and seven positive NDI scores (Figs. 6 and 9). However, the removal of just two mt data sets collapses this group (Fig. 8). As for the Giraffidae + Cervidae clade, mt cytochrome *b* provides most of the character support for Ziphiidae + Delphinoidea (Figs. 6 and 9).

The simultaneous analysis suggests that Cetacea is nested within a paraphyletic Artiodactyla (Fig. 4). Cetacea + Hippopotamidae, Cetacea + Hippopotamidae + Ruminantia, and Cetacea + Hippopotamidae + Ruminantia + Suina are consistent with previous molecular studies (Fig. 10, nodes G, H, and I, respectively; Graur and Higgins, 1994; Gatesy *et al.*, 1996; Gatesy, 1997, 1998; Shimamura *et al.*, 1997; Milinkovitch *et al.*, 1998). These three nodes have BS scores of +12 to +17,

FIG. 9. NDI, HNDI, and the difference between NDI and PBS for each data set at each node supported by the simultaneous analysis of 17 artiodactyl data sets. Because of missing taxa for several data sets, some BS scores are ambiguous. That is, BS cannot be unambiguously assigned to one node. In these cases, the range of HNDI scores (HNDI = NDI minus BS) is given (e.g., "0-1" = HNDI from zero to positive one). Black (+), gray (0), and white (-) boxes to the left and abbreviations for data sets are as in Fig. 6. Abbreviations for indices are as in Table 1.



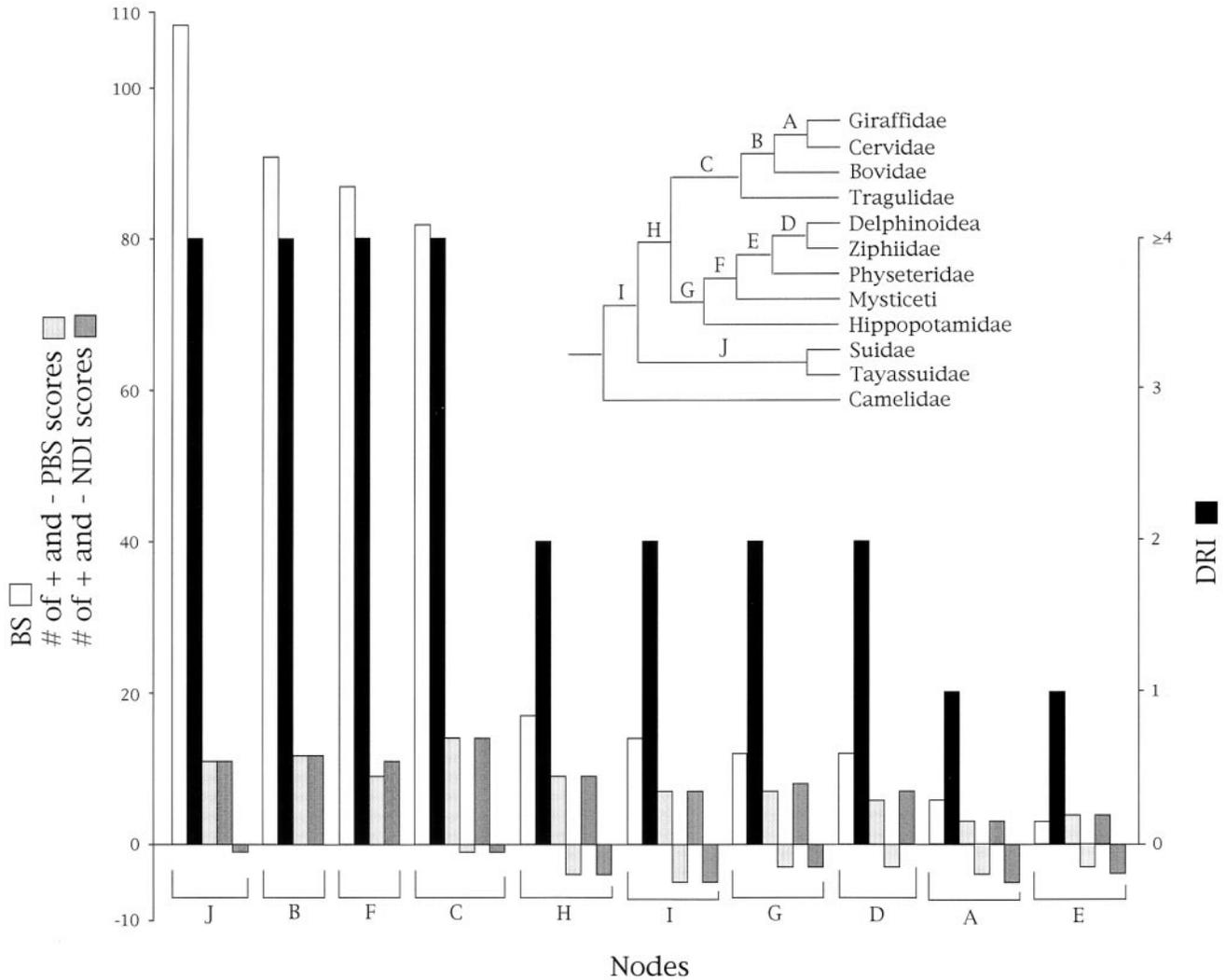


FIG. 10. A summary of support at each node of the total data tree. For each node (A–J) in the total data tree, BS (white bars), the DRI (black bars), the number of positive and negative PBS scores (light gray bars), and the number of positive and negative NDI scores (dark gray bars) are shown. Abbreviations for indices are as in Table 1.

are stable to the removal of any single data set, and are at least weakly corroborated by minimally one mtDNA data set, at least one amino acid data set, and minimally four nu DNA data sets (Figs. 6, 8, 9, and 10). The Hippopotamidae + Cetacea + Ruminantia clade is also supported by three independent retropositional insertions (Shimamura *et al.*, 1997), and Cetacea + Hippopotamidae + Ruminantia + Suina is weakly favored by the morphological characters in the simultaneous analysis (Figs. 6 and 9). All three nodes that cluster Cetacea as an artiodactyl subclade are characterized by an abundance of hidden support (Figs. 6 and 9; Table 2).

Although Cetacea + Hippopotamidae, Cetacea + Hippopotamidae + Ruminantia, and Cetacea + Hippopotamidae + Ruminantia + Suina are well supported by most measures, DRIs show that all three nodes collapse with the removal of two data sets from the same nu gene,  $\beta$ -casein (Fig. 8). Based on the DRI, this gene clearly has the most influence in the simultaneous analysis. This point is reinforced by the high DIs, scaled DIs, and sums of PBS for the  $\beta$ -casein data sets (Fig. 7). Furthermore, no extra character steps are required to fit these two data sets to the total data tree (Fig. 4). The importance of  $\beta$ -casein in the simultaneous

analysis may indicate that  $\beta$ -casein is a particularly informative locus for resolving relationships within Artiodactyla + Cetacea. Alternatively, if  $\beta$ -casein is a systematically misleading data set, its control over the simultaneous analysis is troubling. Either way, the  $\beta$ -casein data require further examination.

A cladistic analysis of all available  $\beta$ -casein exon 7 sequences (Gatesy, 1998) is shown in Fig. 11. The addition of 55  $\beta$ -casein exons has little effect on phylogenetic results. The nine groups supported by the analysis of 13  $\beta$ -casein exons (Fig. 4) also are supported in the analysis of 68  $\beta$ -casein exons (Fig. 11). BS scores for the three nodes that define a paraphyletic Artiodactyla are similar in both analyses (Fig. 11), and the retention index (Farris, 1989) is higher in the analysis of 68 sequences (0.7778) than in the analysis of 13  $\beta$ -casein sequences (0.7163). Relationships supported by the 13  $\beta$ -caseins used in the simultaneous analysis (Appendix 1) are not altered by increased taxonomic sampling.

There are few, additional, published sequences for  $\beta$ -casein intron 7. So, at this point it is not possible to test whether increased taxonomic sampling alters relationships supported by the 13  $\beta$ -casein introns in this study (Appendix 1). However, the topologies derived from  $\beta$ -casein intron 7 are stable to differential character weighting according to fit (Goloboff, 1993b). Goloboff weighting at  $k = 0$  does not rearrange topologies implied by  $\beta$ -casein intron 7,  $\beta$ -casein exon 7, or both data sets combined. Heuristic searches were as described in Materials and Methods using PAUP\*, but the "use Goloboff fit criterion" was invoked (Swofford, in prep.).

The  $\beta$ -casein data appear to be internally consistent. The signal from exon 7 agrees with the signal from intron 7 (Fig. 4). Consistency indices and retention indices are reasonably high for both sequences (Figs. 4 and 7), optimal topologies for the exon are stable to increased taxonomic sampling (Fig. 11), and both separate and combined  $\beta$ -casein data sets are stable to Goloboff weighting with a low  $k$ .

It is possible that an undetected gene duplication in  $\beta$ -casein is confounding our results (Goodman *et al.*, 1979). However, at this point there is no empirical evidence for this hypothesis (i.e., two copies of  $\beta$ -casein within a haploid artiodactyl genome). Alternatively, there may be some other systematic bias in the  $\beta$ -casein data. Perhaps nucleotide substitution asymmetries (Collins *et al.*, 1994), branch length differences

(Felsenstein, 1978), differential lineage sorting (Pamilo and Nei, 1988), gene conversion (Rozas and Aguade, 1994), horizontal transfer (Clark *et al.*, 1994), or directional selection pressures (Stewart *et al.*, 1987) have made this gene a poor indicator of phylogeny. Because  $\beta$ -casein intron 7 is highly congruent with  $\beta$ -casein exon 7 (ILD = 0), any systematic biases would have to extend across both the exons and the introns of this gene.

The speculations above are contradicted by the congruence from independent data sets for all of the controversial relationships implied by  $\beta$ -casein (Figs. 4, 5, 6, 9, and 10). It is difficult to imagine a common bias that is present across nu exons, nu introns, a mt protein-coding gene, nu amino acid sequences, 5'/3' noncoding regions, genes that code for functional RNAs, A + T and G + C biased DNA regions, morphological characters, and insertions of transposons. Therefore, the simplest explanation for the common hierarchical pattern among characters would seem to be phylogeny.

Proponents of taxonomic congruence suggest that replicated clades from separate analyses of different data sets are the strongest evidence for phylogeny (e.g., Miyamoto and Fitch, 1995). However, in the artiodactyl analysis, much of the corroboration among data sets is derived from hidden support that emerges in simultaneous analysis. With the taxonomic congruence approach, this support is ignored.

### *Hidden Support: The Missing Element of Taxonomic Congruence*

**Hidden support in mt cytochrome b.** A remarkable amount of hidden support emerges in the simultaneous analysis (Figs. 6–9, Table 2). The sum of HBS scores at the 10 nodes recovered in the simultaneous analysis is +196. The total of BS scores for these same relationships among the separate analyses is only +236. For relationships supported by the total combined data set, there is almost as much HBS in simultaneous analysis as there is BS from each of the separate data sets (Figs. 6 and 7C).

According to DI (Fig. 7A) and the DRI (Fig. 8), mt cytochrome *b* is one of the more influential data sets in the simultaneous analysis. Mt cytochrome *b* is also characterized by more HBS than any of the other 16 data sets (Fig. 7C). The HBS from mt cytochrome *b* that

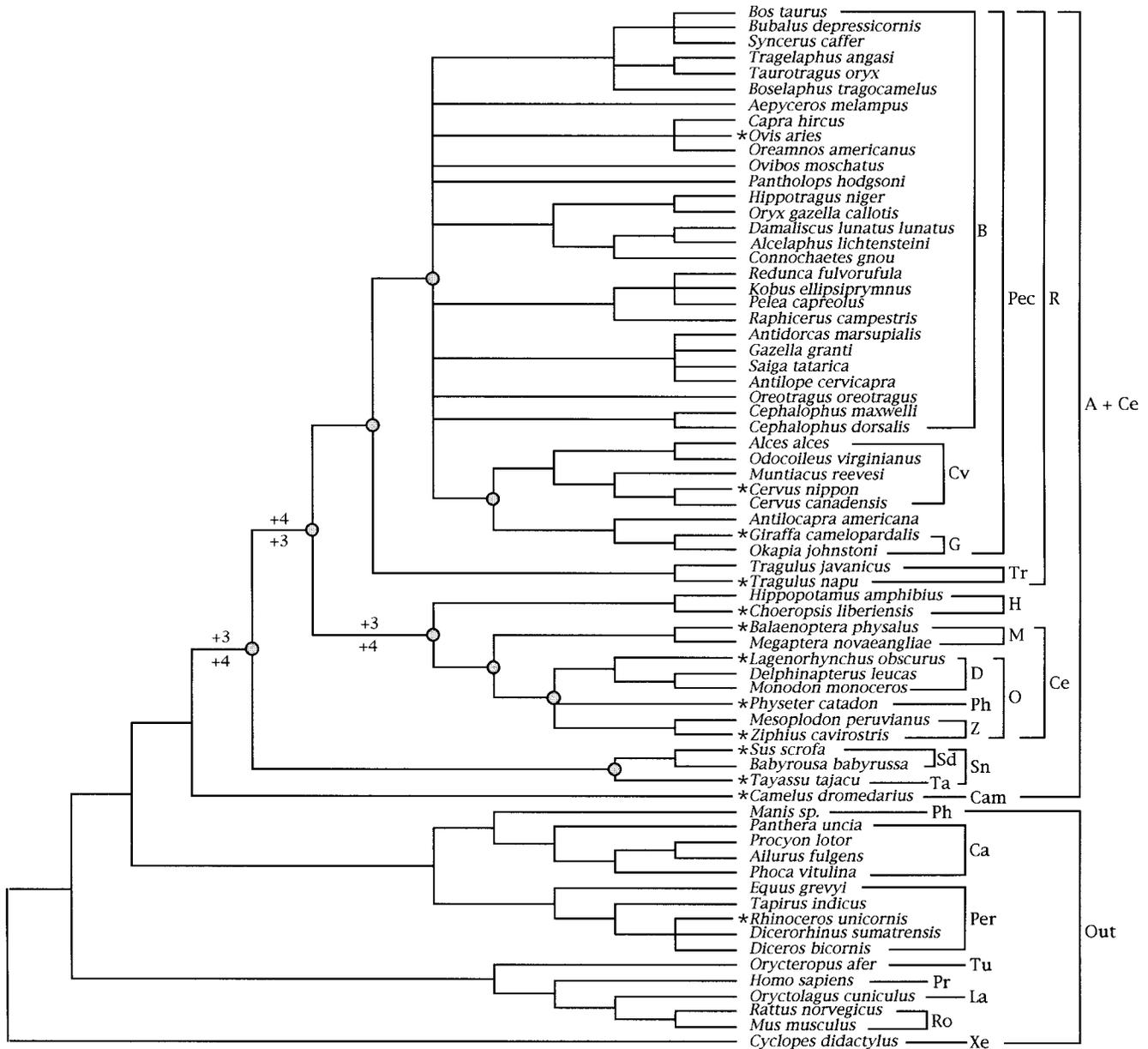


FIG. 11. A cladistic analysis of  $\beta$ -casein exon 7 from 68 mammalian taxa. Six hundred and forty-eight optimal trees were found with PAUP\*, and 172 optimal topologies were discerned with NONA. The strict consensus for both sets of optimal topologies is shown. BS scores for three nodes are indicated. BS above internodes is from the analysis of 68 taxa, and BS below internodes is for the reduced data set of 13 taxa (see Appendix 1). These 13 taxa are marked by \*. Gray dots mark the nine nodes supported by the analysis of 68  $\beta$ -caseins that are also supported in the reduced analysis of 13  $\beta$ -caseins and in the simultaneous analysis of 17 data sets (Fig. 4). There are 107 informative characters in the  $\beta$ -casein data set and 1202 steps in minimum length trees. For these topologies, the consistency index is 0.5238, and the retention index is 0.7778. Higher level taxa are delimited by brackets to the right of the tree: B = Bovidae, Cv = Cervidae, G = Giraffidae, Pec = Pecora, Tr = Tragulidae, R = Ruminantia, H = Hippopotamidae, M = Mysticeti, D = Delphinoidea, Ph = Physeteridae, Z = Ziphiidae, O = Odontoceti, Ce = Cetacea, Sd = Suidae, Ta = Tayassuidae, Sn = Suina, Cam = Camelidae, A = Artiodactyla, Ph = Pholidota, Ca = Carnivora, Per = Perissodactyla, Tu = Tubulidentata, Pr = Primates, La = Lagomorpha, Ro = Rodentia, Xe = Xenarthra, and Out = Outgroups.

emerges in simultaneous analysis exceeds the BS from mt cytochrome *b* in its separate analysis (Fig. 7C).

Support for relationships favored by the simultaneous analysis can be brought out of mt cytochrome *b* in at least three different ways:

(1) By adding 86 mt cytochrome *b* sequences to the 13 cytochrome *b* sequences analyzed above, positive character support emerges for two of the three nodes that define artiodactyl paraphyly in the simultaneous analysis. Figure 12 shows an analysis of 99 published mt cytochrome *b* sequences. These data support a Cetacea + Hippopotamidae clade (BS = +5) and a Hippopotamidae + Cetacea + Ruminantia + Suina clade (BS = +6). However, the reduced analysis of 13 mt cytochrome *b* sequences does not support Cetacea + Hippopotamidae (BS = -9), and support for Hippopotamidae + Cetacea + Ruminantia + Suina is weak (BS = +1, Figs. 4 and 12). With improved taxonomic sampling, the increases in BS for Cetacea + Hippopotamidae (+14) and Hippopotamidae + Cetacea + Ruminantia + Suina (+5) are substantial (Figure 12).

(2) By sampling different taxonomic exemplars, there is increased support for some relationships supported by the simultaneous analysis. For example, the 13 mt cytochrome *b* sequences in the combined data set do not support Hippopotamidae + Cetacea (BS = -9, Fig. 4). However, BS is +3 for this clade if the following exemplars are sampled: *Ovis aries*—Bovidae, *Odocoileus hemionus*—Cervidae, *Giraffa camelopardalis*—Giraffidae, *Tragulus javanicus*—Tragulidae, *Lagenorhynchus albirostris*—Delphinoidea, *Ziphius cavirostris*—Ziphiidae, *Kogia breviceps*—Physeteridae, *Megaptera novaeangliae*—Mysticeti, *Choeropsis liberiensis*—Hippopotamidae, *Sus scrofa*—Suidae, *Tayassu tajacu*—Tayassuidae, *Camelus dromedarius*—Camelidae, and *Equus grevyi*—Perissodactyla.

(3) By adding a single nu data set to the mt cytochrome *b* data set, hidden support in mt cytochrome *b* is apparent for Hippopotamidae + Cetacea. Hippopotamidae + Cetacea is not supported by separate analyses of either mt cytochrome *b* (BS = -9) or  $\beta$ -casein intron 7 (BS = 0, Figs. 4 and 6). Regardless, when these two data sets are combined in simultaneous analysis, Cetacea + Hippopotamidae is supported (BS = +1, HBS = +10, and HNDI for mt cytochrome *b* = +1).

**Twelve wrongs make one right?** The Hippopotamidae + Cetacea node (H + C) illustrates the difference

between the quantification of support among data sets in taxonomic congruence versus simultaneous analysis. H + C is only supported in four of the 17 separate analyses (Figs. 4 and 13A), but there is extensive hidden support in data sets that do not individually recover this clade (Figs. 6 and 9, Table 2). Figure 13B shows a simultaneous analysis of 12 of the 13 data sets that, in isolation from each other, do not support H + C. Examination of optimal topologies for each of these “wrong” data sets suggests that H + C is very poorly supported (sum of BS = -28). However, in combination these data sets show a BS score of +3 for H + C (Fig. 13B). As in the hypothetical examples of Fig. 3, homoplasy is dispersed in the simultaneous analysis. The peculiarities of each data set are cancelled out by the unique peculiarities of the others, and the remaining common signal emerges (Barrett *et al.*, 1991; Nixon and Carpenter, 1996). This hidden information is overlooked in the taxonomic congruence approach.

Within the context of character information from all 17 data sets, net positive support for H + C is recognized in 7 data sets by PBS (Fig. 6) and in 8 data sets by NDI (Fig. 9). Twelve data sets provide unambiguous synapomorphies for H + C in the simultaneous analysis (Table 2). These inferences contrast with the amount of corroboration among data sets recognized with taxonomic congruence; only 4 individual data sets recover H + C (Fig. 4). For the reduced analysis of six taxa common to 16 of the data sets, results are similar. Mysticeti (Cetacea) + Hippopotamidae is supported in only three of the separate analyses, but in simultaneous analysis, there are seven positive PBS scores at this node (Fig. 5E). Finally, for the 8 data sets with complete taxonomic sampling, H + C is not present in strict, semistrict, and 50% majority rule consensus trees (Fig. 5G), but in simultaneous analysis, 6 data sets have positive PBS scores for H + C (Fig. 5H). Because HBS is ignored, taxonomic congruence underestimates corroboration among data sets when there is much hidden support (e.g., Fig. 3—scenario e) and overestimates corroboration among data sets when there is an abundance of hidden conflict (e.g., Fig. 2—scenario d).

Taxonomic congruence provides information on the distribution of support among data sets, but the approach is basically replicated in the DRI analysis. That is, 13 of the 17 data set removals of size 16 (77%) collapse the H + C clade. The DRI subsumes taxonomic congruence and is an extension of this method to the

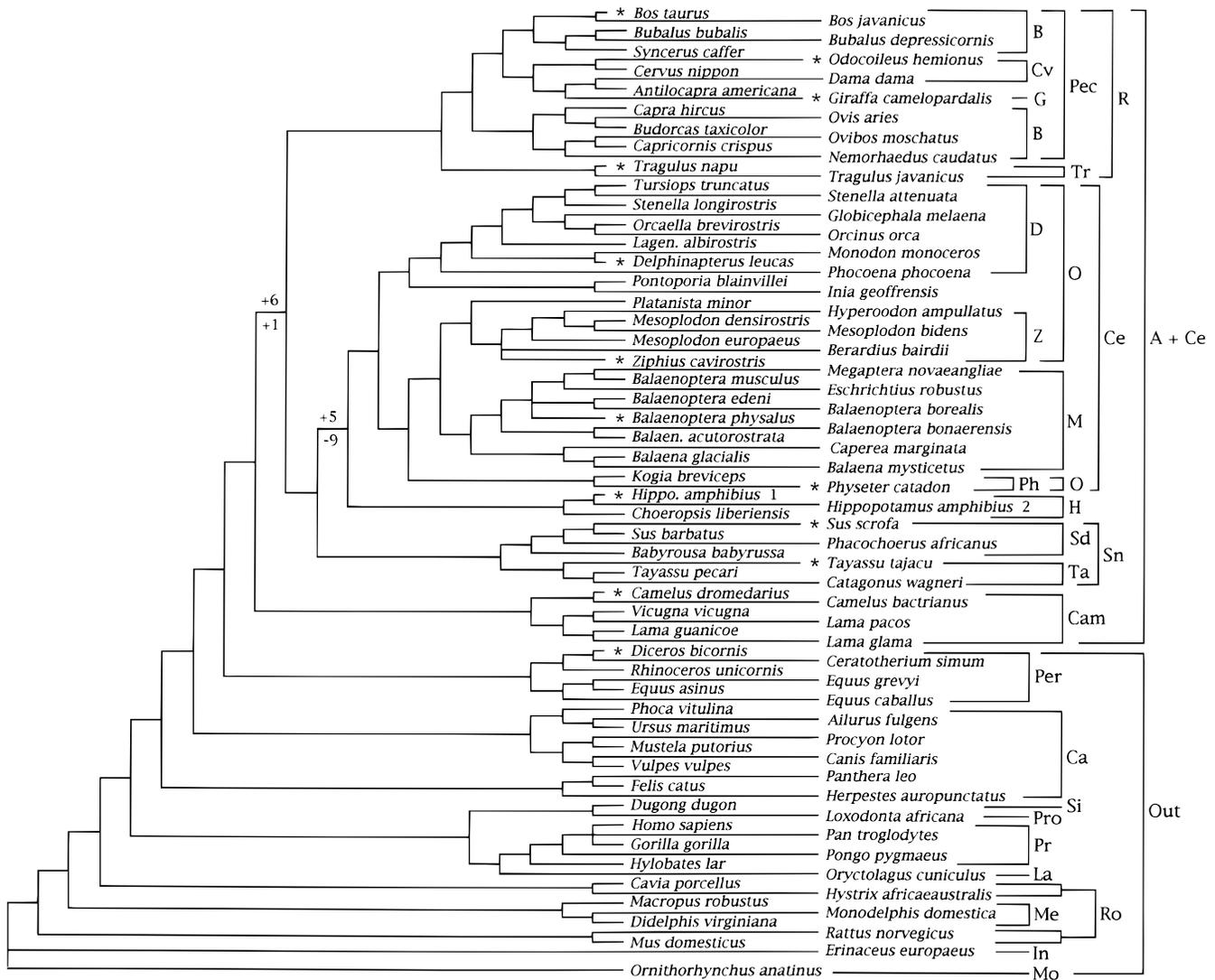
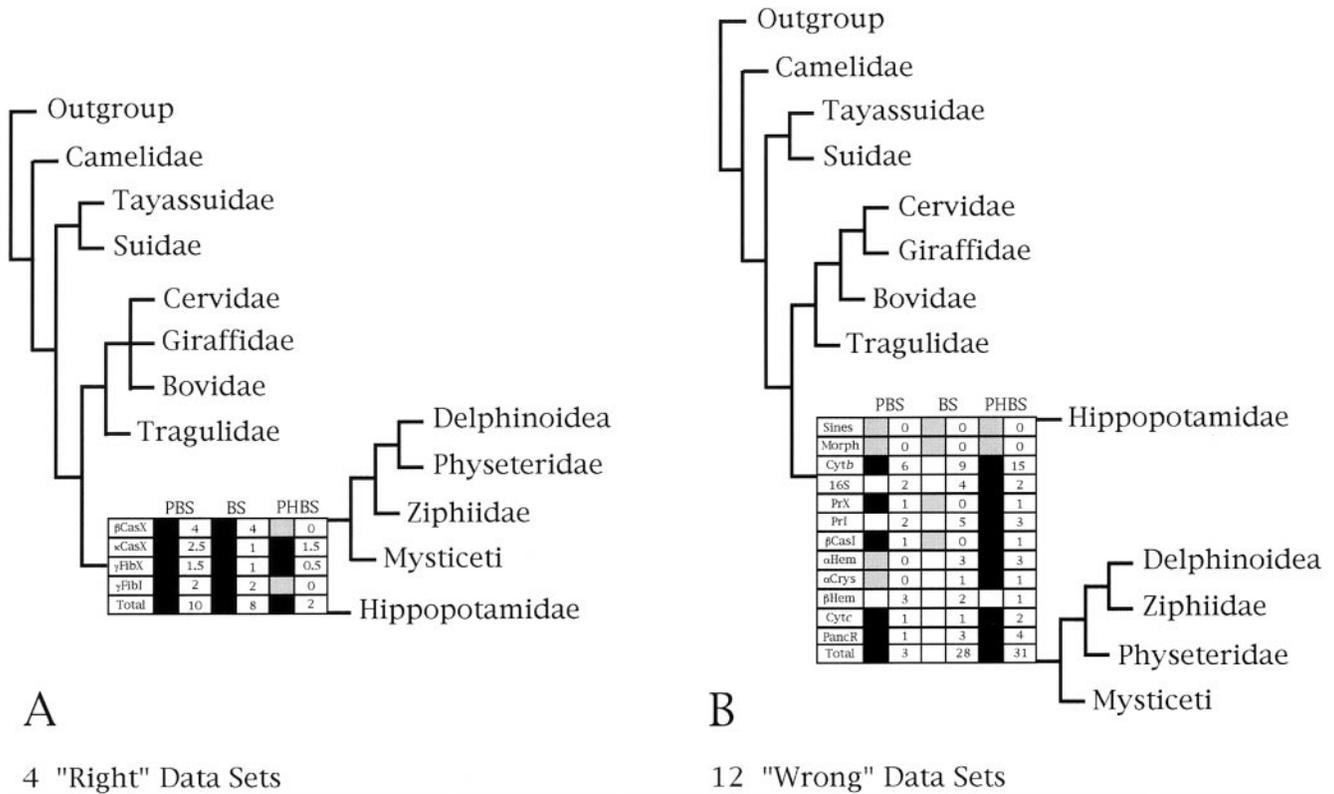


FIG. 12. A cladistic analysis of mt cytochrome *b* from 99 mammalian taxa. Eight optimal trees were found with PAUP\* and with NONA; the strict consensus of these optimal trees is shown. BS scores are indicated for two nodes. BS scores above internodes are from the analysis of 99 mt cytochrome *b* sequences, and BS scores below internodes are for the reduced data set of 13 mt cytochrome *b* sequences (see Appendix 1). These 13 sequences are marked by \*. There are 637 informative characters in the mt cytochrome *b* data set and 9306 steps in minimum length trees. For these topologies, the consistency index is 0.1407, and the retention index is 0.4923. Higher level taxa are delimited by brackets to the right of the tree: B = Bovidae, Cv = Cervidae, G = Giraffidae, Pec = Pecora, Tr = Tragulidae, R = Ruminantia, H = Hippopotamidae, M = Mysticeti, D = Delphinoidea, Ph = Physeteridae, Z = Ziphiidae, O = Odontoceti, Ce = Cetacea, Sd = Suidae, Ta = Tayassuidae, Sn = Suina, Cam = Camelidae, A = Artiodactyla, Per = Perissodactyla, Ca = Carnivora, Si = Sirenia, Pro = Proboscidea, Pr = Primates, La = Lagomorpha, Ro = Rodentia, Me = Metatheria, In = Insectivora, Mo = Monotremata, and Out = Outgroups. *Hippo.* = *Hippopotamus*, *Lagen.* = *Lagenorhynchus*, and *Balaen.* = *Balaenoptera*.

simultaneous analysis framework. In combination with PBS and NDI scores, the DRI offers a more complete assessment of support among data sets than taxonomic congruence.

**HBS and tests of data set incompatibility.** The ILD

test was designed to assess the null hypothesis of congruence among data sets (Farris *et al.*, 1994a). Despite compelling philosophical reasons for combining all relevant character evidence in simultaneous analysis (Miyamoto, 1985; Kluge, 1989, 1997; Brower *et al.*, 1996;



**FIG. 13.** Hidden support that emerges for Cetacea + Hippopotamidae in simultaneous analysis. (A) Four "right" data sets support a Cetacea + Hippopotamidae sister group relationship individually and in combination. (B) Separate analyses of twelve "wrong" data sets do not support Hippopotamidae + Cetacea, but simultaneous analysis of these data sets does support Hippopotamidae + Cetacea. PBS, the sum of all PBS scores (= BS), positive, zero, or negative BS for each partition, the sum of these BS scores, PHBS, and the sum of all PHBS scores (= HBS) are shown for the Hippopotamidae + Cetacea node. Black (+), gray (0), and white (-) boxes to the left and abbreviations for data sets are as in Fig. 6. Abbreviations for indices are as in Table 1. For the combined data set in A, there are two optimal trees (1134 steps) with consistency index of 0.6841 and retention index of 0.7067. For the combined data set in B, there is one optimal tree (3330 steps) with consistency index of 0.5157 and retention index of 0.4504.

Nixon and Carpenter, 1996; DeSalle and Brower, 1997; Farris, 1997; Siddall, 1997; Siddall and Kluge, 1997), several authors have suggested that the ILD test be used to exclude data sets from simultaneous analysis (e.g., Shaffer *et al.*, 1997). Similarly, the Wilcoxon ranked sums test of character support (Templeton, 1983) has been offered as a measure of data set incompatibility (Larson, 1994). Data sets that fail these tests have been interpreted as significantly incongruent. Proponents of total evidence usually ignore such incongruence and combine all data sets in simultaneous analysis (e.g., Baker and DeSalle, 1997). Proponents of conditional combination either separate the incongruent data sets

and then proceed with simultaneous analysis (e.g., Miyamoto, 1996), remove problematic taxa and then proceed with simultaneous analysis (e.g., deQueiroz *et al.*, 1995), or differentially weight the incongruent character partitions to increase congruence among data sets (e.g., Cunningham, 1997).

The relationship between HBS and these tests has not been explored. However, simple hypothetical examples suggest that HBS may confound interpretations of data set incompatibility. Figure 14 shows a simple case in which the combination of two data sets, I and II, increases support relative to either of the separate analyses. These data sets are incongruent according to

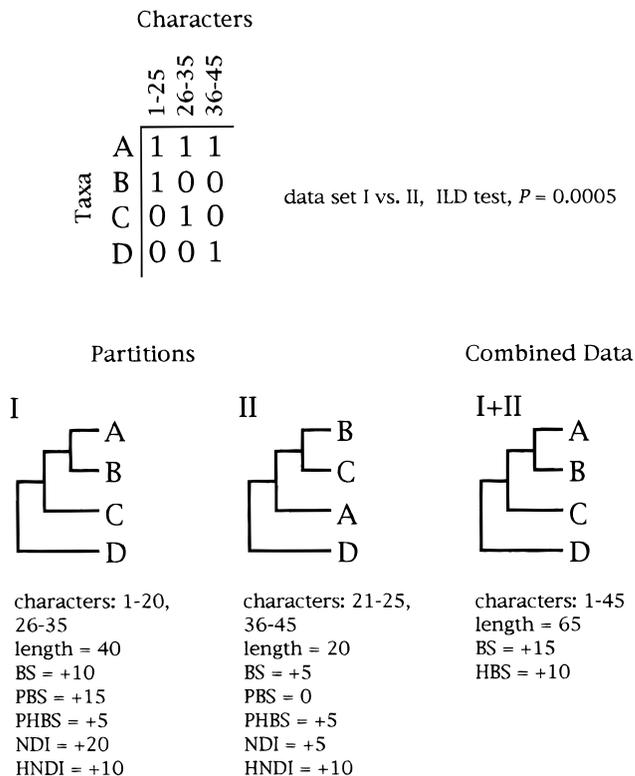


FIG. 14. A simple case in which the combination of two data sets that are significantly incongruent provides greater topological support than do separate analyses of each data set. A hypothetical combined data set (45 characters for four taxa) is composed of two data sets (I and II). These data sets reject congruence according to the test of Farris *et al.* (1994a). However, the combination of these data sets results in increased support. For each separate data set, the following are shown: minimum length topology, characters in that partition, tree length, BS, PBS, PHBS, NDI, and HNDI. Minimum length topology, characters in the combined data set, tree length, BS, and HBS are shown for the simultaneous analysis of data sets I + II. The ILD test was calculated as in the Materials and Methods, but 9999 random data partitions were generated instead of 999.

the ILD test of Farris *et al.* (1994a,  $P = 0.0005$ ). In data set I, BS for clade A + B is +10. In data set II, BS for clade B + C is +5 (Fig. 14). When these data sets are combined in simultaneous analysis, BS for A + B jumps to +15 because of HBS in both data sets. The data sets are significantly incongruent, but it would be difficult to argue that the data sets should not be analyzed simultaneously. There is no guarantee that the combination of incongruent data sets will yield a weaker phylogenetic result.

An empirical example reinforces this conclusion. Figure 15 shows a case where the combination of two significantly incongruent data partitions increases support and resolution. For Artiodactyla, the nu partition ( $\kappa$ -casein +  $\beta$ -casein +  $\gamma$ -fibrinogen) and mt cytochrome *b* are significantly incongruent ( $P = 0.0005$ ) according to the test of Farris *et al.* (1994a). Regardless, because of extensive HBS in mt cytochrome *b*, BS is much higher in optimal topologies for the combined nu + mt data set than for either separate analysis (Fig. 15). With the addition of mt cytochrome *b* to the nuclear partition, BS increases by +84, and two additional nodes are resolved (Fig. 15). The limited conflicts between the data sets in simultaneous analysis are indicated by negative PBS scores for mt cytochrome *b* at two nodes in the total data tree, Cetacea + Hippopotamidae + Ruminantia (PBS = -0.5) and Odontoceti (PBS = -3). However, at both of these nodes, there is PHBS from both mt cytochrome *b* (Cetacea + Hippopotamidae + Ruminantia = +8, Odontoceti = +4.5) and the nuclear data (Cetacea + Hippopotamidae + Ruminantia = +2, Odontoceti = +0.5). Much of the apparent conflict between the data sets is offset by the HBS in mt cytochrome *b* (total HBS = +49). The mt cytochrome *b* partition and the nu partition are surely incongruent, but this does not necessarily mean that the data sets should be analyzed separately or reweighted according to a detailed model of molecular evolution.

The cases in Figs. 14 and 15 (also see Sullivan, 1996) may be anecdotal, but at this point, we are not convinced that a significant ILD test is reason enough to separate data sets or to differentially weight characters to correct for incongruence. When incongruent characters from one data set conflict with incongruent characters from another data set, the negative characteristics of each data set often cancel out (Figs. 3, 13, 14, and 15).

In this paper, data sets such as mt cytochrome *b* and 12S mt rDNA were included in the overall simultaneous analysis. According to some of the ILD tests, these data sets are significantly incongruent with the other data sets. However, given the amount of HBS in mt cytochrome *b* and 12S mt rDNA (Figs. 6 and 7C), combining these data with the other 15 data sets does not seem very controversial to us.

The 12S mt rDNA data set is certainly at odds with the other data sets. For 12S mt rDNA, ILDs are extreme, DI is 0, the sum of PBS scores is -0.75, and net BS for

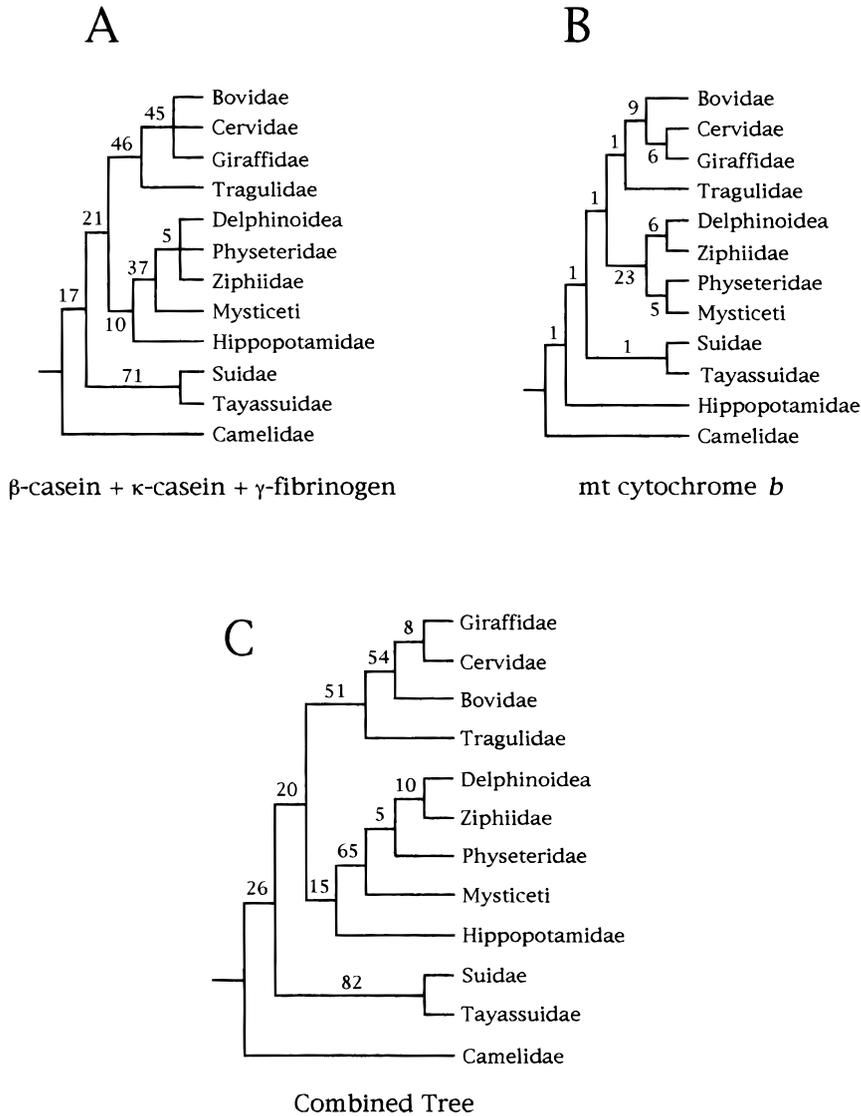


FIG. 15. An empirical case in which the combination of data sets that are significantly incongruent provides greater support for relationships than do separate analyses of each data set (A) The strict consensus of four minimum length topologies for a nu data set. (B) The minimum length topology for mt cytochrome *b*. (C) The minimum length topology for the nu data set plus mt cytochrome *b*. Positive BS is shown at internodes for each topology. According to the ILD test of Farris *et al.* (1994a), the two data sets reject congruence ( $P = 0.0005$ ). The ILD test was calculated as in Fig. 14.

groups in the total data tree is  $-32$  (Fig. 7). However, the addition of the 12S mt rDNA data set to the other 16 data sets has no impact on the topology of the total data tree. The DRI analysis shows that the inclusion or exclusion of 12S mt rDNA makes no difference (Fig. 8). The significant ILD for 12S mt rDNA *versus* the remaining data sets *does* suggest that some of the more ambiguous sequence alignment regions in the 12S mt

rDNA data set should be reexamined (Gatesy and O'Grady, in prep.).

### Alternative Phylogenetic Hypotheses

In the approach advocated here, the support among data sets for suboptimal hypotheses is quantified with the same methods that are used to assess optimal

hypotheses. For example, the Cervidae + Giraffidae node is especially weakly supported in the simultaneous analysis (Figs. 6, 8, 9, and 10). Alternatives can be assessed by examining BS scores and PBS scores of less parsimonious hypotheses. Bovidae + Cervidae has a lower BS score (−6) than the Cervidae + Giraffidae clade (+6) but the Bovidae + Cervidae clade is corroborated by four different data sets and is contradicted by only three (i.e., PBS is positive for four data sets and negative for three). Although the Cervidae + Giraffidae clade has higher BS, this group is corroborated by only three data sets and is contradicted by four (Fig. 6).

For Cervidae + Giraffidae, the PBS scores suggest that corroboration among data sets is weak. Most of the support for Cervidae + Giraffidae is concentrated in mt cytochrome *b*. For this node, BS is only +6, but PBS for mt cytochrome *b* is +8, and NDI for mt cytochrome *b* is also +8 (Figs. 6 and 9); the removal of mt cytochrome *b* collapses the Cervidae + Giraffidae node (Fig. 8). This distribution of support among data sets illustrates the need for further systematic investigations of this node. Indeed, with increased taxonomic sampling, mt cytochrome *b* does not even support a monophyletic Cervidae (Fig. 12). PBS, NDI, DRI, BS, and increased taxonomic sampling all agree that Cervidae + Giraffidae is weakly supported by the data.

Figure 16 shows another poorly supported clade, Artiodactyla. This is a weak alternative to the three well supported nodes that define a paraphyletic Artiodactyla (Fig. 10). By any measure, artiodactyl monophyly receives little support from the 17 data sets analyzed here. The DRI is 0. None of the separate analyses of the 17 data sets support a monophyletic Artiodactyla (Fig. 4), and BS for this clade summed over the individual analyses is −77 (Fig. 16). In the shortest topology that recovers Artiodactyla, there is some HBS for this group (+11), but 10 of 17 data sets have negative PBS scores, and BS is an abysmal −66 (Fig. 16).

In 1993, Prothero argued that, artiodactyl paraphyly is a “grossly unparsimonious” hypothesis. In 1998, artiodactyl monophyly has become a grossly unparsimonious hypothesis. There is very little molecular evidence for this traditionally recognized clade. Furthermore, because the morphological data set utilized in this paper was not coded for Cetacea (Gentry and Hooker, 1988), this character matrix is inadequate for testing the unity of Artiodactyla. At this point, an explicit quantification of the morphological evidence

for artiodactyl monophyly is necessary (see recent abstracts by Theodor, 1996; Geisler and O'Leary, 1997).

### *The Stability of Relationships to the Addition of Taxa*

Wheeler (1992) and Philippe and Douzery (1994) showed that inadequate taxonomic sampling can lead to spurious phylogenetic results. Robustly supported relationships should be stable to the addition of taxa.

Only 13 taxa were included in the simultaneous analysis of 17 artiodactyl data sets (Appendix 1). A more complete taxonomic sample was used to test the stability of nodes supported in that analysis (Fig. 4). Results for a cladistic analysis of 34 artiodactyls, 17 cetaceans, and 25 outgroup taxa are shown in Fig. 17. The topology is based on alignments of DNA sequences from seven genes (mt cytochrome *b*, 12S mt rDNA, 16S mt rDNA,  $\beta$ -casein,  $\kappa$ -casein,  $\gamma$ -fibrinogen, and protamine P1). Each taxon is represented by information from at least two genes (Fig. 17).

Numerous, traditional higher level taxa are supported by the simultaneous analysis of seven genes. These groups are noted in Fig. 17. Cervidae + Giraffidae and Delphinoidea + Ziphiidae are not favored, but Odontoceti, Hippopotamidae + Cetacea, Hippopotamidae + Cetacea + Ruminantia, and Hippopotamidae + Cetacea + Ruminantia + Suina are supported and have BS scores that are comparable to the analysis of 17 data sets and 13 taxa (Fig. 4). Pecora, Ruminantia, Cetacea, Suina, and cetaceans + artiodactyls are also solidly supported in the expanded analysis. Eight of 10 groups supported in the simultaneous analysis of 17 artiodactyl data sets are stable to increased taxonomic sampling of the DNA data sets (Fig. 17).

## CONCLUSIONS

### *Summary of Artiodactyl Relationships*

Pecora, Ruminantia, Cetacea, Cetacea + Hippopotamidae, Cetacea + Hippopotamidae + Ruminantia, Suina, and Cetacea + Hippopotamidae + Ruminantia + Suina are robustly supported in the simultaneous analysis of 17 artiodactyl data sets (Fig. 10, nodes B, C, F, G, H, J, and I). This conclusion is based on BS

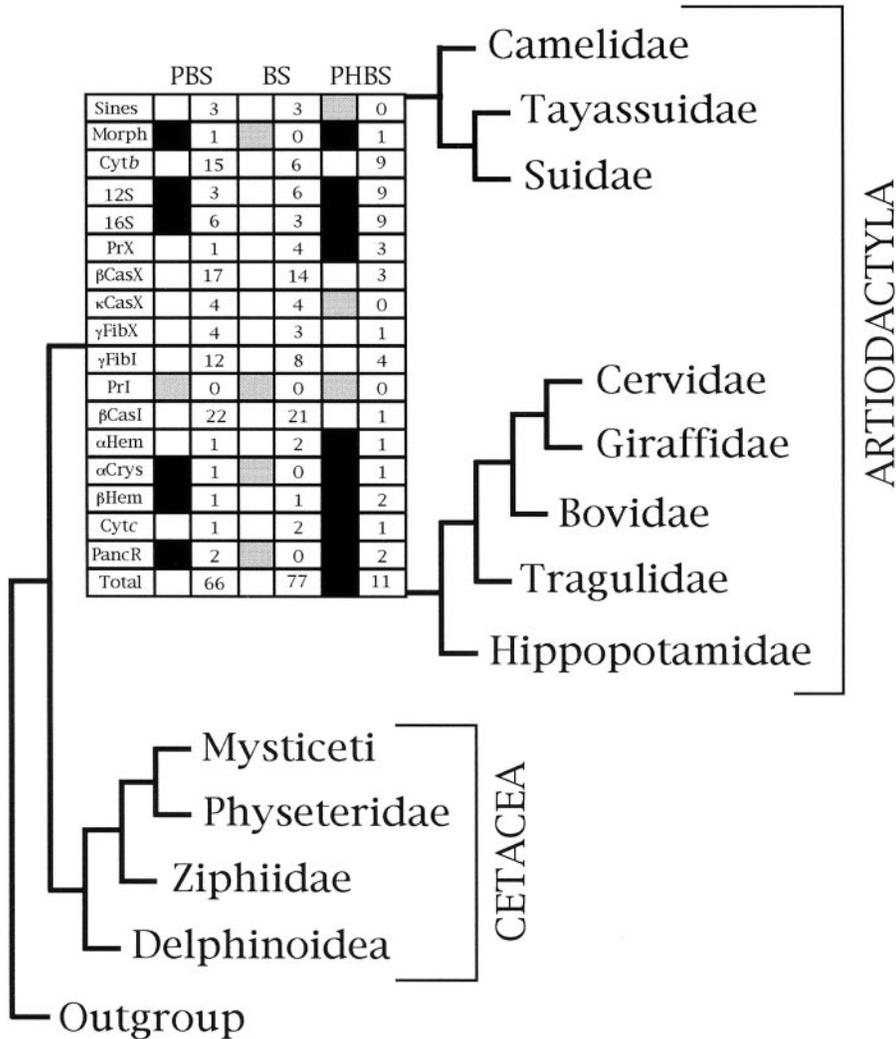


FIG. 16. An example of an extremely weakly supported node in the simultaneous analysis of 17 artiodactyl data sets. The shortest topology that supports a monophyletic Artiodactyla is shown (tree length = 4888, consistency index = 0.5317, and retention index = 0.4775). PBS, the sum of all PBS scores (= BS), positive, zero, or negative BS for each partition, the sum of these BS scores, PHBS, and the sum of all PHBS scores (= HBS) are shown. Black (+), gray (0), and white (-) boxes to the left and abbreviations for data sets are as in Fig. 6. Abbreviations for indices are as in Table 1.

scores  $\geq +12$  (Fig. 4), corroboration by multiple data sets (Figs. 6 and 9; Table 2), stability to the removal of any single data set (Fig. 8), and stability to more complete taxonomic sampling for nine of the DNA data sets (Fig. 17). However, removal of the  $\beta$ -casein exon 7 data set and the  $\beta$ -casein intron 7 data set does result in the collapse of the three nodes that cluster Cetacea with some artiodactyls to the exclusion of other artiodactyls (Fig. 8). Cervidae + Giraffidae, Delphinoidea + Ziphiidae, and Odontoceti are also recovered in the simultaneous analysis of 17 data sets (Fig. 10, nodes

A, D, and E), but are not as well supported as the clades listed above.

### Summary of Indices Defined in this Paper

The indices defined in this report measure hidden support and conflict, the distribution of support among data sets for a particular node, and the relative importance of different data sets in simultaneous analysis (Table 1).

HBS, PHBS, HCS, and HNDI are variations of BS

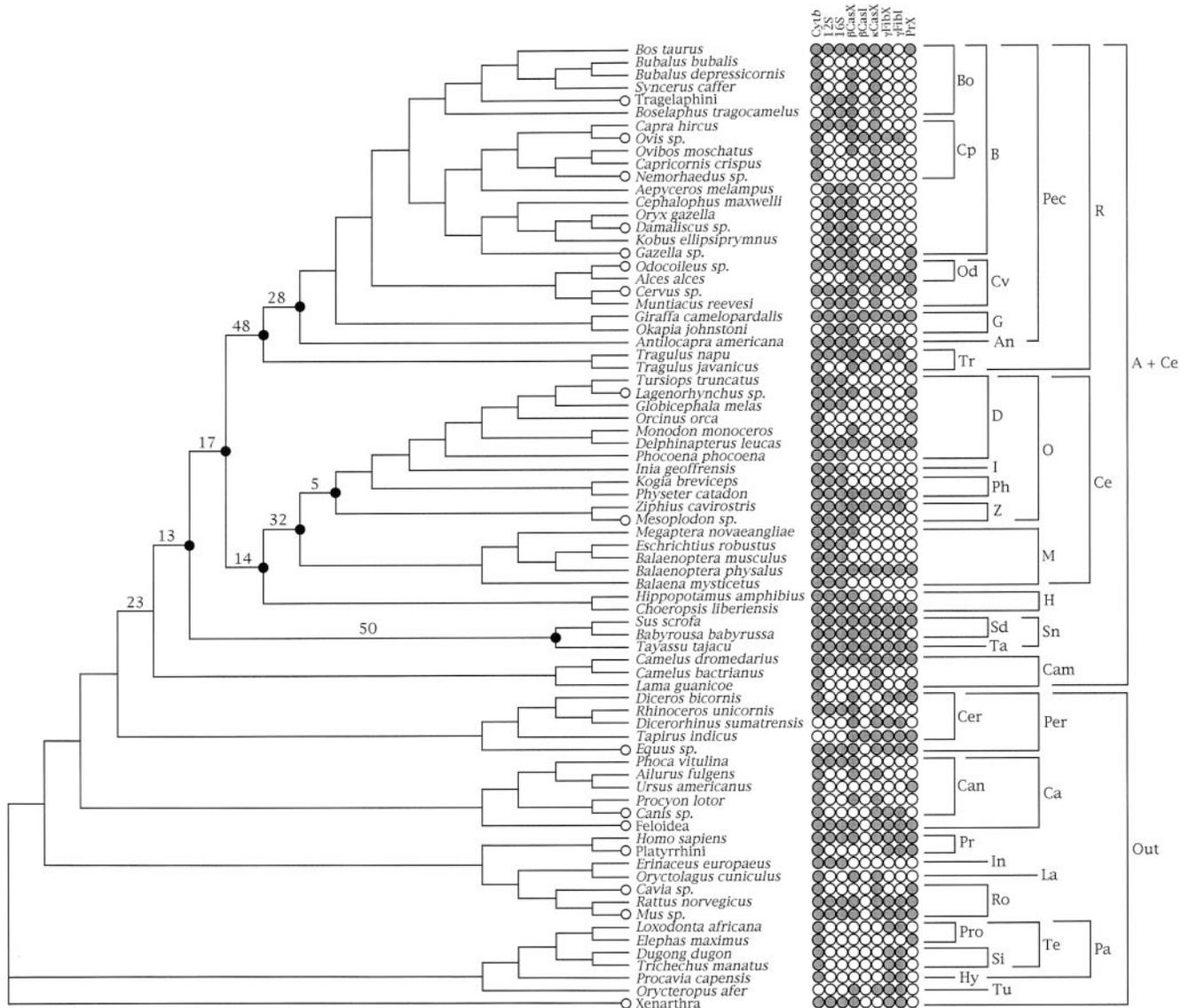


FIG. 17. A simultaneous cladistic analysis of DNA sequences from seven genes for 76 mammalian taxa. A single most parsimonious tree was found with both PAUP\* and NONA. Black dots mark nodes that are also supported by the simultaneous analysis of 17 data sets for 13 taxa (Fig. 4). BS scores for selected clades are shown at internodes. Given that the combined data set has much missing data and many local optima ("islands" of trees *sensu* Maddison, 1991), BS scores may be lower than indicated. There are 2056 informative characters in the combined data set and 12,343 steps in the minimum length tree. For this topology, the consistency index is 0.3155, and the retention index is 0.5429. Each terminal taxon is represented by information from at least two genes. DNA sequences sampled for each taxon are marked by gray dots to the right of each taxon. Abbreviations for different genic regions are as in Figure 6. Hybrid taxa composed of sequences from different species are marked by white dots at terminals: Tragelaphini: 12S/16S = *Tragelaphus imberbis*,  $\beta$ CasX/ $\kappa$ CasX = *Taurotragus oryx*; *Ovis sp.*: Cytb/ $\beta$ CasX/ $\beta$ CasI/ $\kappa$ CasX = *O. aries*,  $\gamma$ FibX/ $\gamma$ FibI = *O. dalli*; *Nemorhaedus sp.*: Cytb = *N. caudatus*,  $\kappa$ CasX = *N. goral*; *Damaliscus sp.*: 12S/16S = *D. dorcas*,  $\beta$ CasX = *D. lunatus*; *Gazella sp.*: 12S/16S = *G. thomsoni*,  $\beta$ CasX = *G. granti*, PrX = *G. dorcas*; *Odocoileus sp.*: Cytb = *O. hemionus*, 12S/16S/ $\beta$ CasX/ $\kappa$ CasX/PrX = *O. virginianus*; *Cervus sp.*: Cytb/ $\beta$ CasX/ $\kappa$ CasX = *C. nippon*, 12S/16S = *C. unicorn*, PrX = *C. elaphus*; *Lagenorhynchus sp.*: Cytb = *L. albirostris*, 12S/16S/ $\beta$ CasX/ $\kappa$ CasX/PrX = *L. obscurus*; *Mesoplodon sp.*: Cytb/12S/16S = *M. europaeus*,  $\beta$ CasX = *M. peruvianus*; *Equus sp.*: Cytb/ $\beta$ CasX/ $\kappa$ CasX = *E. grevyi*, 12S/16S = *E. asinus*,  $\gamma$ FibX/ $\gamma$ FibI = *E. przewalskii*, PrX = *E. caballus*; *Canis sp.*: Cytb = *C. familiaris*,  $\gamma$ FibX/ $\gamma$ FibI/ $\kappa$ CasX = *C. latrans*, Feloidea: Cytb/12S/16S/PrX = *Felis catus*,  $\beta$ CasX/ $\kappa$ CasX = *Panthera uncia*,  $\gamma$ FibX/ $\gamma$ FibI = *Crocuta crocuta*; Platyrrhini: Cytb = *Saimiri sciureus*,  $\gamma$ FibX/ $\gamma$ FibI = *Saguinus oedipus*, PrX = *Alouatta seniculus*; *Cavia sp.*: Cytb/PrX = *C. porcellus*,  $\kappa$ CasX = *C. cutleri*; *Mus sp.*: Cytb = *M. domesticus*, 12S/16S/ $\beta$ CasX/ $\kappa$ CasX/ $\gamma$ FibX/ $\gamma$ FibI/PrX = *M. musculus*; *Xenarthra*: Cytb = *Dasyopus novemcinctus*, 12S/16S = *Choloepus didactylus*,  $\beta$ CasX/ $\gamma$ FibX/ $\gamma$ FibI = *Cyclopes didactylus*. The monophyly of these terminals was assumed. Higher level taxa are delimited

(Bremer, 1988, 1994) that measure hidden support and conflict in simultaneous analysis. At a particular node, HCS measures hidden support and conflict of individual characters, PHBS measures hidden support and conflict in a particular data partition, and HBS measures the net hidden support and conflict among all data partitions. HNDI is an index of hidden support and conflict that is quantified through the removal of data. At a particular node, HNDI for a particular data set is the PHBS for that data set plus the net HBS that the data set brings out of other data sets in simultaneous analysis. In contrast to HBS, PHBS, HCS, and HNDI, HS is a measure of hidden support that is defined in terms of synapomorphy.

NDI and PBS (Baker and DeSalle, 1997) are measures of support among data sets at a particular node. An NDI score for a particular data set is made up of BS from that data set, HBS in that data set, and HBS that the data set brings out of other data sets in simultaneous analysis. In contrast, PBS measures the BS from that data set plus the HBS in that data set. PBS scores do not require the removal of any data for their calculation, and as measures of corroboration among data sets are more consistent with the total evidence approach to systematics (Kluge, 1989).

For a particular combined data set, the DRI is a record of phylogenetic results from all possible combinations of data sets. The DRI quantifies hidden support and conflicts, measures the relative strength of support from different data sets, specifies which combinations of data sets are critical for the resolution of particular nodes, and also basically replicates the taxonomic congruence approach.

DI and scaled DI indicate which data sets provide the most character support when combined with other data sets in simultaneous analysis. Unlike the sum of PBS scores for a data set, DI recognizes the influence of a particular data set on the levels of HBS in other data sets.

## *Corroboration among Data Sets in Simultaneous Analysis*

Within a simultaneous analysis framework, corroboration among different data sets can be quantified with the DRI, NDI, and PBS. These indices offer complementary measures of support that in combination have three clear advantages over the taxonomic congruence approach:

(1) Simultaneous analyses that incorporate these measures are oftentimes more resolved than consensus trees derived from separate analyses of each component data set.

(2) With these measures, the relative strength of support rendered by different data sets is taken into account in assessments of support among data sets.

(3) With these measures, hidden support and conflict are recognized in the quantification of support among data sets.

The simultaneous phylogenetic analysis of Artiodactyla clearly demonstrates these three principles. First, no taxonomic groupings are supported by all 17 separate analyses of individual data sets (Fig. 4). Consensus trees derived from the separate analyses are generally poorly resolved (Fig. 5). In contrast, the optimal topology for the simultaneous analysis is fully resolved (Fig. 10). Second, certain data sets suggest anomalous relationships that differ radically from the total data tree (Fig. 4). These taxonomic incongruences are usually only weakly supported in relation to the weight of the total evidence (Figs. 6 and 9). Third, and most importantly, hidden support that emerges in simultaneous analysis has profound effects in the combined analysis (Figs. 6, 7, 9, and 13; Table 2). Individual data sets that support unique relationships often show net positive support for the total data topology when combined with other data sets in simultaneous analysis. Some data sets, such as mt cytochrome *b*, have more HBS than BS (Fig. 7C), and HBS accounts for 45% of the

---

by brackets to the right of the tree: B = Bovidae, Bo = Bovinae, Cp = Caprinae, Od = Odocoileinae, Cv = Cervidae, G = Giraffidae, An = Antilocapridae, Pec = Pecora, Tr = Tragulidae, R = Ruminantia, M = Mysticeti, D = Delphinoidea, I = Iniidae, Ph = Physeteridae, Z = Ziphiidae, O = Odontoceti, Ce = Cetacea, H = Hippopotamidae, Sd = Suidae, Ta = Tayassuidae, Sn = Suina, Cam = Camelidae, A = Artiodactyla, Cer = Ceratomorpha, Per = Perissodactyla, Can = Caniformia, Ca = Carnivora, Pr = Primates, In = Insectivora, La = Lagomorpha, Ro = Rodentia, Pro = Proboscidea, Si = Sirenia, Te = Tethytheria, Hy = Hyracoidea, Pa = Paenungulata, Tu = Tubulidentata, and Out = Outgroups.

overall character support in the combined artiodactyl cladogram (Fig. 6). Ignoring this much hidden information is inadvisable.

With taxonomic congruence, a consensus of fundamental cladograms derived from separate data sets determines the preferred scheme of relationships. In contrast, simultaneous analysis stresses character congruence (Miyamoto, 1985; Kluge, 1989; Nixon and Carpenter, 1996). When different data sets are combined in simultaneous analysis, the distribution of evidence among data sets has no bearing on the choice of optimal topologies. Regardless, a detailed description of conflict, support, and congruence *among* data sets in simultaneous analysis can only lead to a better understanding of the character evidence at hand. Congruence among characters *and* among data sets is readily interpreted in simultaneous analysis.

## APPENDIX 1

Taxa for each data set. Higher level groups are abbreviated Bovidae, B; Cervidae, Ce; Giraffidae, G; Tragulidae, Tr; Delphinoidea, D; Zhiphiidae, Z; Physeteridae, P; Mysticeti, M; Hippopotamidae, H; Suidae, S; Tayassuidae, Ta; Camelidae, Ca; outgroup/Perissodactyla, O.

SINE retroposons: *Bos taurus* (B), *Axis axis* (Ce), *Giraffa camelopardalis* (G), *Tragulus napu* (Tr), *Tursiops truncatus* (D), *Berardius bairdii* (Z), *Physeter catadon* (P), *Balaenoptera acutorostrata* (M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Camelus bactrianus* (Ca), *Equus caballus* (O).

Skeletal/dental characters. In most cases, character states are for primitive members of each clade (Gentry and Hooker, 1988): Leptomerycidae (B), Leptomerycidae (Ce), Leptomerycidae (G), Tragulidae (Tr), Hippopotamidae (H), Suidae (S), Tayassuidae (Ta), Camelidae (Ca), hypothetical ancestor (O).

mt cytochrome *b*: *Bos taurus* (B), *Odocoileus hemionus* (Ce), *Giraffa camelopardalis* (G), *Tragulus napu* (Tr), *Delphinapterus leucas* (D), *Ziphius cavirostris* (Z), *Physeter catadon* (P), *Balaenoptera physalus* (M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Tayassu tajacu* (Ta), *Camelus dromedarius* (Ca), *Diceros bicornis* (O).

12S/16S mt rDNA: *Bos taurus* (B), *Cervus unicolor*

(Ce), *Giraffa camelopardalis* (G), *Tragulus napu* (Tr), *Lagenorhynchus obscurus* (D), *Ziphius cavirostris* (Z), *Physeter catadon* (P), *Balaenoptera physalus* (M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Tayassu tajacu* (Ta), *Camelus dromedarius* (Ca), *Rhinoceros unicornis* (O).

Protamine P1 exons/introns: *Bos taurus* (B), *Alces alces* (Ce), *Giraffa camelopardalis* (G), *Tragulus javanicus* (Tr), *Lagenorhynchus obscurus* (D), *Balaenoptera physalus* (M), *Choeropsis liberiensis* (H), *Sus scrofa* (S), *Tayassu tajacu* (Ta), *Lama guanicoe* (Ca), *Diceros bicornis* (O).

$\beta$ -Casein exon 7: *Ovis aries* (B), *Cervus nippon* (Ce), *Giraffa camelopardalis* (G), *Tragulus napu* (Tr), *Lagenorhynchus obscurus* (D), *Ziphius cavirostris* (Z), *Physeter catadon* (P), *Balaenoptera physalus* (M), *Choeropsis liberiensis* (H), *Sus scrofa* (S), *Tayassu tajacu* (Ta), *Camelus dromedarius* (Ca), *Rhinoceros unicornis* (O).

$\beta$ -Casein intron 7: *Ovis aries* (B), *Alces alces* (Ce), *Giraffa camelopardalis* (G), *Tragulus napu* (Tr), *Delphinapterus leucas* (D), *Ziphius cavirostris* (Z), *Physeter catadon* (P), *Balaenoptera physalus* (M), *Choeropsis liberiensis* (H), *Sus scrofa* (S), *Tayassu tajacu* (Ta), *Camelus dromedarius* (Ca), *Tapirus indicus* (O).

$\kappa$ -Casein exon 4: *Ovis aries* (B), *Cervus nippon* (Ce), *Giraffa camelopardalis* (G), *Tragulus javanicus* (Tr), *Lagenorhynchus obscurus* (D), *Ziphius cavirostris* (Z), *Physeter catadon* (P), *Balaenoptera physalus* (M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Tayassu tajacu* (Ta), *Camelus dromedarius* (Ca), *Rhinoceros unicornis* (O).

$\gamma$ -Fibrinogen exons/introns: *Ovis dalli* (B), *Alces alces* (Ce), *Giraffa camelopardalis* (G), *Tragulus napu* (Tr), *Delphinapterus leucas* (D), *Ziphius cavirostris* (Z), *Physeter catadon* (P), *Balaenoptera physalus* (M), *Choeropsis liberiensis* (H), *Sus scrofa* (S), *Tayassu tajacu* (Ta), *Camelus dromedarius* (Ca), *Diceros bicornis* (O).

$\alpha$ -Hemoglobin: *Bos taurus* (B), *Alces alces* (Ce), *Tursiops truncatus* (D), *Physeter catadon* (P), *Balaenoptera acutorostrata* (M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Camelus dromedarius* (Ca), *Ceratotherium simum* (O).

$\beta$ -Hemoglobin: *Bos taurus* (B), *Alces alces* (Ce), *Tursiops truncatus* (D), *Physeter catadon* (P), *Balaenoptera acutorostrata* (M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Camelus dromedarius* (Ca), *Ceratotherium simum* (O).

$\alpha$ -Crystallin A: *Bos taurus* (B), *Giraffa camelopardalis* (G), *Delphinapterus leucas* (D), *Balaenoptera acutorostrata* (M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Camelus dromedarius* (Ca), *Ceratotherium simum* (O).

Cytochrome *c*: *Bos taurus* (B), *Eschrichtius robustus*

(M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Camelus dromedarius* (Ca), *Equus caballus* (O).

Pancreatic ribonuclease: *Bos taurus* (B), *Alces alces* (Ce), *Balaenoptera acutorostrata* (M), *Hippopotamus amphibius* (H), *Sus scrofa* (S), *Camelus dromedarius* (Ca), *Equus caballus* (O).

## ACKNOWLEDGMENTS

A. deQueiroz, C. Hayashi, J. Carpenter, M. Allard, R. DeSalle, and an anonymous reviewer provided constructive comments on various stages of this study. D. Funk is especially thanked for his detailed reading of the manuscript. D. Swofford permitted use of test versions of PAUP\*. This work was funded by a University of Arizona Genetics Department graduate fellowship awarded to P. O'Grady, an AMNH graduate student fellowship awarded to R. Baker, and NSF Systematics Grant DEB-9509551 awarded to J. Gatesy.

## REFERENCES

- Allard, M., and Carpenter, J. (1996). On weighting and congruence. *Cladistics* **12**, 183–198.
- Baker, R., and DeSalle, R. (1997). Multiple sources of character information and the phylogeny of Hawaiian drosophilids. *Syst. Biol.* **46**, 654–673.
- Baker, R., Yu, X., and DeSalle, R. (1998). Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. *Mol. Phylogenet. Evol.* **9**, 427–436.
- Barrett, M., Donoghue, M., and Sober, E. (1991). Against consensus. *Syst. Zool.* **40**, 486–493.
- Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**, 795–803.
- Bremer, K. (1990). Combinable component consensus. *Cladistics* **6**, 369–372.
- Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10**, 295–304.
- Brower, A., DeSalle, R., and Vogler, A. (1996). Gene trees, species trees, and systematics: A cladistic perspective. *Annu. Rev. Ecol. Syst.* **27**, 423–450.
- Bull, J., Huelsenbeck, J., Cunningham, C., Swofford, D., and Waddell, P. (1993). Partitioning and combining data in phylogenetic analysis. *Syst. Biol.* **42**, 384–397.
- Cannatella, D., Hillis, D., Chippindale, P., Weigt, L., Rand, A., and Ryan, M. (1998). Phylogeny of frogs of the *Physalaemus pustulosus* species group, with an examination of data incongruence. *Syst. Biol.* **47**, 311–335.
- Chippindale, P., and Wiens, J. (1994). Weighting, partitioning, and combining characters in phylogenetic analysis. *Syst. Biol.* **43**, 278–287.
- Clark, J., Maddison, W., and Kidwell, M. (1994). Phylogenetic analysis supports horizontal transfer of *P* transposable elements. *Mol. Biol. Evol.* **11**, 40–50.
- Collins, T., Wimberger, P., and Naylor, G. (1994). Compositional bias, character-state bias, and character-state reconstruction using parsimony. *Syst. Biol.* **43**, 482–496.
- Cunningham, C. (1997). Can three incongruence tests predict when data should be combined? *Mol. Biol. Evol.* **14**, 733–740.
- Davis, J. (1993). Character removal as a means for assessing stability of clades. *Cladistics* **9**, 201–210.
- Davis, J., Simmons, M., Stevenson, D., and Wendel, J. (1998). Data decisiveness, data quality, and incongruence in phylogenetic analysis: An example from the monocotyledons using mitochondrial *atpA* sequences. *Syst. Biol.* **47**, 282–310.
- deQueiroz, A., Donoghue, M., and Kim, J. (1995). Separate versus combined analysis of phylogenetic evidence. *Annu. Rev. Ecol. Syst.* **26**, 657–681.
- DeSalle, R., and Brower, A. (1997). Process partitions, congruence, and the independence of characters: Inferring relationships among closely related Hawaiian *Drosophila* from multiple gene regions. *Syst. Biol.* **46**, 751–764.
- Eernisse, D., and Kluge, A. (1993). Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology. *Mol. Biol. Evol.* **10**, 1170–1195.
- Farris, J. (1989). The retention index and the rescaled consistency index. *Cladistics* **5**, 417–419.
- Farris, J. (1997). Combinability vs. congruence—abstract. *Cladistics* **13**, 170.
- Farris, J., Källersjö, M., Kluge, A., and Bult, C. (1994a). Testing significance of incongruence. *Cladistics* **10**, 315–319.
- Farris, J., Källersjö, M., Kluge, A., and Bult, C. (1994b). Permutations. *Cladistics* **10**, 65–76.
- Felsenstein, J. (1978). Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* **27**, 401–410.
- Gatesy, J. (1997). More support for a Cetacea/Hippopotamidae clade: The blood-clotting protein gene  $\gamma$ -fibrinogen. *Mol. Biol. Evol.* **14**, 537–543.
- Gatesy, J. (1998). Molecular evidence for the phylogenetic affinities of Cetacea. In “Advances in Vertebrate Paleobiology” (J. Thewissen, Ed.), pp. 63–111. Plenum, New York.
- Gatesy, J., and O'Grady, P. (in prep.). Character congruence and incongruence at the base of Artiodactyla: An analysis of ten data sets.
- Gatesy, J., Hayashi, C., Cronin, M., and Arctander, P. (1996). Evidence from milk casein genes that cetaceans are close relatives of hippopotamid artiodactyls. *Mol. Biol. Evol.* **13**, 954–963.
- Geisler, J., and O'Leary, M. (1997). A phylogeny of Cetacea, Artiodactyla, Perissodactyla, and archaic ungulates: The morphological evidence. *J. Vert. Paleo. Abstracts* **17**, 48A
- Gentry, A., and Hooker, J. (1988). The phylogeny of the Artiodactyla. In “The Phylogeny and Classification of the Tetrapods” (M. Benton, Ed.), Vol. 2, pp. 235–272. Clarendon Press, Oxford.

- Gilbert, D. (1992). "SeqApp." Version 1.9a. Indiana University, Bloomington.
- Goloboff, P. (1991). Homoplasy and the choice among cladograms. 1991. *Cladistics* **7**, 215–232.
- Goloboff, P. (1993a). "NONA." Version 1.16. American Museum of Natural History, New York.
- Goloboff, P. (1993b). Estimating character weights during tree search. *Cladistics* **9**, 83–91.
- Goodman, M., Czelusniak, J., Moore, G., Romero, A., and Matsuda, G. (1979). Fitting the gene lineage into its species lineage, a parsimony strategy illustrated by cladograms constructed from globin sequences. *Syst. Zool.* **28**, 132–163.
- Graur, D., and Higgins, D. (1994). Molecular evidence for the inclusion of cetaceans within the order Artiodactyla. *Mol. Biol. Evol.* **11**, 357–364.
- Irwin, D., and Arnason, U. (1994). Cytochrome *b* gene of marine mammals: Phylogeny and evolution. *J. Mammal. Evol.* **2**, 37–55.
- Kluge, A. (1989). A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Syst. Zool.* **38**, 7–25.
- Kluge, A. (1997). Testability and the refutation and corroboration of cladistic hypotheses. *Cladistics* **13**, 81–96.
- Kluge, A., and Farris, J. (1969). Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18**, 1–32.
- Kluge, A., and Wolf, A. (1993). Cladistics: What's in a word? *Cladistics* **9**, 183–199.
- Larson, A. (1994). The comparison of morphological and molecular data in phylogenetic systematics. In "Molecular Ecology and Evolution: Approaches and Applications" (B. Schierwater, B. Streit, G. Wagner, and R. DeSalle, Eds.), pp. 371–390. Birkhauser Verlag, Basel.
- Maddison, D. (1991). The discovery and importance of multiple islands of most-parsimonious trees. *Syst. Zool.* **40**, 315–328.
- Maddison, W., and Maddison, D. (1992). "MacClade 3.03." Sinauer, Sunderland.
- Margush, T., and McMorris, F. (1981). Consensus n-trees. *Bull. Math. Biosci.* **43**, 239–244.
- Mickevich, M., and Farris, J. (1981). The implications of congruence in *Menidia*. *Syst. Zool.* **30**, 351–370.
- Milinkovitch, M., Orti, G., and Meyer, A. (1993). Revised phylogeny of whales suggested by mt ribosomal DNA sequences. *Nature* **361**, 346–348.
- Milinkovitch, M., Meyer, A., and Powell, J. (1994). Phylogeny of all major groups of cetaceans based on DNA sequences from three mt genes. *Mol. Biol. Evol.* **11**, 939–948.
- Milinkovitch, M., Orti, G., and Meyer, A. (1995). Novel phylogeny of whales revisited but not revised. *Mol. Biol. Evol.* **12**, 518–520.
- Milinkovitch, M., LeDuc, R., Adachi, J., Farnir, F., Georges, M., and Hasegawa, M. (1996). Effects of character weighting and species sampling on phylogeny reconstruction: A case study based on DNA sequence data in cetaceans. *Genetics* **144**, 1817–1833.
- Milinkovitch, M., Bérubé, M., and Palsbøll, P. (1998). Cetaceans are highly derived artiodactyls. In "Advances in Vertebrate Paleobiology" (J. Thewissen, Ed.), pp. 113–131. Plenum, New York.
- Miyamoto, M. (1985). Consensus cladograms and general classifications. *Cladistics* **1**, 186–189.
- Miyamoto, M. (1996). A congruence study of molecular and morphological data for eutherian mammals. *Mol. Phylogenet. Evol.* **6**, 373–390.
- Miyamoto, M., and Goodman, M. (1986). Biomolecular systematics of eutherian mammals: Phylogenetic patterns and classification. *Syst. Zool.* **35**, 230–240.
- Miyamoto, M., and Fitch, W. (1995). Testing species phylogenies and phylogenetic methods with congruence. *Syst. Biol.* **44**, 64–76.
- Montgelard, C., Catzeflis, F., and Douzery, E. (1997). Phylogenetic relationships of artiodactyls and cetaceans as deduced from the comparison of cytochrome *b* and 12S rRNA mt sequences. *Mol. Biol. Evol.* **14**, 550–559.
- Nelson, G. (1979). Cladistic analysis and synthesis: Principles and definitions, with a historical note on Adanson's Familles des Plantes (1763–1764). *Syst. Zool.* **28**, 1–21.
- Nixon, K., and Carpenter, J. (1996). On simultaneous analysis. *Cladistics* **12**, 221–241.
- Novacek, M. (1989). Higher mammal phylogeny: The morphological-molecular synthesis. In "The Hierarchy of Life" (B. Fernholm, K. Bremer, and H. Jornvall, Eds.), pp. 421–435. Elsevier Science Publishers, B.V.
- Novacek, M. (1992). Mammalian phylogeny: Shaking the tree. *Nature* **356**, 121–125.
- Olmstead, R., and Sweere, J. (1994). Combining data in phylogenetic systematics: An empirical approach using three molecular data sets in the Solanaceae. *Syst. Biol.* **43**, 467–481.
- Pamilo, P., and Nei, M. (1988). Relationships between gene trees and species trees. *Mol. Biol. Evol.* **5**, 568–583.
- Philippe, H., and Douzery, E. (1994). The pitfalls of molecular phylogeny based on four species, as illustrated by the Cetacea/Artiodactyla relationships. *J. Mammal. Evol.* **2**, 133–152.
- Pickford, M. (1983). On the origins of Hippopotamidae together with descriptions of two new species, a new genus, and a new subfamily from the Miocene of Kenya. *Geobios* **16**, 193–217.
- Prager, E., and Wilson, A. (1988). Ancient origin of lactalbumin from lysozyme: Analysis of DNA and amino acid sequences. *J. Mol. Evol.* **27**, 326–335.
- Prothero, D. (1993). Ungulate phylogeny: Molecular versus morphological evidence. In "Mammal Phylogeny" (F. Szalay, M. Novacek, and M. McKenna, Eds.), Vol. II, pp. 173–181. Springer-Verlag, New York.
- Prothero, D., Manning, E., and Fischer, M. (1988). The phylogeny of the ungulates. In "The Phylogeny and Classification of the Tetrapods" (M. Benton, Ed.), Vol. 2, pp. 201–234. Clarendon, Press, Oxford.
- Rozas, J., and Aguade, M. (1994). Gene conversion is involved in the transfer of genetic information between naturally occurring inversions of *Drosophila*. *Proc. Natl. Acad. Sci. USA* **91**, 11517–11521.
- Sarich, V. (1993). Mammalian systematics: Twenty-five years among

- their albumins and transferrins. In "Mammal Phylogeny" (F. Szalay, M. Novacek, and M. McKenna, Eds.), Vol. II, pp. 103–114. Springer-Verlag, New York.
- Schuh, R., and Polhemus, J. (1980). Analysis of taxonomic congruence among morphological, ecological, and biogeographic data sets for the Leptopodomorpha (Hemiptera). *Syst. Zool.* **30**, 309–325.
- Shaffer, H., Meylan, P., and McKnight, M. (1997). Tests of turtle phylogeny: Molecular, morphological, and paleontological approaches. *Syst. Biol.* **46**, 235–268.
- Shimamura, M., Yasue, H., Ohahima, K., Abe, H., Kato, H., Kishiro, T., Goto, M., Munechika, I., and Okada, N. (1997). Molecular evidence from retroposons that whales form a clade within even-toed ungulates. *Nature* **388**, 666–670.
- Shoshani, J. (1986). Mammalian phylogeny: Comparison of morphological and molecular results. *Mol. Biol. Evol.* **3**, 222–242.
- Siddall, M. (1997). Prior agreement: Arbitration or arbitrary. *Syst. Biol.* **46**, 765–769.
- Siddall, M., and Kluge, A. (1997). Probabilism and phylogenetic inference. *Cladistics* **13**, 313–336.
- Stanhope, M., Smith, M., Waddell, V., Porter, C., Shivji, M., and Goodman, M. (1996). Mammalian evolution and the interphotoreceptor retinoid binding protein (IRBP) gene: Convincing evidence for several superordinal clades. *J. Mol. Evol.* **43**, 83–92.
- Stewart, C., Schilling, J., and Wilson, A. (1987). Adaptive evolution in the stomach lysozymes of foregut fermenters. *Nature* **330**, 401–404.
- Sullivan, J. (1996). Combining data with different distributions of among-site rate variation. *Syst. Biol.* **45**, 375–380.
- Swofford, D. (1993). "PAUP: Phylogenetic Analysis using Parsimony." Version 3.1.1. Illinois Natural History Survey, Champaign.
- Swofford, D. (in prep.). "PAUP\*."
- Templeton, A. (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37**, 221–244.
- Theodor, J. (1996). Postcranial evolution in early non-ruminant artiodactyls. *J. Vert. Paleo. Abstracts* **16**, 15A.
- Theodor, J. (1997). Why do molecules and morphology conflict? Examination of the artiodactyl–cetacean relationship. *J. Vert. Paleo. Abstracts* **17**, 80A.
- Thewissen, J. (1994). Phylogenetic aspects of cetacean origins: A morphological perspective. *J. Mammal. Evol.* **2**, 157–184.
- Webb, S., and Taylor, B. (1980). The phylogeny of the hornless ruminants and a description of the cranium of *Archaeomeryx*. *Bull. Amer. Mus. Nat. Hist.* **167**, 117–158.
- Wheeler, W. (1992). Extinction, sampling, and molecular data. In "Extinction and Phylogeny" (M. Novacek and Q. Wheeler, Eds.), pp. 205–215. Columbia University Press, New York.
- Wheeler, W., and Gladstein, D. (1994). "MALIGN." Version 2.1. American Museum of Natural History, New York.
- Wheeler, W., Cartwright, P., and Hayashi, C. (1993). Arthropod phylogeny: A combined approach. *Cladistics* **9**, 1–39.
- Whiting, M., Carpenter, J., Wheeler, Q., and Wheeler, W. (1997). The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Syst. Biol.* **46**, 1–68.
- Xu, X., Janke, A., and Arnason, U. (1996). The complete mt DNA sequence of the greater Indian rhinoceros, *Rhinoceros unicornis*, and the phylogenetic relationship among Carnivora, Perissodactyla, and Artiodactyla (+ Cetacea). *Mol. Biol. Evol.* **13**, 1167–1173.
- Zardoya, R., and Meyer, A. (1996). Phylogenetic performance of mt protein-coding genes in resolving relationships among vertebrates. *Mol. Biol. Evol.* **13**, 933–942.