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Molecules, Morphology and the Phylogeny of Falconiform Birds

by

Carole S. Griffiths

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INTRODUCTION

History is hidden. Pathways to history remain as traces in the variations among species. Phylogenetic hypotheses link those pathways and provide the patterns that are the history of species. These patterns are an essential framework for comparative research in evolutionary biology (Pagel and Harvey 1988, Gittleman and Luh 1992). For many orders of birds, these hypotheses don't exist. When phylogenies do exist, conflict in hypotheses renders the history ambiguous.

The order Falconiformes is one of the avian orders with unresolved higher level systematics. The Falconiformes currently consists of 76 genera and 290 species divided into four families (Stresemann and Amadon 1979); the Accipitridae (60 genera), the Falconidae (10 genera); the Cathartidae (5 genera); and Sagittariidae (monotypic). The primary goals of my research were to infer phylogenies: for the order Falconiformes using variations in morphology, and for the family Falconidae, using variations in morphology and gene sequences.

Morphological data have been the traditional source of information used to reconstruct phylogenies. However, within the last twenty years, the predominate source of data has turned from morphology to molecules (Moritz and Hillis 1990, Barrowclough 1992), and focused, within the last five years, on gene sequence data (Miyamoto and Cracraft 1991). The increase in molecular systematics has brought with it concerns about the quality of molecular and morphological data and the appropriate analyses of these data.

In order to address those concerns, I collected morphological and molecular data for the Falconidae, and asked the following questions. (1) Are there advantages to either morphological or molecular data for phylogenetic

research? (2) Are there differences in the analysis of these two different kinds of data? (3) Are the patterns of divergence and of homoplasy similar for these data?

As a final goal, I wanted to use the phylogenies produced by my research to trace the evolution of the different structures used as the source of characters.

The morphological data I collected were variations in syringeal morphology. At the end of the 19th century, the syrinx was an important source of data for classifying the major subdivisions of the Passeriformes (Ames 1971). Syringeal data have become important again in the systematics of oscines and suboscines (e.g., Ames 1971, Lanyon 1984, Prum 1990), but haven't influenced the systematics of other orders. I wanted to assess the usefulness of the syrinx for systematics of non-passerines. I also wanted to examine the variation in syringeal anatomy at different hierarchical levels; specifically, at the ordinal, familial and generic levels.

As the second data set for the Falconidae phylogeny, I collected variations in sequences of the mitochondrial protein coding gene, cytochrome *b*. This gene has been characterized as one of the slowest evolving in the mitochondrial genome. Thus, variation in sequences would be appropriate for analyzing relationships at the family level (Moritz et al. 1987, Desjardins and Morais 1990, Arctander 1991). As with all protein coding genes, however, patterns of substitution, and subsequent phylogenetic information, vary at different codon positions (Moritz et al. 1987). At the third codon position, transitions do not cause amino acid replacement and these occur most frequently. Transversions, which generally result in amino acid substitutions at all three codon positions, occur more slowly. Thus, transitions are considered useful for resolving generic relationships within families, and transversions appropriate for resolving relationships at the familial and

ordinal level (Swofford & Olson 1990, Arctander 1991, Irwin et al. 1991, Helm-Bychowski & Cracraft 1993). This information was the starting point for my investigation of the evolution of cytochrome *b* within the Falconidae.

The first two chapters of this dissertation present the results of the morphological analysis of the Falconidae and of the Falconiformes. The last presents the results of the molecular analysis of the Falconidae, describes a method for analyzing sequence data, and discusses the analysis of molecular and morphological data.

CHAPTER 1.

MONOPHYLY OF THE FALCONIFORMES INFERRED FROM SYRINGEAL MORPHOLOGY.

ABSTRACT.- The systematic relationships of the diurnal birds of prey (Falconiformes) are unresolved. The monophyly of the order has not been established, and the relationships of the families within the order, and of genera within the three polytypic families are unclear. I analyzed variation in syringeal morphology of genera within each of the families in the order as well as among four orders of outgroups to derive a phylogeny. An additional goal of this research was to assess the usefulness of the syrinx for resolving the systematics of non-passerines. The phylogeny derived from these syringeal data support the monophyly of the Falconiformes. In addition, syringeal data provide strong support for the monophyly of three clades within the Falconiformes: the Cathartidae, positioned basal to the Falconidae, and an Accipitrinae-Sagittariidae-Pandioninae cluster. Overall results of this analysis indicate that syringeal morphology is conservative, with most of the informative variation occurring at higher systematic levels.

The order Falconiformes currently consists of 76 genera and 290 species divided into four families (Stresemann and Amadon 1979); Accipitridae, (including Accipitrinae, hawks and eagles, 59 genera, 217 species, and Pandioninae, osprey, monotypic); Falconidae (falcons, 10 genera, 62 species); Cathartidae (New World vultures, 5 genera, 7 species); and Sagittariidae (the secretary bird, monotypic).

Comprehensive reviews of the history of falconiform classification have been published previously (Jollie 1976, Sibley and Ahlquist 1990), and only a summary will be presented here. The order historically has been united by several external morphological characters (hooked beak and curved talons) and by several internal characters (biceps slip absent, ambiens present, caeca rudimentary; Beddard 1898). However, four current classifications disagree on the monophyly of the order, and on subordinal and familial relationships (Fig. 1). Two consider the order monophyletic (Stresemann and Amadon 1979, Storer 1971), while the other two propose either removal of the Cathartidae from the order (Sibley and Ahlquist 1990) or inclusion of Strigiformes (owls) within the order (Cracraft 1981).

The idea that the Cathartidae may not belong in the order has been proposed previously, based on the morphological and behavioral differences of this family from others in the Falconiformes. In addition, similarities of cathartids to other groups have been noted, including the Ciconiidae (Garrod 1873, Beddard 1898, Ligon 1967, Rea 1983), Pelecaniformes (Beddard 1898, Jollie 1976), Procellariformes (Jollie 1976), and Gruiformes (Pycraft 1902). Friedmann (1950: 6) stated that the Cathartidae were a primitive group not differentiated from the "primitive stock from which the Ciconiiformes, Pelecaniformes and Procellariformes have been developed."

Hypotheses of a falconiform-strigiform relationship have been advanced at various times, based on similarities of palatal and myological characters (reviewed in Sharpe 1891, Cracraft 1981, McKitrick 1991). Pandioninae (Sharpe 1891, Pycraft 1902) and Falconidae (Beddard 1898, Brown and Amadon 1968) have been suggested as links between falconiforms and strigiforms.

Relationships of the Falconiformes to other orders have also been proposed, including the orders Pelecaniformes, Ciconiiformes and Psittaciformes (reviewed in Sharpe 1891, Shufeldt 1909), Gruiformes (Shufeldt 1909), Cuculiformes and Columbiformes (Verheyen 1950, reviewed in Jollie 1977). The AOU (1983) Checklist places the Falconiformes between Anseriformes and Galliformes whereas Sibley and Ahlquist (1990) position the order (with Cathartidae removed) as sister taxon to a group including the Podicipedidae, Sulidae, Phalacrocoracidae and Phaethontidae.

Syringeal morphology had been used in the classification of the major subdivisions of the Passeriformes at the end of the 19th century (Ames 1971). Within the last 20 years, syringeal data have again become important in the systematics of oscines and suboscines (e.g. Ames 1971, Warner 1972, Lanyon 1984, Prum 1990, 1992), but have not strongly influenced the systematics of other orders. There have been no detailed reports on falconiform syringeal anatomy since Beddard (1903), and no systematic analysis of that anatomy.

I examined patterns of variation in syringeal morphology within the Falconiformes to assess the usefulness of the syrinx for systematics of non-passerines; that is, are there phylogenetically informative syringeal characters; and to answer two primary systematic questions: (1) Is the order monophyletic? (2) What are the relationships of the major clades within the order?

MATERIALS AND METHODS

Specimens. - I examined syringes from collections at the American Museum of Natural History (AMNH), the National Museum of Natural History (USNM), the Royal Ontario Museum (ROM), the University of Kansas Museum of Natural History (KUMNH), the Museum of Vertebrate Zoology, University of California at Berkeley (MVZ), and the Louisiana State University Museum of Natural Science (LSUMNS), some of which I dissected from fresh or alcohol preserved specimens. These were cleared and double-stained to distinguish cartilaginous and ossified tissue (Cannell 1988). Observations were made using a Wild M5A dissecting microscope and drawings made with a camera lucida. Drawings were scanned into a Macintosh computer and final illustrations prepared using Aldus Freehand 2.0.

I analyzed 124 falconiform syringes and 66 syringes from purported outgroups (Appendix 1). Three of the five cathartid genera and all falconid genera were included. Within the Accipitridae, genera were chosen to represent each of the previously proposed subgroups; these totaled 60% of the currently recognized genera. In general, sampling within the ingroup was constrained by the availability of specimens. Two or more individuals from 27 species were analyzed to assess variation at the intra-specific level.

Analysis. - Variation in morphology was coded using both binary and multistate characters. Multistate characters were ordered if either of two criteria was satisfied. Similarity of derived states was the primary criterion used (Patterson 1982). Thus, transformation series were proposed if adjacent derived states were similar, and each succeeding state was a modification of the previous state; that is, the derived states formed a nested set of synapomorphies. Transformation series were also proposed using

ontogenetic information (character 7). Justifications used for ordering are discussed in the character descriptions (Appendix 2). Multistate characters were coded as unordered if states were alternative variations of a character.

I used outgroup information to polarize characters (Maddison et al. 1984). However, the relationships of other orders to the Falconiformes are unclear, and the choice of an appropriate outgroup is not readily apparent. Species from four orders of birds were included in the analysis. A comprehensive subset of genera in the Ciconiiformes and Strigiformes was examined because there are phylogenetic hypotheses of relationships of these orders to the Falconiformes. In addition, species within the Pelecaniformes were added since several authors suggested a relationship of this order to the Falconiformes (Beddard 1898, Shufeldt 1909, Friedmann 1950, Jollie 1976). Because monophyly of the Falconiformes could not be assumed, species from these other orders were not treated as outgroups in the analysis, nor was there a constraint on monophyly imposed in the analysis (Maddison et al. 1984). Species within the Galliformes were used to root the cladogram. I used PAUP 3.0s (Swofford 1991) to derive the most parsimonious resolution of the data. The size of this data set precluded the use of exact algorithms for resolving the data; therefore, the heuristic algorithm was used. However, this option does not guarantee optimality and may identify a solution that is only locally optimal. To increase the probability of finding solutions that were globally optimal, analyses were repeated varying both the branch-swapping and taxa-addition options.

Three indices were used to assess the congruence of the characters hypothesized as synapomorphies (Farris 1989): (1) consistency index, which is the minimum amount of change of a character divided by the amount observed on the tree; (2) rescaled consistency index, which is a linear rescaling

to allow the consistency index to vary between 0 and 1; and (3) retention index, which is the proportion of original characters remaining as synapomorphies. Consensus methods were used to summarize information from the set of most parsimonious trees. I used strict consensus trees, which include groups found in each of the most parsimonious cladograms, and majority rule trees, which include groups found in a defined proportion of cladograms. Consensus trees must be interpreted with care as they may not be parsimonious reconstructions of the original data (Swofford 1992). Nevertheless, consensus trees remain an efficient way for highlighting congruent clades, and the characters and taxa causing incongruence.

RESULTS

Syringeal Morphology. - The main components of a typical accipitrid syrinx, the supporting elements, membranes and muscles, are illustrated in Figure 2. Definitions of syringeal structures follow Ames (1971) and include four types of supporting elements, *A* and *B* ringlike elements, the pessulus and accessory cartilaginous structures. Ames (1971) used three criteria for defining *A* and *B* elements, composition, cross-sectional shape and orientation of concavity. *A* elements are ossified and flattened in cross-section, occurring on the trachea as single rings but sometimes extending onto the bronchi as paired double rings. They may be complete (forming a closed ring) or incomplete. In addition, they may be fused partially or completely near the tracheo-bronchial junction, forming a drum or tympanum. *B* elements are generally cartilaginous and D-shaped in cross-section, occurring as paired rings on the bronchi. These may be complete rings, or may have an opening on the medial surface of each bronchus. The pessulus, a cartilaginous or ossified bar, is located on the mid-sagittal plane

between the bronchi. The dorsal and ventral ends of the pessulus may be free or may fuse to *A* or *B* elements. Finally, accessory cartilaginous structures occur on the craniad edge of the internal membranes.

The definitions of *A* and *B* elements differ from traditional terminology, in which ringed structures are tracheal or bronchial rings (King 1989), based on the relative position of the structures to the tracheo-bronchial junction. There are no homologies that can be postulated using traditional names. For example, the external membrane in falconid genera is located between the second and third, third and fourth, or fourth and fifth bronchial rings. When these rings are recognized as *A* and *B* elements, however, this membrane is always between A1 and B1, a synapomorphy for the family. Definitions of rings as *A* and *B* elements have been used in systematic studies of oscines and suboscines (Ames 1971, Lanyon 1984, Prum 1990, 1992, 1993) and, in limited detail, to a broad range of orders (Cannell 1986). My analysis is the first application of these definitions to detailed structures in orders other than Passeriformes.

Ames' (1971) criteria for defining *A* and *B* elements are sufficient for most of the families used in this analysis. A1 and B1 can be differentiated by cross-sectional shape and composition in the Cathartidae, Ciconiidae, Ardeidae, Falconidae, and Strigiformes. However, because the first *B* elements in several Accipitridae species are highly ossified, these criteria alone could not always differentiate *A* and *B* elements. I used an additional criterion, the distinctive shape of the B1 element, for the accipitrids in this analysis. The first *B* element is wider medially than other *A* and *B* elements, with narrower ends. This was corroborated by the congruence of muscle insertion; the M. tracheolateralis always inserted on B1 when B1 was defined using these criteria.

In addition to structural elements, the syrinx is composed of two kinds of syringeal membranes. The internal membranes are located on the medial surface of the bronchi between the dorsal and ventral ends of incomplete *A* and *B* elements. These are considered the sound producing structures (Gaunt and Gaunt 1985) and occur in all species examined except the ciconiids. The external membranes are on the lateral walls of the bronchi, usually between A1 and one or two of the first four B elements. Although the location of an external membrane is a synapomorphy for the Falconidae, the existence of external membranes is variable within most of the other families examined and may be correlated with structural modifications in elements bordering the membranes. This character was, therefore, of limited use in this analysis.

There are also two classes of syringeal muscles. Intrinsic muscles originate and insert on syringeal elements and are important in the systematics of the Passeriformes. These do not exist in falconiform species. The two extrinsic muscles originate outside the syrinx. The M. sternotrachealis originates on the internal surface of the coracoid or costal process of the sternum and inserts on several *A* elements on the trachea. The M. tracheolateralis originates on the lateral surface of the cricoid cartilage of the larynx and descends laterally down the trachea. In many taxa it is contiguous with, or underlies the M. sternotrachealis, and the insertion of this muscle can be somewhat obscured because of this. Because muscle fibers are damaged in cleared specimens, the insertions of these muscles were not always apparent in specimens I examined and were of limited use phylogenetically. Where visible, the M. tracheolateralis inserts on A1, B1 and possibly B2 in the Ardeidae, on B1 in the accipitrids (including *Pandion* and *Sagittarius)* and in the Strigiformes, and on the lateral membrane in the falconids. This muscle has been claimed to be lost in *Struthio, Casuarius,*

Dromaius, Rhea, Apteryx, the Cathartidae, Ciconiidae, most Pelecaniformes and some Galliformes (Beddard 1898, King 1989). Within the ratites, however, the muscle exists but ends where the M. sternotrachealis begins (King 1989). Similarly, I observed muscle fibers in a *Coragyps* syringeal specimen, laterally on the trachea, ending cranial to the junction. These muscles have also been described in *Cathartes* (Maynard 1897) and are probably the M. tracheolateralis, but this diagnosis must be confirmed in intact specimens.

Intraspecific Variation in morphology. - Assessment of intraspecific variation revealed several characters that were polymorphic in species within the Falconidae and Accipitridae and were not used in this analysis. The number of *A* elements fused ventrally in the tympanum varied in *Buteo platypterus* (5, 6, or 7), in *Accipiter striatus* (5 or 6) and in *Falco sparverius* (3 or 4). The size of the ventral ossified patch covering the fused tympanum rings varied within *Buteo* and *Leucopternis* species. Finally, the B1 element, although normally cartilaginous, was partially to totally ossified within several species, the amount of ossification varying intraspecifically.

I found no sexual dimorphism in the species for which this information was available. General patterns in ontogenetic variation were found. Ossification of the tympanum and *A* elements increased in adults compared to juveniles in *Tyto, Buteo, Falco, Pandion, Accipiter* and *Cathartes*. Fusion of the elements in the tympanum also increased slightly in adults compared to juveniles of *Pandion, Tyto* and *Falco*. Both of these patterns are consistent with observations in the Passeriformes (Ames 1971). An additional pattern was found in the Cathartidae. In nestling *Cathartes*, single *A* elements immediately cranial to the tracheo-bronchial junction are

complete rings. In adults, these are incomplete, with gaps dorsally (character 7).

Phylogenetic analysis of Falconiformes. - Variation in syringeal morphology was coded initially for 103 taxa as 49 characters, of which 33 were binary and 16 were multistate (Appendix 2). Redundant species (those with identical character states) were then deleted, resulting in a final matrix of 88 taxa (Table 1). Analysis of these data resulted in more than 5,000 most-parsimonious cladograms, which is not unexpected for a matrix with more taxa than characters. The shortest trees found had a length of 195, consistency index of 0.401, rescaled consistency index of 0.358, and retention index of 0.865.

The strict consensus tree (Fig. 3) indicates support for the monophyly of the order Falconiformes. Within the order, Cathartidae is basal to a clade of two sister taxa, the Falconidae and the Accipitridae (including *Sagittarius* and *Pandion)*. Two derived characters unite the three clades in the order. All species have complete, double *A* elements on the bronchi; these also occur in several strigiform genera (character 5). Species also have an ossified pessulus which is connected dorsally and ventrally to *A* elements; the dorsal attachment is lost in *Vultur* (character 19). Four characters unite the Falconidae and the Accipitridae as sister taxa. Species in these families have a tympanum composed of *A* elements fused ventrally and dorsally, to which the pessulus is attached (characters 20, 22). In addition, these species have sheets of cartilaginous tissue forming a border cranially on the internal membranes (character 44). The tympanum is ossified totally in falconid species and in the most basal accipitrid species, but an alternative pattern, lack of ossification dorsally, exists in most of the other accipitrids (character 21).

There is strong support for the monophyly of the Falconidae. The falconid syrinx (Fig 4) is characterized by a completely ossified tympanum, an

ossified pessulus fused dorsally and ventrally to the tympanum, a lateral membrane located between A1 and B1 on which the M. tracheolateralis inserts, B1 elements evenly wider than other *B* elements, and fusion of the ends of incomplete A1 and B1 elements (characters 26, 28, 33, 37, 41).

The three genera of cathartids included in my study are united by three unambiguous synapomorphies. *Coragyps* is the sister taxon to *Cathartes* (Fig. 5) and *Vultur*. Cathartid species are commonly considered as lacking a syrinx (Ligon 1967, Rea 1983, Gill 1990). Although the cathartid syrinx does lack some modifications occurring in other falconiform syringes, it has internal membranes, an ossified pessulus, characteristic ossification of *A* elements, and probably, as noted above, the M. tracheolateralis. The unique modifications characterizing cathartid syringes include dorsal gaps in the most caudal single *A* elements, and a minimum of four complete, medially thin, double A elements on the bronchi (characters 6, 7, 8).

Phylogeny of Accipitridae. - Syringeal data group *Sagittarius* and *Pandion* with the accipitrid genera; this clade is supported by three derived characters (characters 22.2, 23, 24.3). In these genera, A1 is not fused ventrally to the tympanum, although fusion and ossification of the tympanum is more extensive ventrally than dorsally. In addition, the dorsal attachment of the pessulus to the tympanum forms characteristic patterns different from the falconid pattern.

Limited sampling of genera and the lack of phylogenetically informative variation of the syrinx preclude deriving a fully resolved phylogeny for the Accipitridae and impose constraints on the inferences that can be derived from this analysis. Nonetheless, there are some findings of interest, illustrated in the majority rule tree (Fig. 6), and some results which indicate the need for further research.

Syringeal data support the polyphyly of the kites. The 17 genera currently considered to be kites have been grouped based on their predatory and social behavior and have been considered primitive to the other accipitrids (Brown and Amadon 1968), but the monophyly of this group has been questioned (Amadon and Bull 1988). There is support for the relationship of the old world milvine kites, *Haliastur* and *Milvus*, sister taxa in 99% of the trees (Fig. 6). Two kites, *Elanus* and *Gampsonyx*, currently grouped together with *Chelictinia* in the Elaninae (Brown and Amadon 1968) are not sister taxa, but are among the most basal accipitrid genera.

Butastur and *Kaupifalco* are thought to be closely related (Amadon and Bull 1988), and they are sister taxa in this analysis. Several other species groupings within the Accipitridae are not supported by syringeal data. The serpent eagles (*Terathopius, Spilornis,* and *Circaetus*) are in a clade with four other species and are not sister taxa. The five genera of sub-buteonines included in this analysis are widely separated. However, two taxa, *Parabuteo* and one of the *Leucopternis* species, form a monophyletic group with *Buteo jamaicensis*.

There are no syringeal characters uniting the four Old world vultures included in this analysis. *Aegypius* is separated from a clade of three genera, *Gypaetus, Necrosyrtes* and *Neophron*, which are united by one unambiguous synapomorphy (character 47). Similarly, there are no derived characters supporting the monophyly of the two speciose genera, *Accipiter* and *Buteo*. Three of the *Accipiter* species are clustered together, while the fourth, *Accipiter cooperii*, is in a sister clade with *Circus, Busarellus* and *Aviceda*. There are also no characters supporting the sister taxa relationship of *Heterospizias* and *Buteogallus*, two species that have recently been synonymized (Amadon 1982).

Outgroup Relationships. - Sampling of genera within the four orders of outgroups was not comprehensive enough, nor were enough orders included to derive a phylogeny at the ordinal level. Findings of my study, therefore, should be interpreted with care. Nevertheless, as a result of the more extensive examination of genera within the Ciconiiformes and Strigiformes, some conclusions can be drawn. Syringeal characters support the monophyly of each of the two families of Ciconiiformes included in the analysis but do not support the monophyly of the order. Three derived characters cluster the three genera of Ciconiidae (characters 3, 4, 47; Fig. 7) while one derived character (character 20) unites the six genera of the Ardeidae examined.

Syringeal data also support the monophyly of the Strigiformes. Of the two currently recognized families, Tytonidae and Strigidae, there are derived characters uniting only one, the Strigidae (characters 6, 12, 48, 52). Tyto and Phodilus are in unresolved positions relative to the Strigidae.

DISCUSSION

Systematics and the Syrinx. - Syringeal data, in particular the variation in intrinsic musculature, have been important in the systematics of the oscines and suboscines, but these data have been virtually ignored in the systematics of most other orders of birds. This may have been the result of a perception that the structural elements of the syrinx offer minimal phylogenetic information, and that only the intrinsic muscles are informative. One goal of my research was to assess the usefulness of syringeal data in resolving phylogenetic relationships for orders other than the Passeriformes. This analysis has demonstrated that there is enough

variation in the morphology of syringeal structural elements to derive phylogenetic hypotheses for the Falconiformes.

There are, however, a limited number of structures comprising the syrinx and a limit to the variation. Thus, the presence of major structural elements, e.g. the presence of a tympanum, the presence and location of lateral membranes, or different pessulus morphologies, provides synapomorphies defining orders or families of birds. Minor structural variants provide characters that define genera and resolve some generic relationships within families. These include changes in the shape of B1 or A1 elements, the shape of the ends of incomplete elements, and variations in the degree of ossification or fusion of the tympanum. Although there are not enough characters to resolve relationships within the Accipitridae, a family of approximately 60 genera and more than 200 species, there is sufficient variation to produce a robust phylogeny for the Falconidae, a family with 10 genera.

Phylogenetic Relationships. - Estimates of phylogenetic relationships of the five genera within the Cathartidae are ambiguous, but there is consensus that the smaller cathartids (*Cathartes* and *Coragyps*) are sister taxa to the other genera (Fisher 1944, Emslie 1988). Syringeal data support the basal position of *Coragyps*, whereas *Cathartes* and *Vultur* are sister taxa. A more complete taxonomic sampling is needed to resolve the phylogeny of the cathartids.

One surprising result is the inclusion of *Sagittarius* and *Pandion* within the Accipitridae. *Sagittarius* is divergent in external morphology, behavior, and osteology from the other accipitrid genera, and has been considered closer to the Gruiformes than the Falconiformes (Pycraft 1902, Mayr and Amadon 1951). This divergence has been the justification for elevating *Sagittarius* to a monotypic family separate from the other clades

within the order. *Pandion* has received similar taxonomic treatment. The distinctive treatment of these two species has not been supported by my phylogenetic analysis. Thus, the characters suggesting the distinctiveness of these two species may be autapomorphies and may suggest high rates of phenotypic evolution in these species relative to other accipitrids.

Monophyly of Falconiformes. - The composition of the Falconiformes has been a point of contention since the order was defined. At present, the most problematic taxon is the family of New World vultures (Cathartidae). There is a growing consensus that this family is the sister taxon to the Ciconiidae (Holdaway 1991, Emslie 1988), with three studies offered as support: Ligon (1967), Rea (1983), and Sibley and Ahlquist (1990).

Ligon (1967) and Rea (1983) each reviewed a range of characters for taxa in the Ciconiiformes and Falconiformes, and each concluded that Ciconiidae and Cathartidae were sister taxa. However, these studies were produced before cladistic methodology was in general use in ornithological analyses, and the data in these studies were not analyzed phylogenetically. Each study simply lists the taxa and the character states diagnosing the taxa.

There are additional problems with their conclusions because of the limited number of taxa sampled. Ligon examined three genera within the Cathartidae, three within the Accipitridae and six within the Ciconiidae, and listed 49 osteological characters. Of these, 27 cluster Ciconiidae with Cathartidae, and only six cluster Accipitridae with Cathartidae. I examined skeletons of 10 genera (*Ciconia, Leptotillus, Sarcorhamphus, Vultur, Hieraaetus, Buteo, Aquila, Accipiter,* and *Falco*) and researched additional anatomical studies. At best, only eight of his 27 characters support clustering the Ciconiidae and Cathartidae. Several characters he used to unite ciconiids and cathartids, or to separate cathartids and accipitrids, occur in accipitrids or

cathartids that he did not sample. Thus, he lists basipterygoid processes as not present in the Accipitridae. However, they exist in several taxa (Shufeldt 1909, pers. obs.), and his illustration on page 5 shows these processes in *Necrosyrtes*. He lists a prominent foramen on the humeral shaft, and one pair of sternal manubrial fenestrae as occurring in Accipitridae only. These also occur in the Cathartidae.

Some of the described character states grade into one another. The curvature of the pelvic girdle is described as having a slight angle above the antitrochanter in the Ciconiidae and Cathartidae, and a 45 degree angle in the Accipitridae. However, my observations indicate that the curvature in the Cathartidae is intermediate between the other two families. Using this character to unite the cathartids with either of the other two groups requires an extremely subjective decision. Similarly, the location of the anterior iliac crest is intermediate in the Cathartidae compared to the other two families, as is the crossing of the coracoidal sulci, the shape of the ilioischiatic fenestra and the angle at which the bicipital crest joins the humeral shaft. In addition, several of Ligon's characters are composites which oversimplify variation or ignore variation in the accipitrids, e.g. the cathartids and ciconiids have a stocky or stout and sigmoidal humeral shaft. Within the accipitrids, the shaft is "often slender;" it appeared to be stocky in my observation of Hieraaetus.

Rea (1983) presented lists which are diagnoses of the Ciconiiformes (including the Cathartidae) and major groups within that order. His choice of characters has similar problems to Ligon's. For example, he lists characters uniting Ciconiidae and Cathartidae, which should be absent in Accipitridae, but are present in that family. These include the presence of M. ambiens (George and Berger 1966, McKitrick 1991), the presence of full spread wing postures in sunning (Simmons 1986) and the absence of the accessory M.

femorocaudal (George and Berger 1966). Some of his characters ignore or oversimplify variation. Thus, a 'variably degenerate' syrinx unites Ciconiidae and Cathartidae. However, the syrinx is not one organ but a series of modifications of structures, and my analysis indicates that the variation in syringeal morphology cannot be described in one character. When fully described, the variations do not unite Ciconiidae and Cathartidae.

Finally, he lists characters uniting Ciconiidae and Cathartidae, which are, presumably, unique to those to families. However, these also can be found in other orders of birds. Thus, urohydrosis (urination used as a cooling mechanism), listed as occurring only in Ciconiidae and Cathartidae, also occurs in the Sulidae (order Pelicaniformes; Carboneras 1992). The pattern of macrochromosomes shared by Ciconiidae and Cathartidae are, in fact, more closely shared by the Cathartidae, Galliformes, Gruiformes and Phoenicopteriformes (de Boer 1976). M. pectoralis major is described as double in storks and cathartids. However, it is also double in Gruidae, Procellariiformes and Pelecaniformes (George and Berger 1966). The most serious problem in both studies is that, without a phylogenetic analysis, none of the characters described by Ligon and Rea can be used to demonstrate a relationship between the Ciconiidae and Cathartidae; the characters may all be plesiomorphic or convergent. Whether any of their data are derived characters shared by these two families awaits an extensive survey of genera within each of these two families and other families within the two orders, and a phylogenetic analysis of the resulting data.

Sibley and Ahlquist (1990) used DNA-DNA hybridization data to generate phylogenies for all birds. Although widely cited, there are problems with the methodology, analysis and results of these data (e.g. Barrowclough 1992, Cracraft 1992, Lanyon 1992, Mindell 1992). One primary claim of these

data is the sister taxa relationship of the cathartids and ciconiids. Sibley and Ahlquist (1990) present two different hypotheses of cathartid relationships: a Fitch tree (their fig. 338), which does not assume rate constancy, places the cathartids with the strigiforms as a sister clade to the other Falconiformes, while UPGMA analysis, which does assume rate constancy, places the cathartids as sister taxa to the ciconiids. I reanalyzed the data in their figure 338 using the same method they mention, the Fitch option of Phylip (Felsenstein 1990). My reanalysis produced a tree different from the one they reported, and one which is congruent with the results of my syringeal analysis; the cathartids are sister taxa to the falconids and accipitrids (see also Mindell 1992). Sibley and Ahlquist (1990) dismiss their Fitch tree by concluding that different ages at first breeding cause problems. However, the additional assumptions, data and corrections used to produce the UPGMA tree are not presented, and there is no way to assess the rigor of that hypothesis. In summary, the tree derived from my syringeal analysis fits the DNA hybridization data better than the tree reported by Sibley and Ahlquist (1990).

Although the notion that the cathartids should be removed from the Falconiformes is becoming acceptable (Emslie 1988, Snyder and Snyder 1991), the data and analysis offered in support of this notion are ambiguous at best. On the other hand, my phylogenetic analysis of syringeal data strongly supports inclusion of the cathartids within the Falconiformes. In addition, syringeal data provide support for the monophyly of three major clades within the Falconiformes: the Cathartidae, the Falconidae, and an Accipitrinae-Sagittarius-Pandion group.

APPENDIX 1. Syringeal specimens examined. Specimens cleared and double stained unless designated as unstained (UNS.). Abbreviations for institutions from which specimens were borrowed are given in the Materials and Specimens section. Uncataloged specimens identified by a collector's number in parentheses.

ACCIPITRIDAE: Accipiter striatus, AMNH 18761, 18762, 8686, 8482, 15938 immat. female, (CSG 9212, 9215), 1 Dec 85 immat. female, (UNS) AMNH unnum. 1985 male, (CSG 9213). A. gentilis, AMNH 17 Dec 84 male, 11 Apr 91 immat. male . A. virgatus, AMNH 8030. A. cooperii, AMNH 20007, 20623, (CSG 16, 9217, 9218, 9264 immat. female). Aegypius tracheliotus, KUMNH 81668. Aquila audax, USNM 289389. A. chrysaetos, LSUMNS 126432. Aviceda subcristata, AMNH unnum. 1950. Busarellus nigricollis, LSUMNS 120424. Butastur indicus, AMNH 8497. Buteo albicaudatus, AMNH 8683. B. buteo, USNM 541690. B. jamaicensis, AMNH 18764, 20546, unnum. 1985. B. magnirostris, ROM 104270. Buteo platypterus, AMNH 18763, 8687, 20008, (CSG 9211), (UNS) AMNH 21464. B. regalis, AMNH 19629. Buteogallus urubitinga LSU 114340. Circus approximans, AMNH 4-24. C. macrourus, USNM 615215. C. cyaneus, USNM 226415. Elanoides forficatus, AMNH (ROP 270). Elanus leucurus, KU 56804. Gampsonyx swainsonii, AMNH 8529. Geranospiza caerulescens, LSUMNS 120423. Gypaetus barbatus, AMNH 6398. Haliaeetus leucocephalus, KUMNH 46189, ROM 132599. Haliastur indus, AMNH 8496. Harpagus bidentatus, LSUMNS 114345. Henicopernis longicauda , USNM 615210. Heterospizias meridionalis, AMNH unnum. Hieraaetus morphnoides, AMNH unnum. Ichthyophaga nana, AMNH 8399. Ictinia plumbea, AMNH (ROP271). I. mississippiensis, KUMNH 049239. Kaupifalco monogrammicus, USNM 615217. Leptodon cayanensis, LSUMNS 120426. Leucopternis albicollis,

AMNH 8492, (ROP377). L. kuhli, LSUMNS 114338. Melierax canorus, USNM 615216. Milvus migrans, USNM 615213. Necrosyrtes monachus, USNM 34631. Neophron percnopterus, USNM 615217. Parabuteo unicinctus, AMNH 19590. Pithecophaga jefferyi, AMNH 6396. Rostrhamus sociabilis, USNM 615212, AMNH (CSG 232, 711). Spilornis cheela, AMNH 8616. Spizaetus ornatus, LSUMNS 3053. Terathopius ecaudatus, AMNH unnum.

PANDIONINAE: Pandion haliaetus, AMNH 8488 immat. male, 18808 female, (PFC445 female), USNM 615209.

SAGITTARIIDAE: Sagittarius serpentarius, YPM 3721, MVZ 4611.

FALCONIDAE: Daptrius americanus, AMNH 8667, (ROP266). D. ater, KU068951. Falco berigora, AMNH 193358. F. biarmicus, AMNH 15927. F. cenchroides, AMNH 193394. F. columbarius, AMNH 19752, 14713. F. femoralis, LSUMNS 123309. F. mexicanus, KUMNH 053827. F. peregrinus, AMNH 8499, 19751. F. rufigularis, KUMNH 041874. F. sparverius, AMNH 8430 male, 8688 female, 8413 male, 15808, 15931, 16307, (CSG9210), (UNS) AMNH (CSG 21, 1216 male, 1217 immat. female). Herpetotheres cachinnans, AMNH unnum. Micrastur gilvicollis, LSUMNS 98021. M. semitorquatus, USNM 507797. Microhierax erythrogonys, AMNH 8623. Milvago chimachima, LSUMNS 120427. M. chimango, USNM 346421. Phalcoboenus australis, USNM 511795, LSUMNS 120728. Polihierax semitorquatus, USNM 615218. P. insignis, AMNH 8627. Polyborus plancus, AMNH 9094. Spiziapteryx circumcinctus, LSUMNS 8 Sept 90.

CATHARTIDAE: Coragyps atratus, AMNH 19607, (UNS) (PRS 245). Cathartes aura, AMNH 20933, unnum. Dec 85, (PFC443), nestling. C. melambrotus, LSUMNS 114336. C. burrovianus, USNM 227269. Vultur gryphus, AMNH 8498.

ARDEIDAE: Ardea herodias, AMNH 8933. Bubulcus ibis, AMNH 8624. Butorides striatus, AMNH 20736. Egretta caerulea, AMNH (PFC 427 immat. female). Ixobrychus sinensis, AMNH 8621. Nycticorax nycticorax, AMNH 8625, 20335, (UNS) AMNH 8432.

CICONIIDAE: Ciconia ciconia, AMNH unnum. 1936. C. nigra, AMNH 6377. Ephippiorhyncus asiaticus, USNM 510447 (incomp.). Mycteria americana, AMNH 8513, (UNS) AMNH 9038, 9062, 9063, (MYC 85003, 85004, 85006-85009).

TYTONIDAE: Tyto alba, AMNH 14715, 8680, 8682, 20624, (CSG 9216). T. tenebricosa, AMNH 7495. Phodilus badius, AMNH 6419.

STRIGIDAE: Aegolius acadicus, AMNH 8489, (CSG 9214). Asio otus, AMNH 8312. A. stygius, AMNH 7466. A. flammeus, AMNH 8684. Bubo bubo, AMNH 7450. B. shelleyi, AMNH 8414. B. virginianus, AMNH 16092, (SC 957 immat.). Ciccaba huhula, AMNH SC 407. C. virgata, (UNS) AMNH 7408. Ketupa ketupu, BM A1981. Glaucidium brasilianum, AMNH (SC 302, 410, 268). G. gnoma, AMNH 7404. Ninox jacquinoti, AMNH 7422. N. odiosa, AMNH 7423. N. connivens, AMNH 7442. N. philippensis, AMNH 8490. N. scutulata, AMNH 8615. Nyctea scandiaca, AMNH 8836. Otus asio, AMNH 8310, 20625. O. watsonii, AMNH 8685. O. nudipes, AMNH 7438. O. guatemalae, AMNH 7437. Pulsatrix perspicillata, AMNH 2784. Speotyto cunicularia, AMNH 8679, 7447. Strix varia, AMNH 7439.

PHALACROCORACIDAE: Phalacrocorax auritus, (UNS) AMNH unnum.

SULIDAE: Sula bassanus, AMNH 8618, 8846. Sula nebouxi, (UNS) AMNH 8618.

PELECANIDAE: Pelecanus roseus, (UNS) AMNH 8619.

CRACIDAE: Crax alector, AMNH 15006, (UNS) (PFC 412).

PHASIANIDAE: Tetrao parvitostris, (UNS) AMNH 14100.

APPENDIX 2. Descriptions of 49 syringeal characters used in the analysis. Derived states are described first. Characters 7, 26, 28, 38, 40 are multistate, ordered characters; justification for ordering follows descriptions. Characters 1, 13, 15, 21, 22, 24, 29, 30, 34, 37, 45 multistate unordered. Distribution of character states shown in Table 1. Characters illustrated in Figures 2, 4, 5, 7.

A elements

1. Ossification of A elements cranial to tracheo-bronchial junction. - (1) Completely ossified in all genera in the Falconidae and Cathartidae, in most genera in the Strigiformes, Ardeidae, and in several accipitrids.
(2) Ossified ventrally and laterally, but cartilaginous dorsally in most accipitrid genera. (0) No ossification. Elements cartilaginous in *Pelecanus*, the two species of Galliformes and in juveniles in the Strigiformes, Accipitridae, Cathartidae and Falconidae.

2. A elements immediately cranial to tracheo-bronchial junction thinner than subsequent elements and incomplete laterally. - (1) In the Ciconiidae. (0) Not present. *A* elements complete rings in lateral view.

3. Incomplete double *A* elements on bronchi caudal to tracheo-bronchial junction. - (1) Yes. One or two present in species in all families examined except the Ciconiidae. Gaps between ends of incomplete elements on medial walls of the bronchi, forming lateral borders of internal membranes. (0) No *A* elements on bronchi in ciconiid genera.

4. More than three incomplete double *A* elements. - (1) At least 4 incomplete double *A* elements; in several genera in the Ardeidae; in all genera within the Strigidae. (0) No. A4 either single element or complete double elements.

5. Complete double *A* elements on bronchi caudal to tracheo-bronchial junction. - (1) Yes. In all genera in the Cathartidae, Accipitridae and Falconidae; in several genera in the Strigiformes. (0) Not present.

6. More than 3 complete double A elements. - (1) Yes. All cathartid genera have at least 4 complete double *A* elements. (0) No.

7. Single A elements immediately cranial to tracheo-bronchial junction incomplete, with gap in element dorsally. - (1) One or two *A* elements incomplete with small gaps between ends in *Coragyps* (2) More than three *A* elements incomplete with large gaps between the ends of each element, in *Cathartes* and *Vultur*. Unique to the Cathartidae. Change from state 1 to 2 observed in the ontogeny of *Cathartes*. (0) No. In all other families single A elements complete when viewed dorsally.

8. Medial cartilaginous section of complete *A* elements narrower than lateral, ossified section. - (1) Yes. In the Cathartidae. (0) No.

9. Incomplete A1 elements wider laterally, thicker and more ossified than other double A elements. - (1) Yes. In the Strigiformes. (0) No.

10. Dorsal ends of incomplete A1 elements connected medially forming ridge of ossified tissue. - (1) In several *Falco* species and in *Microhierax*. (0) Not present.

11. Ventral ends of incomplete A1 elements extend onto internal membrane forming amorphous, ovoid accessory cartilaginous structures. - (1) In strigid genera. (0) Not present.

12. Ventral ends of incomplete A1 and A2 elements extend onto cranial surface of internal membrane. - (1) In accipitrid genera. (0) Not present.

13. Modifications of caudal edges of dorsal ends of incomplete A1 elements. - (1) Slight pointed cartilaginous extension of ends. (2) Large cartilaginous extension borders edge of internal membrane. (3) Ends fused to

ends of B2 elements forming rings, in *Pandion*. (0) A1 ends unmodified or single element .

14. Dorsal ends of incomplete A1 elements flattened and enlarged forming a paddle shape. - (1) In *Micrastur* species. (0) Not present.

Pessulus

15. Pessulus composition. - (1) Ossified. (2) Cartilaginous in several strigid genera. (0) No pessulus in *Pelecanus, Phalacrocorax, Podiceps, Spheniscus, Struthio, Casuarius, Dromaius,* or *Apteryx*.

16. Pessulus an extension of *A* elements. - (1) Yes. (0) No. Pessulus not present in *Pelecanus*. In Ciconiidae, pessulus an extension of B elements, an autapomorphy for that family and not included in this analysis.

17. Ossified ridges at dorsal and ventral ends of pessulus. - (1) In Ardeidae. (0) Not present.

18. Dorsally, pessulus ends medially. - (1) In Strigidae, *Vultur; Cathartes* polymorphic for this character (see character 19). (0) No.

19. Dorsal and ventral ends of Pessulus extend caudally from *A* elements. - (1) Yes. In all species within Falconidae and Accipitridae. In *Coragyps*, in some adult *Cathartes* and in juvenal *Cathartes*. (0) No.

Tympanum

20. Fusion of *A* elements cranial to tracheo-bronchial junction. - (1) In accipitrid and falconid genera and in *Sagittarius*, tympanum formed from lateral, ventral, and dorsal fusion of *A* elements. Patterns of fusion and ossification vary among families; described in following characters. (0) No fusion of *A* elements.

21. Degree of dorsal ossification of tympanum. - (1) Ossified medially and laterally only in most accipitrid genera. (2) Ossified completely in all falconid genera and several accipitrids. (0) Tympanum not present.

22. Dorsal pessulus attachment to tympanum - (1) Narrow, more highly ossified medial strip extends cranially connecting several *A* elements; in falconid genera. (2) Ossified narrowly, ends at or just above tracheo-bronchial junction; in some accipitrids and *Sagittarius*. (3) Ossified broadly, ends at or just above tracheo-bronchial junction in ovoid or diamond shaped pattern; in some accipitrids. (4) Ossified broadly, extends cranially connecting one or two single *A* elements medially; in some accipitrids. (5) Ossified broadly, extends cranially and laterally forming a cross-like pattern, in *Pandion*.
(0) Tympanum not present.

23. A1 not fused ventrally to tympanum. - (1) In all accipitrids and *Sagittarius*. (0) A1 fused in the Falconidae. In other taxa, there is no tympanum.

24. Pattern of partial dorsal fusion of A elements - (1) Slight fusion medially along caudal and cranial margins of two elements. (2) Medial, triangular-shaped cartilaginous plug fuses several elements. (3) Medial plug ossified, elements also fused somewhat along margins. Derived unordered states describe accipitrid tympanum. (0) Total fusion in Falconidae; in other taxa, no fusion of elements

25. More than seven rings fused to dorsal cartilaginous plug. - (1) In five accipitrid genera. (0) Six or fewer rings fused in all other accipitrids and falconids. No dorsal fusion in other families.

26. Shape of ossified and fused tympanum. - (1) Graduated, widens caudally. (2) Almost cylindrical. (3) Almost cylindrical, A1 flattened laterally. In the Falconidae. State 3 modification of state 2. (0) No systematic variation in accipitrid tympanum shape. In other taxa, no tympanum present.

27. Dorsal fusion of first two single *A* elements medially by ossified bar.- (1) Yes. In *Micrastur*. (0) Not present.

28. Dorsal fusion of first three or four *A* elements along their margins. - (1) Margins apparent along the edges of each ring. (2) Margins somewhat obliterated and only light sutures apparent medially. Derived states in falconid genera except *Micrastur*. State 2 modification of state 1; fusion increased. (0) No. Partial fusion in the accipitrids (character 29), and in Micrastur (character 27). In other taxa, no dorsal fusion.

29. Pattern of partial ventral fusion of tympanum. - (1) Slight medial fusion of *A* elements along margins. (2) Triangular shaped ossified patch covers medial fusion of *A* elements. (3) Irregular, small ovoid ossified patch covers medial fusion. (4) Large ossified patch covers fusion of *A* elements medially and laterally. In Accipitridae, *Sula* and some Strigiformes. (0) Fusion total in falconids, lacking in other taxa.

30. Pattern of total ventral fusion of tympanum. - (1) First three or four *A* elements fused along margins. Spaces apparent between elements. (2) First three or four *A* elements fused lightly, sutures apparent along margins. (3) At least five *A* elements fused entirely along margins, sutures apparent only laterally. In falconids. (0) Fusion partial in accipitrids, lacking in other taxa.

B elements

31. All *B* elements complete rings. - (1) In Ciconiidae. (0) In other taxa, *B* elements with medial gaps.

32. B1 at oblique angle transversely. - (1) In Strigidae. (0) In other taxa, B1 perpendicular to transverse plane.

33. B1 evenly wider than other *B* elements, concave caudally.- (1) In all falconids; in *Butorides*. (0) B1 not concave caudally in any other genera; same width as other *B* elements in all other taxa except accipitrids (character 35).

34. Modifications of shape of incomplete B1 elements - (1) Wider at dorsal ends. (2) Slightly wider laterally, with narrowed ends. (3) Small arc-

shaped extension on cranial margin laterally. (4) Large arc-shaped extension on cranial margin laterally. (5) Very wide dorsally, slight narrowing at ventral ends. In accipitrids and some strigids. (0) Not present.

35. B1, B2 and B3 concave cranially.- (1) In *Sagittarius*. (0) No.

36. B1 overlaps A1 laterally. - (1) In accipitrid genera. (0) Membrane separates A1 and B1 in other taxa.

37. Modification of shape of dorsal ends of B1 elements fusing with A1 elements. - (1) Ends very thick and wide, ascend sharply in L shape to fuse with A1 ends. (2) Ends thin, ascend gradually to fuse with A1 ends. (3) Craniad edges knobbed; craniad extension fuses with A1 ends. (4) Ends thick, rounded, ascend gradually to fuse with A1. Derived states in falconid genera. (0) B1 elements complete in Ciconiidae; ends not modified in other species.

38. Fusion of A1 and *B* ventral ends. - (1) A1 and B1 ends fused. (2) B2 ends also fused. State 2 modification of state 1. (3) B3 ends also fused. State 3 modification of state 2. In falconid genera.(0) Not present.

39. Fusion of B1 and B2 dorsal ends.- (1) In *Accipiter* and 3 other accipitrid genera. (0) Not present.

40. Fusion of B ventral ends forming ridge bordering internal membranes. - (1) B1 and B2 ends fused. (2) B3 ends fused also. State 2 modification of state 1. (0) Not present.

Membranes and Muscles

41. External membrane between A1 and B1 elements. - (1) In all falconid genera, in *Pandion* which has membrane between B1 and B2 also.
(0) Not present.

42. External membranes between B2-4 elements. - (1) In several accipitrids and *Sagittarius*. These have external membrane between B1 and B2, also occurring in many accipitrid genera and not used as a character because

generally small, with intra-specific and individual variation in its occurrence; may be an artifact of preservation. (0) Not present.

43. M. tracheolateralis inserts laterally on A1. - (1) In the Ardeidae and in Sula. (0) In Falconidae, M. tracheolateralis inserts on lateral membrane, in Accipitridae and Strigiformes, inserts on B1. Not coded in analysis because correlated with other characters. See text for discussion of M. tracheolateralis in Galliformes, Cathartidae and Ciconiidae.

Accessory Structures

44. Cartilaginous or border located on cranial edge of internal membrane, extending from dorsal to ventral ends of membrane. - (1) Present. (0) Not present.

45. Shape of cartilaginous border. - (1) Narrow, thicker dorsally than ventrally. (2) Wide, thicker dorsally than ventrally. (3) Wide and even. (4) Narrow and even. (0) Not present.

46. Border ossified. - (1) In three taxa in Accipitridae. (0) Not present.

47. Small cartilaginous paddle-shaped accessory structures extend onto internal membranes from dorsal A1 ends. (1) In several accipitrid species. (0) Not present.

48. Small cartilaginous peaks extend caudally onto internal membranes medially. (1) In several accipitrid species. (0) Not present.

49. Internal membranes almost parallel. - (1) In *Accipiter* (0) No internal membranes in the Ciconiidae. Internal membranes at an angle to each other when viewed dorsally in all other species.

TABLE 1. Distributions in 88 taxa of states of 49 syringeal characters used in phylogenetic analysis. See Appendix 2 for descriptions.

	Character																			
	1									2										
Taxon	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	
ACCIPITRIDAE																				
Accipiter cooperii	1	0	1	0	1	0	0	0	0	0	0	1	2	0	1	1	0	0	1	1
A. gentilis	1	0	1	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	1	1
A. virgatus	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	1	1
A. striatus	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	1	1
Aegypius	1	0	1	0	1	0	0	0	0	0	0	1	2	0	1	1	2	0	0	0
Aquila	1	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	3	0	0
Aviceda	1	0	1	0	1	0	0	0	0	0	0	2	0	1	1	0	0	0	3	0
Busarellus	2	0	1	0	1	0	0	0	0	0	0	1	2	0	1	1	0	0	1	1
Butastur	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	1	1
Buteo albicaudatus	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	2	0	0	0
B. buteo	0	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	2	0	0	0
B. jamaicensis	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	2	0	0	0
B. magnirostra	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	2	0	0	0
Buteogallus	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	3	0	0	0
Circaetus	1	0	1	0	1	1	0	0	1	0	0	1	1	0	0	0	3	0	0	0
Circus	1	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	4	0	0
Elanoides	1	0	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	3	0	0
Elanus	2	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0
Geranospiza	2	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	3	0	0
Gampsonyx	2	0	1	0	0	0	0	1	1	0	0	0	1	1	3	0	0	0	0	0
Gypaetus	1	0	1	0	1	1	0	0	1	0	0	1	1	0	0	0	3	0	0	0
Haliaeetus	1	0	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	3	0	0
Haliastur	1	0	1	0	1	1	0	0	1	1	0	1	2	1	0	0	0	3	0	0
Harpagus	1	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	3	0	0
Henicopernis	1	0	1	0	0	1	1	0	0	1	0	0	1	1	0	0	0	3	0	0
Heterospizias	1	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	0	3	0	0
Hieraaetus	1	0	1	0	1	1	0	0	0	1	0	0	1	1	0	0	0	3	0	0
Ichthyophaga	1	0	1	0	0	1	2	0	0	1	1	2	0	1	0	0	0	3	0	0
Ictinia	1	0	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	3	0	0
Kaupifalco	1	0	1	0	1	1	0	0	1	1	2	0	0	0	1	0	1	0	0	0

Leptodon	201010000 0010011001 1231300003 0000200000 0000120000
Leucopternis kuhli	101010000 0010011001 1131200003 0000301000 0000120000
L. albicollis	101010000 0010011001 1141200003 0000401000 1000120000
Melierax	101010000 0010011001 1121200003 0000501000 0000120001
Milvus	101010000 0011011001 1121210003 0000301000 1000120010
Necrosyrtes	101010000 0010011001 1121200002 0000301000 0000111000
Neophron	101010000 0000011001 1121210002 0000301000 0010111000
Parabuteo	101010000 0011011001 1141200003 0000401000 1000120000
Pithecophaga	101010000 0010011001 1121200003 0000301000 0010110010
Rostrhamus	101010000 0011011001 1221200004 0000401000 1000120001
Spilornis	101010000 0011011001 1121200003 0000301000 2000120000
Spizaetus	101010000 0011011001 1121210003 0000301000 2010120000
Terathopius	101010000 0011011001 1121100003 0000301000 2000120000
Pandion	101010000 0013011001 1150100003 0000100000 2100120000
SAGITTARIIDAE	
Sagittarius	201010000 0000011001 1221300000 2000011000 2010120000
FALCONIDAE	
Daptrius ater	201010000 0000011001 1210001000 3001000410 0100140000
D. americanus	201010000 0000011001 1210001000 3001000410 0100130000
Falco berigora	201010000 1000011001 1210002020 2001000330 0100130000
F. columbarius	201010000 0000011001 1210002010 2001000330 1100130000
F. mexicanus	201010000 0000011001 1210002010 2001000320 0100130000
F. rufigularis	201010000 0000011001 1210002010 2001000330 0100130000
F. sparverius	201010000 1000011001 1210002010 2001000320 0100130000
Herpetotheres	201010000 0000011001 1210001010 2001000300 0100000000
Micrastur gilvicollis	201010000 0000111001 1210001100 1001000110 0100130000
M. semitorquatus	201010000 0000111001 1210002100 1001000110 0100140000
Microhierax	201010000 1000011001 1210003020 2001000210 0100130000
Polihierax insignis	201010000 0000011001 1210001100 2001000320 0100140000
P. semitorquatus	201010000 0000011001 1210003010 2001000210 0100130000
Polyborus	201010000 0000011001 1210001000 3001000410 0100130000
Spiziapteryx	201010000 0000011001 1210001020 2001000300 0100130000
CATHARTIDAE	
Cathartes	201011210 0000011011 0000000000 0000000000 0000000000
Coragyps	201011110 0000011001 0000000000 0000000000 0000000000
Vultur	201011210 0000011010 0000000000 0000000000 0000000000

ARDEIDAE	
Ardea	201100000 0000011100 0000000000 0000001000 0001000000
Bubulcus	201000000 0000011100 0000000000 0000000000 0011000000
Butorides	201000000 0000011100 0000000000 0001000000 0001000000
Egretta	101000000 0000011100 0000000000 0000000000 0001000000
Ixobrychus	201100000 0000011100 0000000000 0000000000 0001000000
Nycticorax	201000000 0000011100 0000000000 0000000000 0001000000
CICONIIDAE	
Mycteria	210000000 0000010000 0000000000 0100000000 0000000000
TYTONIDAE	
Phodilus	201000001 0000021010 0000000000 0000200000 0000000000
Tyto	201010001 0000011010 0000000000 0000200000 0000130000
STRIGIDAE	
Aegolius	201100001 0100011010 0000000000 0010200000 0000000000
Asio otus	201100001 0100021010 0000000000 0010300000 0000000000
A. stygius	201100001 0100011010 0000000000 0010300000 0000000000
Bubo virginiansus	201110001 0100021010 0000000001 0010300000 0000000000
Ciccaba huhula	201100001 0100021010 0000000000 0010200000 0000000000
Ciccaba virgata	101100001 0100021010 0000000000 0010200000 0000000000
Ketupa ketupu	001100001 0100021010 0000000001 0010300000 0000000000
Ninox connivens	201100001 0000011010 0000000000 0010300000 0000000000
Ninox odiosa	201110001 0100011010 0000000001 0010300000 0000130000
Ninos jacquinoti	201100001 0100021010 0000000001 0010300000 0000000000
Nyctea	101100001 0000021010 0000000001 0010300000 0000000000
Otus asio	201110001 0100021010 0000000000 0010300000 0000000000
O. watsonii	201110001 0100011010 0000000000 0010300000 0000000000
SULIDAE	
Sula	201000000 0000011000 0000000001 0000000000 0001000000
PELECANIDAE	
Pelecanus	001000000 0000000000 0000000000 0000000000 0100000000
Galliformes	001000000 0000011000 0000000000 0000000000 0000000000

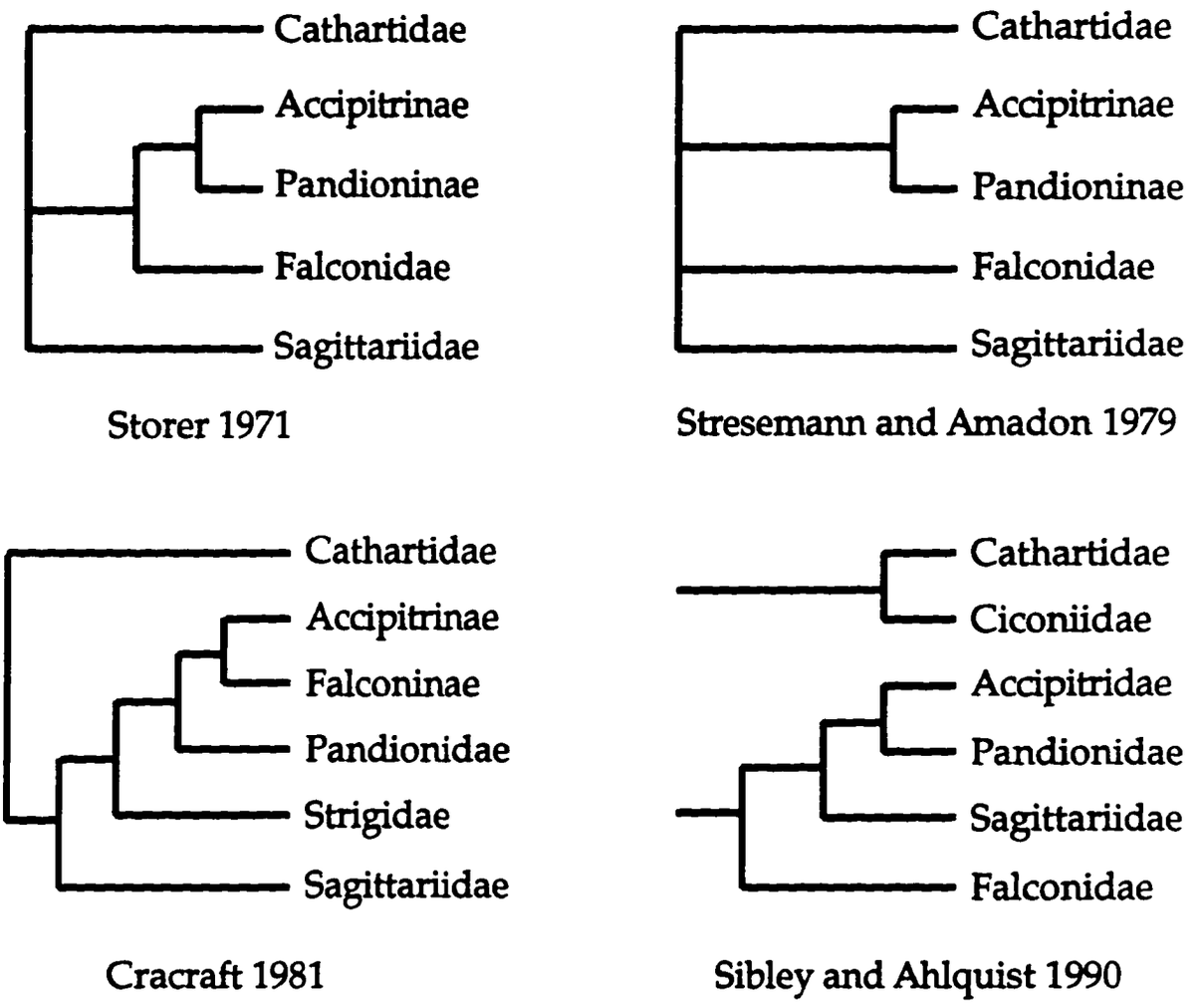

Fig. 1. Four recent conflicting classifications of the Falconiformes.

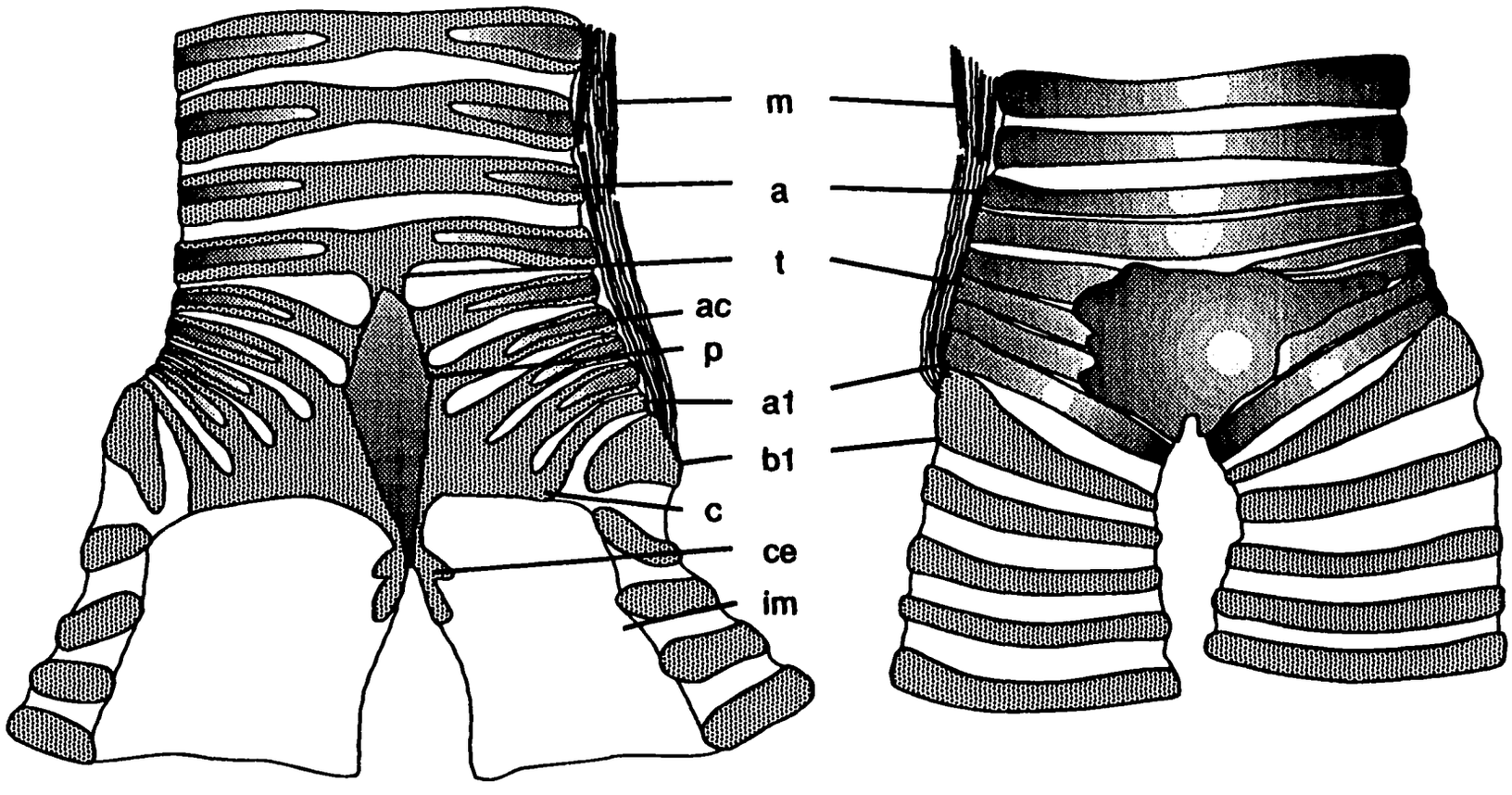

Fig. 2. Accipitridae syrinx (Broad-winged hawk, *Buteo platypterus*, AMNH 20008). Left - dorsal view and right - ventral view. Abbreviations (numbers following refer to characters in Appendix 2): (a) *A* elements (1.2); (a1) A1 elements (3, 23); (ac) complete double *A* elements (5); (b1) B1 elements (34.4, 36); (c) accessory cartilaginous structure (44, 45.2); (ce) cartilaginous extension of A1 and A2 (12); (im) internal membrane; (p) pessulus (22.4); (t) tympanum (20, 21.1 24.2, 29.3); (m) M. tracheolateralis. See text for definitions of structural elements. In all illustrations, stippling indicates

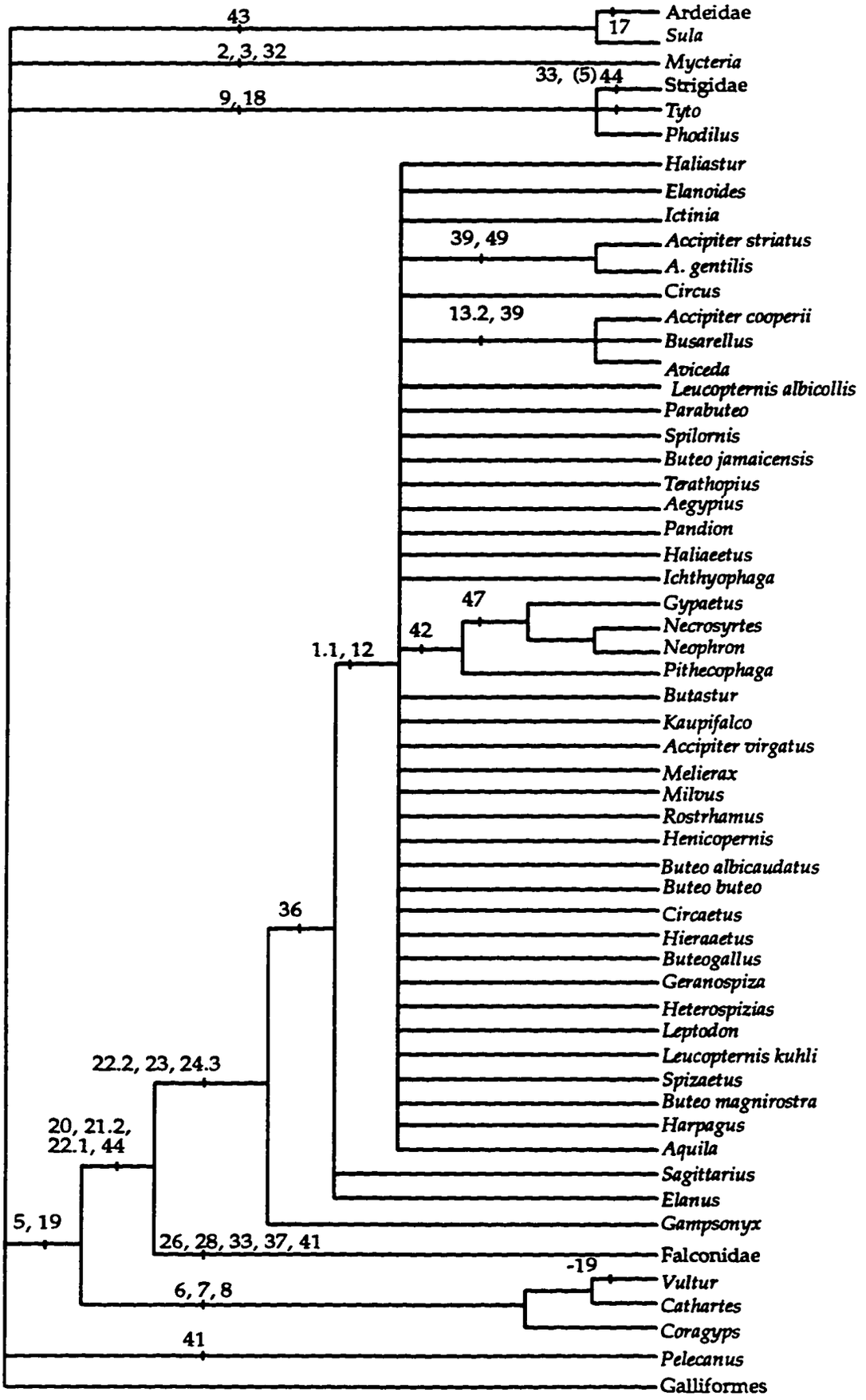

Fig. 3. Falconiform Phylogeny. Strict consensus tree of 5000 most parsimonious trees. Synapomorphies supporting nodes are numbered: these are described in Appendix 1.

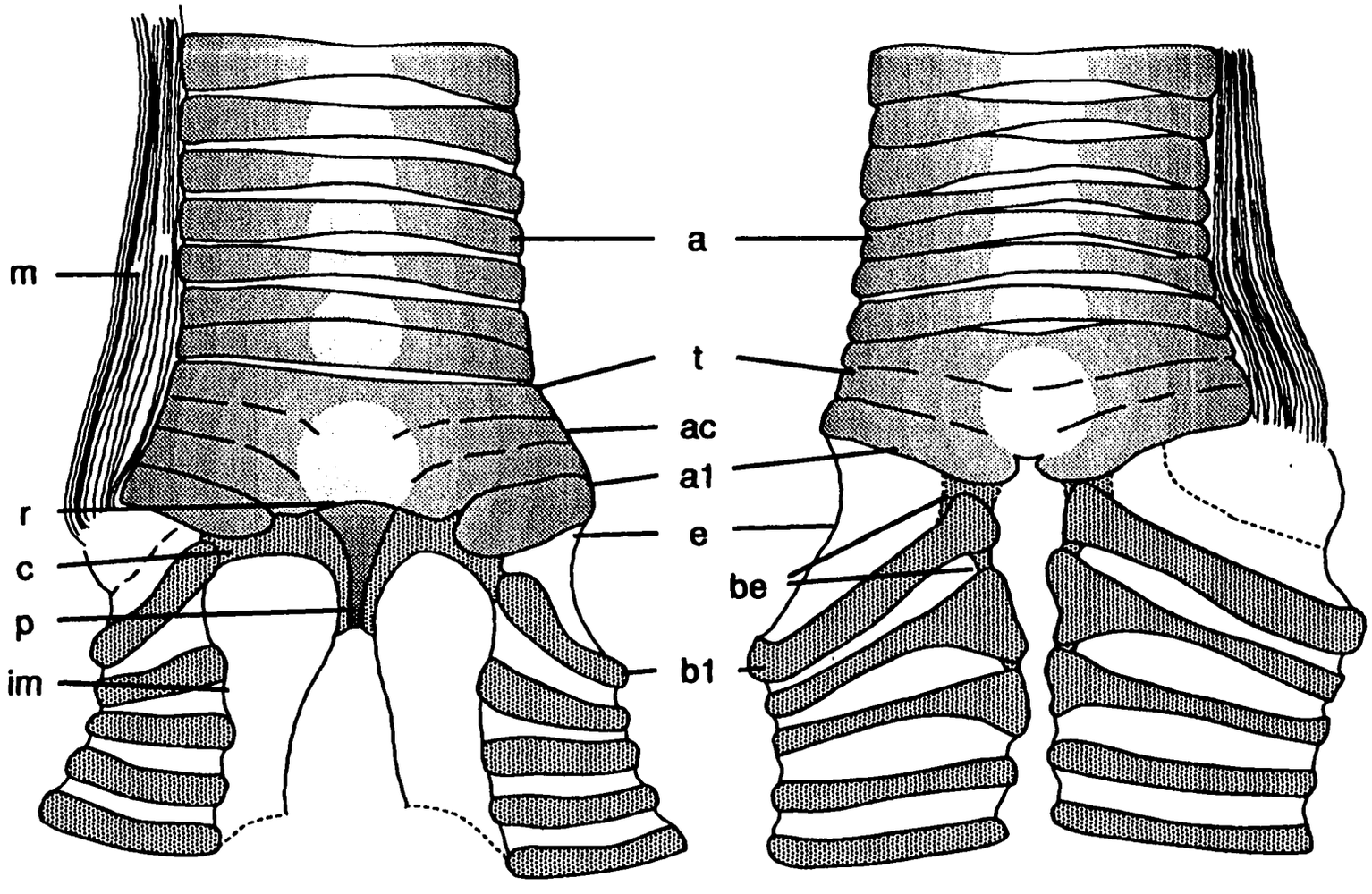

Fig. 4. Falconidae syrinx (Brown falcon, *Falco berigora*, AMNH 193358). Left - dorsal view and right - ventral view. Abbreviations: (a) *A* elements (1.1); (a1) A1 elements (3); (ac) complete double *A* elements (5); (b1) B1 elements (33, 37); (be) Fusion of element ends (38.3); (c) accessory cartilaginous structure (44, 45.3); (e) external membrane (41); (im) internal membrane; (p) pessulus (15, 16); (r) ossified ridge (9); (t) tympanum (20, 21.2, 26.2, 28.2, 30.2).

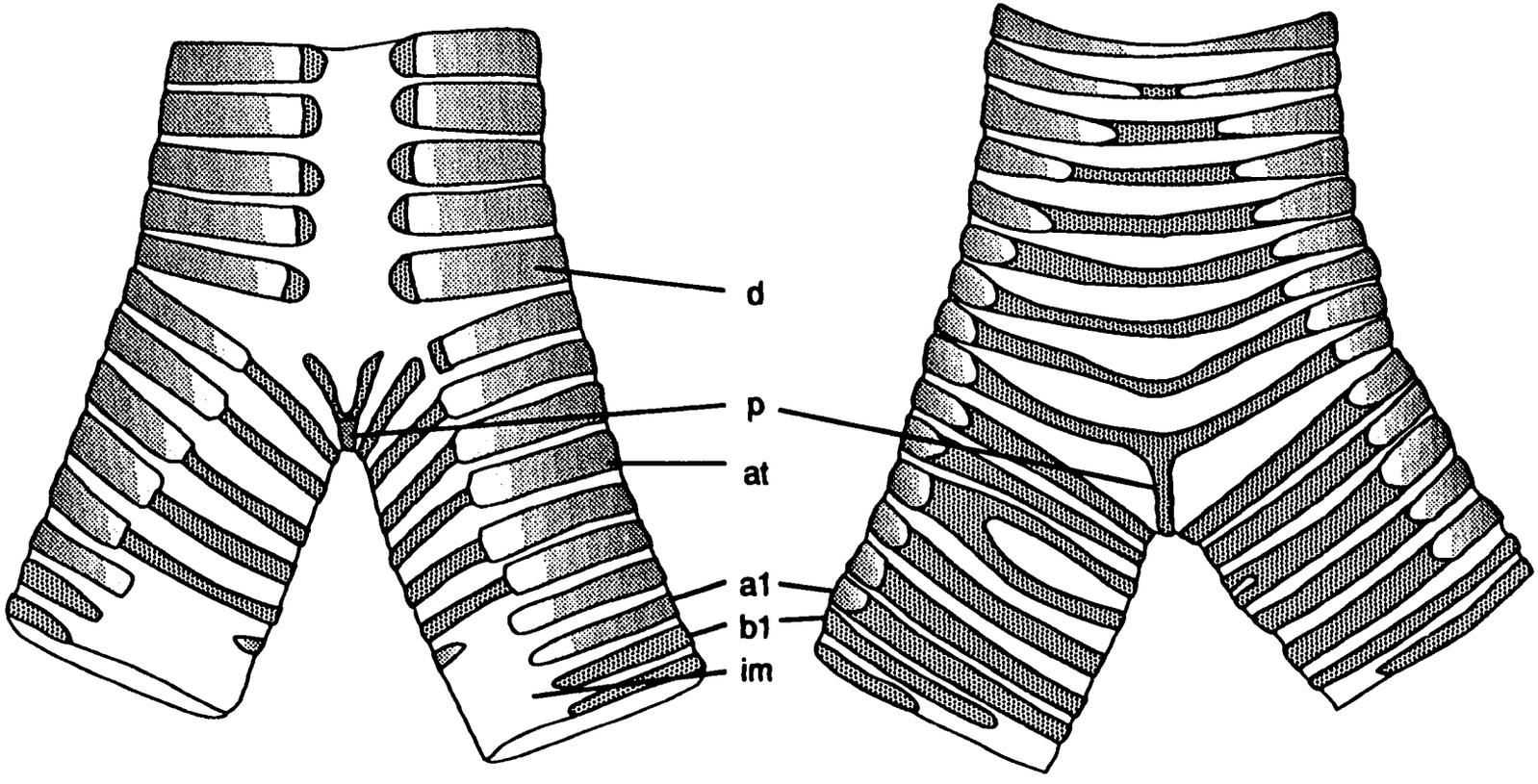

Fig. 5. Cathartidae syrinx (Turkey vulture, *Cathartes aura*, AMNH 20933). Left - dorsal view and right - ventral view. Abbreviations: (a) complete double *A* elements (6, 8); (a1) A1 elements ; (as) incomplete single *A* element (7); (b1) B1 elements; (im) internal membrane; (p) pessulus (16, 18).

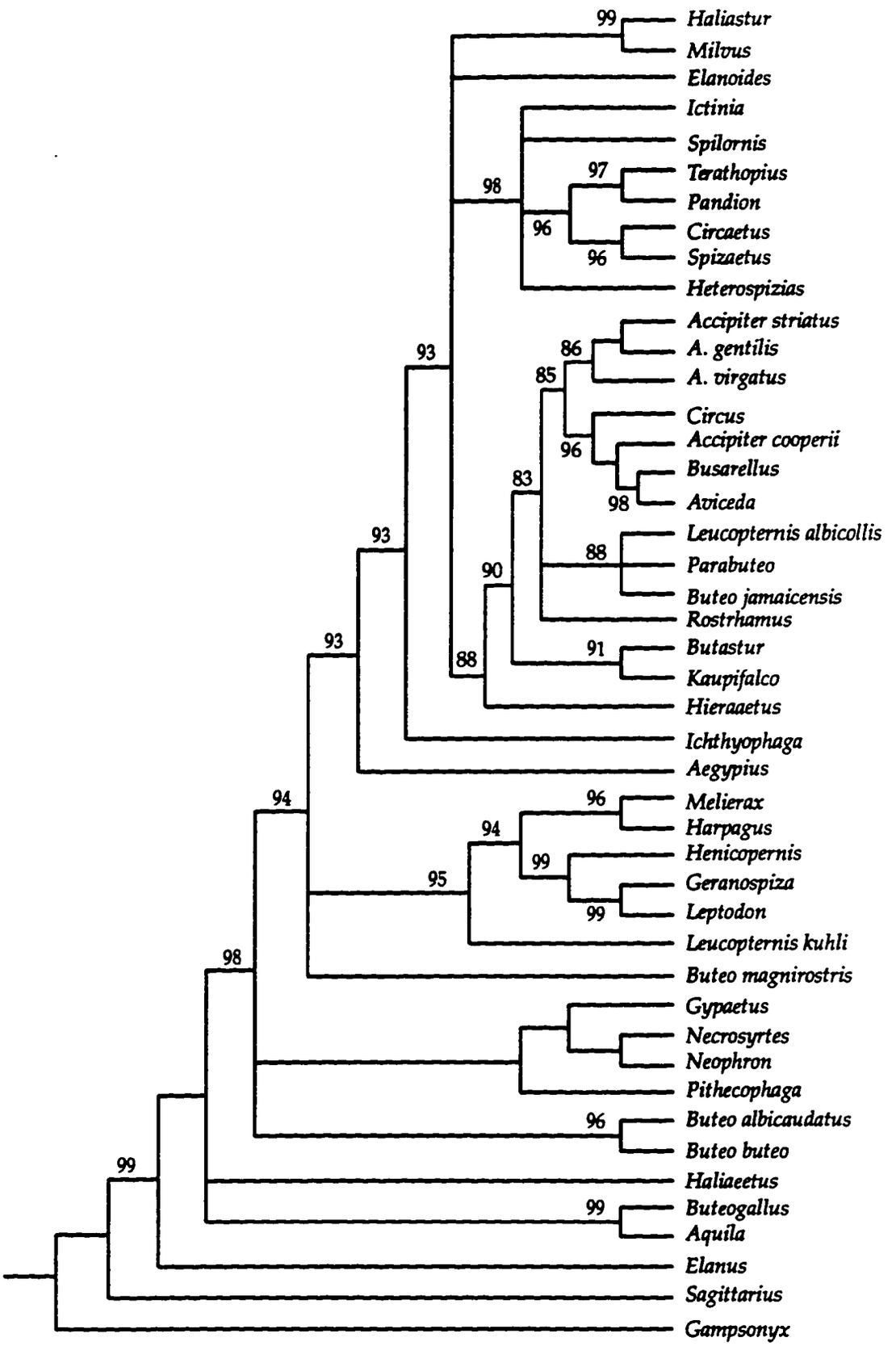

Fig. 6. 80% Majority Rule Consensus tree of the genera in the Accipitridae. Clades illustrated are supported in at least 80% of the most parsimonious trees. Numbers indicate percent support, nodes without numbers are supported in 100% of the most parsimonious trees.

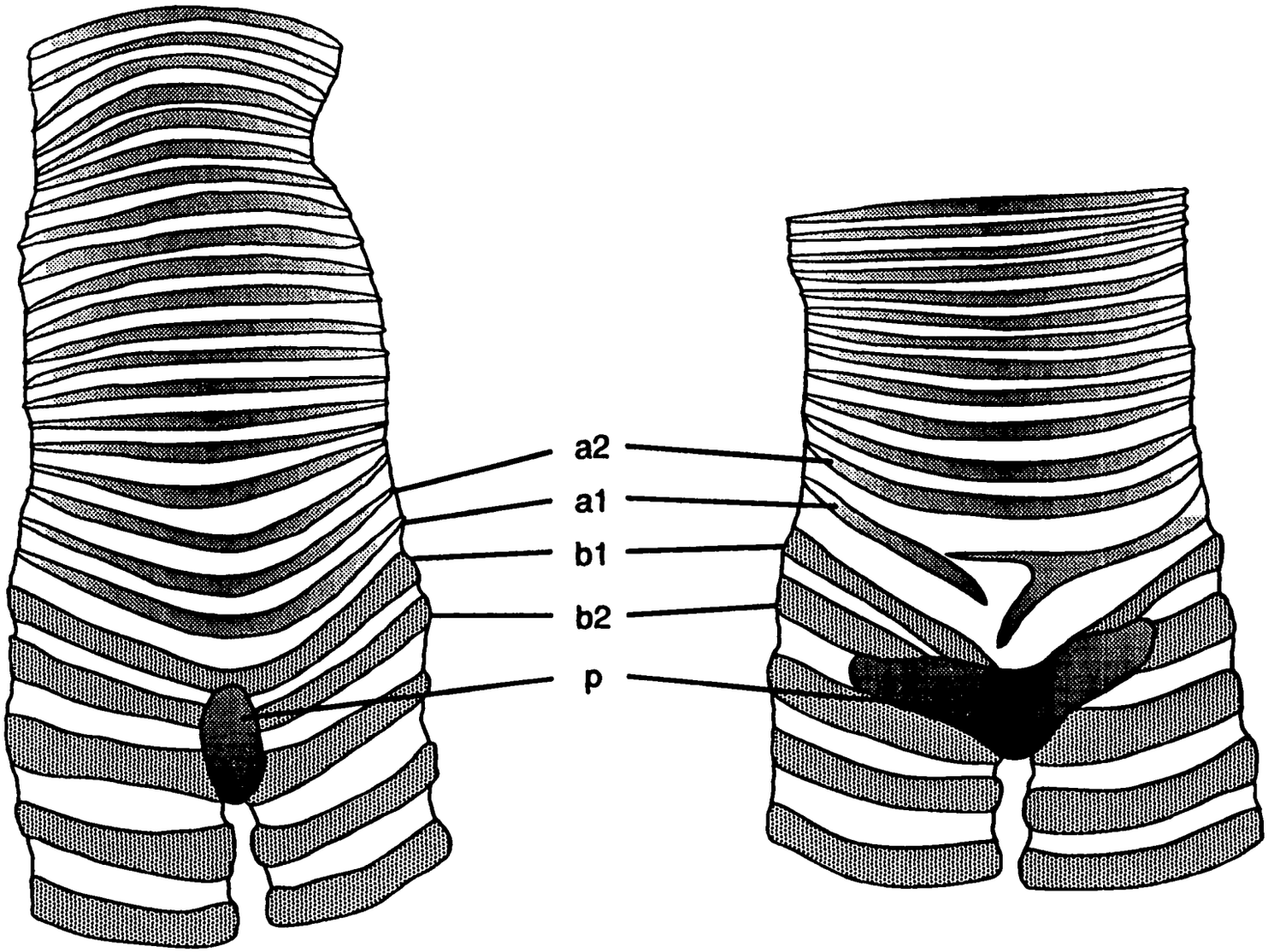

Fig. 7. Ciconiidae syrinx (White stork, *Ciconia ciconia*, AMNH 1936). Left - dorsal view and right - ventral view. Abbreviations: (a1) A1 element; (a2) A2 element (2); (b1) B1 elements; (b2) B2 elements (31); (p) pessulus.

CHAPTER 2

SYRINGEAL MORPHOLOGY AND THE PHYLOGENY OF THE FALCONIDAE

ABSTRACT. - Variation in syringeal morphology was studied to resolve the relationships of species of falcons, falconets, pygmy falcons, and caracaras in the family Falconidae. The phylogeny derived from these data establishes three sub-families within the family: (1) the Polyborinae, containing *Daptrius, Polyborus, Milvago* and *Phalcoboenus* , the four genera of caracaras; (2) the Falconinae consisting of the genus *Falco, Polihierax* (pygmy falcons), *Spiziapteryx* and *Microhierax,* (falconets) and*Herpetotheres* (Laughing Falcon); and (3) the genus *Micrastur* (forest falcons) comprising the third, basal clade in the family. Two genera, *Daptrius* and *Polihierax,* are found to be polyphyletic.

The Falconidae (falcons, caracaras and falconets) is an family whose phylogenetic relationships are in question. Cladistic analyses of syringeal morphology (Griffiths 1994a) and osteology (Becker 1987) support the monophyly of the family, but systematic ambiguities exist at the intrafamilial level. Current classification allocates the 10 genera in the family into two subfamilies (Amadon and Bull 1988):

1. The Polyborinae. This includes seven genera: *Daptrius, Milvago, Polyborus* and *Phalcoboenus* (the caracaras), *Micrastur* (Forest Falcons), *Herpetotheres* (Laughing Falcon) and *Spiziapteryx* (Spot-winged Falconet) and

2. The Falconinae. This includes three genera: *Falco, Polihierax* (Pygmy Falcons) and *Microhierax* (falconets).

Inclusion of the caracaras in the Polyborinae is not questioned (Sharpe 1874, Swann 1922, Peters 1931). The number of named caracara genera, however, has varied from two to four. Three other genera (*Spiziapteryx, Micrastur* and *Herpetotheres*) may not belong in the Polyborinae. *Spiziapteryx* had traditionally been associated with the pygmy falcons and falconets but was placed with the caracaras based on an assessment of osteological similarity (Olson 1976). Once thought to be related to hawks rather than falcons (Sharpe 1874, Swann 1922), *Micrastur* and *Herpetotheres* were later considered to be either one (Peters 1931) or, subsequently, two (Friedmann 1950) separate subfamilies within the Falconidae.

Composition of the Falconinae has also changed. *Polihierax, Spiziapteryx* and *Microhierax* were originally placed with *Falco*(Sharpe 1874, Swann 1922), then placed in a separate subfamily, the Polihieracinae (Peters 1931, Friedmann 1950). More recently, *Microhierax* and *Polihierax* were reunited with *Falco*(Stresemann and Amadon 1979).

Cladistic analyses of the family using osteology (Becker 1987) and allozyme frequencies (Boyce 1989) produced two alternative hypotheses of generic relationships (Figure 1). Osteological data placed *Micrastur* and *Herpetotheres* basal to the other genera, whereas allozyme data supported the falconets as the basal group.

I analyzed the variation in syringeal morphology of the Falconidae to derive a phylogenetic hypothesis for this family. Syringeal myology had been important in the classification of the major subdivisions of the Passeriformes at the end of the 19th century. Within the last 20 years, the use of syringeal data in systematics has intensified. However, this work has centered on the analysis of oscines and suboscines (Ames 1971, Warner 1972, Lanyon 1984, Prum 1990) and "higher birds" (Cannell 1986). There have been no previous analyses of falconid syringes.

MATERIALS AND METHODS

MATERIALS AND SPECIMENS

Syringes were obtained by dissecting fresh specimens and specimens originally preserved in formalin and stored in alcohol at the American Museum of Natural History (AMNH), the National Museum of Natural History (USNM), and the Louisiana State University Museum of Natural Science (LSUNS). These were cleared and double stained to distinguish cartilaginous and ossified tissue (Cannell 1988). Additional cleared and stained specimens prepared by Dr. Peter Cannell were also used; these included specimens borrowed from the University of Kansas Museum of Natural History (KUMNH). Observations were made using a Wild M5A dissecting microscope and drawings made with a camera lucida.

All 10 currently recognized genera within the family (Stresemann and Amadon 1979) and 10 outgroup species were examined, a total of 44 specimens of 32 species (Appendix 1). Species from the monotypic genera *Spiziapteryx, Polyborus* and *Herpetotheres,* and both species in each of the genera, *Daptrius, Milvago* and *Polihierax* were included. Sampling of species within the remaining polytypic genera was limited to the availability of alcohol preserved specimens (one of three species in *Phalcoboenus,* two of five in *Micrastur,* and one of five in *Microhierax*). An additional consideration limiting the number of species examined within the genus *Falco* (nine of 38) was the minimal variation in syringeal morphology in these species.

Multiple individuals from six species were examined to assess variation at the intra-specific level. For the American Kestrel (*Falco sparverius*), collection data were available to allow assessment of both sexual and ontogenetic variation.

ANALYSIS

Variation in syringeal morphology was coded using both binary and multistate characters. The ordering of states for multistate characters were hypothesized using the similarity criterion and tested by examining the relationships of character states to each other in the resulting cladograms (Lipscomb 1992).

Characters were polarized using outgroup information (Maddison et al. 1984). Multiple outgroups were used to ensure global parsimony, that is, that the phylogenetic hypotheses are the most parsimonious both within the Falconidae, and among the Falconidae and its sister taxa (Maddison et al. 1984).

The PAUP 3.0s computer program (Swofford 1991) on a Macintosh IIci was used to derive the most parsimonious resolution of the data. The size of

the data set precluded exact search algorithms; therefore, a heuristic algorithm was used. However, this does not guarantee optimality. To avoid finding a solution that is only locally optimal, analyses were repeated varying both the branch swapping and taxa addition options. In addition, the effect of two different character optimizations was tested, ACCTRAN, which increases reversals, and DELTRAN, which increases parallelisms.

Three indices were used to assess the congruence of characters hypothesized as synapomorphies, (1) the consistency index; the minimum number of character state changes a character may show, divided by the number of changes observed on a particular tree, (2) the rescaled consistency index, a linear rescaling to allow the consistency index to vary between 0 and 1, and (3) the retention index, the proportion of hypothesized synapomorphies retained as homologies (Farris 1989).

Strict consensus trees, which only include groups found in all of the most parsimonious cladograms, were used to summarize the agreement in taxonomic relationships among the set of most parsimonious trees. Consensus trees must be interpreted carefully as they may not be, themselves, parsimonious reconstructions of the original data.

RESULTS

SYRINGEAL MORPHOLOGY

The main structural components of the falconid syrinx are shown in Figure 2; Ames' (1971) definitions of syringeal components were used. These are as follows:

1. *A* elements - occur on the trachea as single rings but may extend onto the bronchi as paired double rings. The rings may be complete or incomplete medially and are generally ossified in birds (Ames 1971, Cannell

1986). In the Falconidae, all genera have at least one incomplete double and one complete double ring and all *A* elements are completely ossified.

2. *B* elements - occur on the bronchi and generally are cartilaginous. These are paired rings that may be either complete or incomplete medially. *B* elements are all incomplete medially (*C* rings) in the Falconidae.

3. Tympanum - fused and ossified *A* elements near the tracheo-bronchial junction. In the Falconidae, from three to eight *A* elements are fused (Appendix 2, Characters 2-7).

4. Pessulus - a dorso-ventrally oriented cartilaginous or ossified bar in the mid-sagittal plane of the trachea between the bronchi. It may be fused to other elements at the dorsal or ventral or both ends. In the Falconidae, the pessulus is always ossified and fused both dorsally and ventrally to the tympanum.

5. Membranes -(a). The internal or medial tympaniform membranes comprise the medial surface of the bronchial tubes, usually close to the tracheo-bronchial junction and supported at the edges by the ends of the divided *A* and *B* elements. The membranes may be continuous from the left to the right bronchus or may be separated by the pessulus. These are generally considered to be the sound producing structures (Gaunt and Gaunt 1985). (b). The external or lateral tympaniform membranes are on the lateral walls of the bronchi, usually between one or two of the first four B elements. In the Falconidae, there is a large external membrane between A1 and B1.

6. Musculature - (a). Intrinsic muscles are short muscles which both originate and insert on syringeal elements. There are no intrinsic muscles within the Falconidae. (b). Extrinsic muscles are longer muscles which originate away from the syrinx and insert on it. M. tracheolateralis originates on the lateral surface of the cricoid cartilage of the larynx and extends down

the lateral surfaces of the trachea, inserting in the syringeal region. In this family, the insertion is on the external membrane. M. sternotrachealis originates on the internal surface of the coracoid or costal process of the sternum, or on the internal surface of one or more ribs. It inserts on the lateral or ventral surface of the trachea or on the tissues surrounding the trachea, and may be continuous with, or overlap the M. tracheolateralis.

PHYLOGENETIC ANALYSIS

Variations in syringeal morphology were examined for 32 taxa; ten of these represented hypothesized outgroups to the Falconidae (five falconiform species and five species from three other orders of birds). After the initial character state coding, redundant outgroup species (those with identical character states) were deleted, resulting in a data matrix containing 25 taxa, 22 falconid species and three outgroup species.

The 25 characters included in this analysis were coded first as 20 binary and five ordered multistate characters. The cladograms resulting from the analysis of this data matrix were examined to assess the congruence of the ordering of character states of the multistate characters. One of the five was highly incongruent; this character was reexamined and then treated as unordered. The final data matrix contained 20 binary, four ordered multistate and one unordered multistate character (Appendix 2, ordered characters 3,4,8,18, unordered character 21).

Analysis of this data matrix (Table 1) resulted in 27 most parsimonious cladograms of 50 steps, consistency index of 0.620, rescaled consistency index of 0.525 and retention index of 0.843. Because the consistency index is negatively correlated with the number of taxa, there is a maximum value expected for different numbers of taxa. The CI for this analysis compares favorably with

the general result expected for 25 taxa (0.483, Sanderson and Donoghue 1989). Congruence among the cladograms produced by this analysis is summarized in the strict consensus tree (Figure 3); there are three polytomies (discussed below) reflecting the conflicting topologies in the 27 most parsimonious cladograms. This tree illustrates the distribution of derived characters and the effect of differing character optimizations (differences in character 10, Figure 3).

CLADES WITHIN THE FALCONIDAE

The phylogeny derived from syringeal data does not support the monophyly of the two currently accepted Falconidae sub-families. Rather, all trees are congruent in their support of three clades: (1.) *Micrastur*, (2.) the four caracara genera, and, (3.) a clade composed of *Herpetotheres, Spiziapteryx, Microhierax, Polihierax* and the *Falco* species.

Four derived characters support the monophyly of the two species of *Micrastur* (Figure 4); three of these are unambiguous synapomorphies. The *Micrastur* tympanum has minimal fusion compared to other genera in the family (dorsal fusion, character 2, CI 0.5; ventral fusion, character 5, CI 1.0). The ends of the A1 elements are flattened and enlarged (character 11, CI 1.0). The B1 elements are straight medially; B1 ends ascend abruptly (character 14, CI 1.0) and fuse totally with A1 ends, forming an inflexible frame for the lateral membrane.

Monophyly of the clade of six species of caracaras also has strong support (three unambiguous synapomorphies, characters 4,7,17). Ventral and dorsal tympanum fusion is most extensive in the caracaras (characters 4 and 7, CI 1.0), in number of elements fused and degree of fusion of each element (Figure 5) . The B1 elements also have a characteristic shape: thick in circumference and concave medially with ends that ascend gradually

(character 17, CI 1.0). Fusion of B1 to A1 ends is not as strong as in *Micrastur*; a cartilaginous bridge connecting the ends allows some flexibility and movement.

The third clade comprises the current sub-family Falconinae (the falcons, pygmy falcons and falconets), with the addition of *Spiziapteryx* and *Herpetotheres*. Two derived characters unite the clade: (1). the pattern of ventral fusion of the tympanum (character 6, CI 1.0), and (2). the broad knobbed ends of the B1 element (character 16, CI 0.5). Within the clade, *Spiziapteryx* is sister taxon to *Falco* and two of the falconet species. *Herpetotheres* is sister taxon to the *Falco*-falconet clade, while *Polihierax insignis* (Asian Pygmy Falcon) is basal.

All trees are also congruent in the resolution of the interrelationship of these three clades. *Micrastur* is supported as the basal clade while one synapomorphy unites the caracara and Falconinae clades (character 21, CI 0.33, Figure 3).

MONOPHYLY OF FALCONID GENERA

Syringeal data suggest that three genera are not monophyletic. Two of the genera, *Polihierax* (Figure 6) and *Daptrius* are polyphyletic; the two species within each of these genera are not sister taxa. *Daptrius americanus* (Red-throated Caracara) is more closely related to *Polyborus plancus* (Crested Caracara), whereas *Daptrius ater* (Black Caracara) is closer to the *Milvago* clade.

Similarly, *Polihierax semitorquatus* (African Pygmy Falcon) forms a clade with *Microhierax erythrogonus* (Philippine Falconet), while P. *insignis* (Asian Pygmy Falcon) is sister taxon to *Herpetotheres, Spiziapteryx, Falco* and the other falconet species.

The monophyly of *Milvago* is also uncertain. In this analysis, *Phalcoboenus* australis occurs in a polytomy with the two *Milvago* species. This clade is supported by two synapomorphies (characters 22 and 24); there are no syringeal characters that distinguish any of these three species (no autapomorphies).

These data support the monophyly of the *Falco* species examined for this study (nine of 38 species), but are unable to resolve the relationships of these species. One clade of four species, *F. mexicanus* (Prairie Falcon), *F. sparverius* (American Kestrel), *F. peregrinus* (Peregrine Falcon) and *F. biarmicus* (Lanner Falcon), is united by two synapomorphy (characters 20 and 21). Relationships of the other five *Falco* species, *F. cenchroides* (Australian Kestrel), *F. columbarius* (Merlin), *F. berigora* (Brown Hawk), *F. rufigularis* (Bat Falcon), and *F. femoralis* (Aplomado Falcon) are ambiguous. In all trees, however, *F. rufigularis* and *F. femoralis* are sister taxa; both have A1 elements that are medially straight and not concave (character 13).

DISCUSSION

SYRINGEAL VARIATION

To resolve phylogeny, the patterns of variation of characters must be informative at the level of the question asked. Thus, characters varying among individuals within a species will not generally be able to resolve generic relationships. Systematists must choose characters with rates of evolution consistent with the taxonomic level of the group in question. However, rates of change of characters may vary among taxa. For example, osteological characters have been used successfully in genus-level analyses in Anseriformes (Livezey 1986), but in the Cathartidae they were useful only at

the family and order level (Emslie 1988). This study examined the levels of usefulness of syringeal data for the Falconidae.

Intraspecific variation was examined in nine specimens of American Kestrel (*F. sparverius*). There was variation among adults only in the number of rings fused in the tympanum. Six specimens (including both males and females) had three rings fused, whereas two (one male and one female) had four. There was additional variation between adult and juvenile specimens. In all adult falconid specimens examined, the *A* rings are completely ossified. However, the juvenile Kestrel had cartilaginous medial sections, both dorsally and ventrally. Similar ontogenetic variation in *A* element ossification has been observed in passerines (Ames 1971) as well as in the Accipitridae and the Tytonidae (Griffiths 1994a). Neither of these two morphological variants was used in this analysis.

Syringeal morphology is relatively conservative within genera and there may not be enough variation within speciose genera to resolve relationships. Thus, among the nine species of *Falco* examined, there is slight variation in the fusion of the tympanum, in the appearance of a ridge over the dorsal pessulus ascent under the tympanum, and in the method of insertion of the M. tracheolateralis. While this amount of variation may be useful in identifying major clades or some small groups of sister taxa, it is not enough to infer the phylogeny of 38 species.

MULTI-STATE CHARACTERS

Although multi-state characters are used extensively in phylogenetic analysis, there is a lack of consensus as to the best method for describing the transformation between the different character states. The most commonly used transformations are ordered and unordered characters, both of which

entail assumptions about character evolution. Unordered character states assume that any state can transform directly into any other, while ordering states implies a (usually linear) relationship among the character states (Swofford & Olsen 1990).

Currently, the use of ordered characters is being questioned. There may be three possible reasons for the trend towards unordered characters: (1). the prevalence of molecular phylogenetic studies which use unordered characters, (2). the conviction that ordering entails a risk that the wrong order may be chosen (Slowinski 1993), and (3). the possible constraining effect that ordering may have on the number of most parsimonious trees found in an analysis (Hauser & Presch 1991). There have been, however, no definitive studies about the effect of these assumptions on phylogenetic analyses and results that have been reported are contradictory. For example, ordering characters may result in phylogenies with greater resolution (Mickevich & Weller 1990, Slowinski 1993) or may have no effect on resolution (Hauser & Presch 1991). Ordering characters may or may not constrain the number of most parsimonious trees found in an analysis; the effects may, in fact, be data-set specific (Hauser & Presch 1991).

The decision to order a multistate character should be made on a character by character basis. If there is evidence for relationships of the states in a multistate character, then to treat that character as unordered is discarding information (Lipscomb 1992, Maddison & Maddison 1992). In this study, similarity of states was the criterion used to hypothesize character state order. For characters 3, 4 and 18 (increases in amount of ossification or fusion of various elements), the ordering patterns were also observed in ontogenetic sequences and are in agreement with general developmental processes. In addition, similar patterns were observed in syringes from juveniles and adults

in species within the Strigiformes, Ciconiiformes, Cathartidae, Falconidae and Accipitridae, and through descriptions in the literature for the Passeriformes (Ames 1971).

MONOPHYLY OF GENERA

There were no discrete characters distinguishing the two *Milvago* species from *Phalcoboenus*. The species composition of caracara genera and the relationships among these genera have always been ambiguous. For example, Sharpe (1874) placed *Daptrius, Phalcoboenus* and *Milvago* in one genus, *Ibycter*. *Milvago* was separated first (Swann 1922) and then all four were recognized as separate genera (Peters 1931). Brown and Amadon (1968) proposed a close relationship of *Polyborus, Phalcoboenus* and *Milvago,* and Vuilleumier (1970) recommended that the three be placed in one genus. This analysis suggests the need for additional study of these genera.

Daptrius was found to be polyphyletic. The two *Daptrius* species have traditionally been united by plumage color and by tarsal and toe characters (Friedmann 1950). Brown and Amadon (1968), however, noted that habitat and behavioral differences suggest that these two should be separated generically and that the genus name *Ibycter* be used again for *D. americanus*. Syringeal data, by demonstrating that *Daptrius americanus* and *Polyborus plancus* are sister taxa, support that conclusion.

The two species of *Polihierax* are not sister taxa. *P. semitorquatus* is in the 'falconet' clade, sister taxon to *Falco*. *P. insignis,* on the other hand, is basal to *Falco* and the other falconet species. Morphological differences (tail length and shape, and 2nd primary length) between the two *Polihierax* species have also been recognized previously, leading to *P. insignis* being placed in a monotypic genus, Neohierax, (Swann 1922). The suggestion that generic

status be accorded to these species has been revived (Brown and Amadon 1968). This is also supported by syringeal data.

PHYLOGENETIC RELATIONSHIPS OF THE FALCONIDAE

Previous designations of Falconid subfamilies have been based on combinations of characters, many of which may be plesiomorphic. Cladistic analysis of syringeal data (Figure 3) results in a division of the family into three comprehensive clades; (1) the genus *Micrastur* basal to the other clades, (2) the Polyborinae (including only the four caracara genera), and (3) the Falconinae (consisting of *Falco*, the pygmy Falcons and Falconets, and *Herpetotheres*) . It does not support the currently accepted (Stresemann and Amadon 1979) designations which divide the family into two subfamilies and place three genera (*Spiziapteryx, Herpetotheres* and *Micrastur*) in the Polyborinae.

Syringeal data also indicate that two genera are not monophyletic, suggesting that more detailed investigations of species relationships in this family must be performed using all extant taxa.

Congruence of these conclusions with the two previous cladistic analyses of the family (Figure 1) cannot be assessed with rigor since neither of these examined all the species analyzed in this research. Nevertheless, some comparisons can be made. Becker's (1987) hypothesis based on osteology (Figure 1B) agrees generally with the syringeal hypothesis: in both, *Micrastur* is basal and *Spiziapteryx* is related to *Falco* and the Falconets, rather than to the caracaras. Only the position of *Herpetotheres* and *Polihierax insignis* differs between the two. The topology derived from allozyme data (Figure 1A, Boyce 1989) differs from the syringeal and osteological phylogenies. However, if the

allozyme hypothesis were rooted at *Micrastur* rather than at the Falconets, there would be substantial congruence among all three.

This study has demonstrated that syringeal data can be used to resolve phylogenetic questions at the generic and family levels of the Falconidae. The value of syringeal morphology for systematics has been known for at least one hundred years (Beddard 1898). Avian systematists, however, have not used syringeal characters to develop phylogenies for orders of birds other than the Passeriformes, and avian biologists, in general, have ignored the syrinx. In 1960, Andrew Berger observed, "There are few anatomical structures throughout the families of birds that need study as badly as the syrinx" (King 1989, page 106). By reinforcing the value of syringeal morphology as a systematic tool, the hope is that this analysis will encourage systematists to explore little known morphological structures as sources of information for phylogenetic reconstruction.

APPENDIX 1. List of specimens examined. Abbreviations for the institutions are in the Materials and Specimens section.

FALCONIDAE

Daptrius americanus	AMNH 8667, unnum 24/7/90
D. ater	KUMNH 041874, AMNH 10128
Falco berigora	AMNH 193358
F. biarmicus	AMNH 15927
F. cenchroides	AMNH 193394
F. columbarius	AMNH 19752, 14713
F. femoralis	LSUMNS 123309
F. mexicanus	KUMNH 053827
F. peregrinus	AMNH 8499, 19751
F. rufigularis	KUMNH 041874
F. sparverius	AMNH 8413, 8430, 8688, 15808, 15931,16307,CSG21,CSG9210.
Herpetotheres cachinnans	AMNH unnum
Micrastur gilvicollis	LSUMNS 98021
M. semitorquatus	USNM 507797
Microhierax erythrogonys	AMNH 8623
Milvago chimachima	LSUMNS 120427
M. chimango	USNM 346421 É
Phalcoboenus australis	USNM 511795; LSUMNS 120728
Polihierax semitorquatus	USNM 615218
P. insignis	AMNH 8627
Polyborus plancus	AMNH 9094
Spiziapteryx circumcinctus	LSUMNS unnum. 8/9/90

OUTGROUPS

Pelecanus roseus	AMNH 8619
Gampsonyx swainsonii	AMNH 8529
Otus asio	AMNH 8310
Mycteria americanus	AMNH 8513
Sula bassanus	AMNH 8618
Buteo jamaicensis	AMNH 18764
Aegypius tracheliotus	AMNH 81668
Accipiter striatus	AMNH 18761
Cathartes aura	AMNH 20933

APPENDIX 2.

DESCRIPTIONS OF 25 SYRINGEAL CHARACTERS

The characters for the syringeal analysis are as follows:

1. Tympanum (characters 1-9)
2. *A* and *B* elements (characters 10-18)
3. Membranes and muscles (characters 19-25)

Characters 3,4, 8 and 18 are ordered multistate characters. Character 21 is an unordered multistate character. The plesiomorphic or primitive state for each character is described either as state [0] for the character, or in the general description for a group of characters.

TYMPANUM

(1). Presence of a tympanum or tracheal drum

[0] There is no tracheal drum.

[1] The first A elements are ossified totally and more extensively than more craniad A elements. The first A elements are fused medially and may be partially or completely fused to each other along their margins, dorsally, laterally and ventrally forming a tracheal drum to which the pessulus is attached. The tracheal drum is always formed from elements A1 and A2, which are double elements. A3, which is usually single, and single elements from A4 to A8 may also be fused to the tympanum (see below).

The following 8 characters describe variations in the tympanum within the Falconidae. The tympanum is either primitively: (a).absent (within the species of Galliformes, Pelecaniformes, Strigiformes and Ciconiiformes examined) or (b). has a different pattern of fusion and different shape (within the Accipitridae species).

<u>Degree of dorsal fusion of tympanum (2-4)</u>

(2).The first 2 single A elements are fused medially only, by an ossified bar which is an extension of the pessulus.

(3).The first 3 or 4 A elements are fused lightly but entirely along their margins. This is an ordered character:

(3.1) Margins are apparent along the edges of each ring.

(3.2) Margins are somewhat obliterated and only light sutures are apparent medially.

(4). At least 5 A elements are fused entirely along their margins. This is an ordered character:

(4.1) Sutures are apparent along the margins of all 5 A elements except medially.

(4.2) Sutures are always obliterated between the first two A single elements (usually A3 and A4).

Degree of ventral fusion of tympanum (5-7).

(5).The first three or four A elements (both double, usually A1 and A2 and single A elements) are fused along their margins. Spaces are apparent between the elements.

(6).The first three or four A elements are fused lightly but entirely along their margins.

(7).At least five A elements are fused entirely along their margins with some sutures apparent along the margins, except medially.

Tympanum Shape (8-9)

(8).The shape of the fused A elements forming the tympanum varies from cylindrical (the most caudal and cranial elements having the same diameter) to a graduated A shape (the most caudal element wider in diameter than the most cranial). This is a partially ordered character: the transformations between states 0,1 and 2 are treated as unordered; the transformation from state 2 to 3 is ordered.

(8.1) Tympanum shape is graduated and widens caudally .

(8.2) Tympanum is cylindrical

(8.3) Tympanum is cylindrical; in addition, A1 laterally is flattened causing a 'pinching in' of the most caudal element.

(9). Dorsal Tympanum shape, medially, at pessulus ascent.

[1] A ridge of ossified tissue forms a medial bridge or connection between the dorsal A1 element ends and covers the dorsal ascent of the pessulus.

A AND *B* ELEMENTS

In all Falconidae genera, A1 and B1 dorsal and ventral ends are fused (A1 left to B1 left, A1 right to B1 right).

A1 Elements (10-13)

In all Falconidae genera, A1 is a double element; both rings are incomplete medially, andA1 ends border the internal membranes.

(10).Separation of dorsal A1 element ends.

[0] A1 dorsal ends are separated.

[1] The dorsal ends of A1 are close medially and are fused together by ossified tissue.

(11).Size of A1 ends

[0] A1 is a single element or the width of the A1 ends is proportionally similar to the width of A1 medially.

[1] The dorsal ends of A1 are very flattened and very enlarged.

(12).Flattening of the paired A1 elements.

[0] A1 is a single element or each half is rounded and forms a C shaped ring.

[1] Each A1 is flattened dorso-ventrally into a parenthetical shape. When viewed laterally, the A1 dorsal and ventral ends protrude out.

(13).Appearance of A1 on lateral view.

[0] A1 is concave up medially.

[1] A1 is flattened medially.

B1 Elements (14-17)

The following 4 characters described modifications of the dorsal ends of the first B element and subsequent variations in the fusion of A1 and B1. The primitive state for these characters is hypothesized to be B1 ends which are rounded and end at the medial membrane without fusing to A1.

(14). B1 ends are very thick, wide and ascend sharply in an L shape to fuse with A1 ends.

(15). B1 ends are thin and ascend gradually to fuse with A1

(16). There is a knobbing of B1 craniad edges; the craniad extension or knob fuses with A1.

(17). B1 ends are thick and rounded and ascend gradually to fuse with A1.

Fusion of *A* and *B* ventral ends

(18). Fusion of additional *B* elements ends to form a ridge bordering the internal membrane. This is an ordered character:

(18.0) There is no fusion of *B* element.

(18.1) B2 ends are fused to B1/A1.

(18.2) B3 ends are also fused.

MEMBRANES AND MUSCLES

(19). Existence of external (lateral) membrane on bronchi.

[0] There is no membrane, or, if one exists, the membrane lies between B1-4 elements.

[1] An external membrane is located between A1 which is concave up and B1 which is concave down.

M. tracheolateralis (20-22)

Within the Falconidae the M. tracheolateralis always inserts on the external (lateral) membrane. The following 3 describe variations in this derived character; the primitive state is hypothesized to be absence of the insertion on the membrane.

(20). A cartilaginous bar exists on the lateral membrane onto which the M. tracheolateralis inserts.

(21). M. tracheolateralis inserts on a membranous extension of the external membrane.

(21.1) There is a thick, bulbous membrane on the dorsal half of the external membrane. The M. tracheolateralis inserts on the bulbous extension and on the external membrane.

(21.2) The bulbous membrane covers the entire width of the external membrane.

(22). The M. tracheolateralis inserts on the dorsal half of the lateral membrane

Cartilaginous Border on Internal Membrane (23-25)

(23). Appearance of the internal (medial) membrane.

[0] Either no membrane or a thin membrane with no cranial border.

[1] The internal (medial) membrane has a thickened cartilaginous cranial border. The following 2 characters describe the appearance of the cartilaginous border, which is primitively hypothesized to be absent.

(24). The cartilaginous border is thick and even, and concave up from A1 dorsal to A1 ventral ends.

(25). Additional thin, amorphous cartilage edges border, forming straight caudal edge from A1 dorsal to A1 ventral ends.

Table 1. Data matrix of 25 syringeal morphological characters for 25 Falconidae and outgroup species

	Characters																								
										1										2					
Taxa	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5
Pelecanus roseus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Otus asio	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gampsonyx swainsonii	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
Daptrius americanus	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	0	1	0	1	0	2	0	1	1	1
D. ater	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0
Falco sparverius	1	0	1	0	0	1	0	2	1	0	0	0	0	0	0	1	0	1	1	1	0	0	1	1	0
F. rufigularis	1	0	1	0	0	1	0	2	0	0	0	0	1	0	0	1	0	2	1	0	2	0	1	1	0
F. biarmicus	1	0	1	0	0	1	0	2	0	0	0	0	0	0	0	1	0	2	1	1	0	0	1	1	0
F. mexicanus	1	0	1	0	0	1	0	2	0	0	0	0	0	0	0	1	0	1	1	1	1	0	1	1	0
F. femoralis	1	0	1	0	0	1	0	2	0	0	0	0	1	0	0	1	0	2	1	0	2	0	1	1	0
F. cenchroides	1	0	1	0	0	1	0	2	1	0	0	0	0	0	0	1	0	1	1	0	2	0	1	1	0
F. berigora	1	0	2	0	0	1	0	2	1	0	0	0	0	0	0	1	0	2	1	0	2	0	1	1	0
F. columbarius	1	0	1	0	0	1	0	2	0	0	0	0	0	0	0	1	0	2	1	0	2	0	1	1	0
F. peregrinus	1	0	1	0	0	1	0	2	0	0	0	0	0	0	0	1	0	2	1	1	0	0	1	1	0
Herpetotheres cachinnans	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0
Micrastur gilvicollis	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0
M. semitorquatus	1	1	0	0	1	0	0	2	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1
Microhierax erythrogonys	1	0	2	0	0	1	0	3	1	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0
Milvago chimachima	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1	0
M. chimango	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1	0
Phalcoboenus australis	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1	0
Polihierax semitorquatus	1	0	1	0	0	1	0	3	0	0	0	0	0	0	1	0	0	0	1	0	2	0	1	1	0
P. insignis	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	1	0	2	0	1	0	1
Polyborus plancus	1	0	0	2	0	0	1	1	0	1	0	1	0	0	0	0	1	0	1	0	2	0	1	1	1
Spiziapteryx circumcinctus	1	0	2	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	2	0	1	1	0

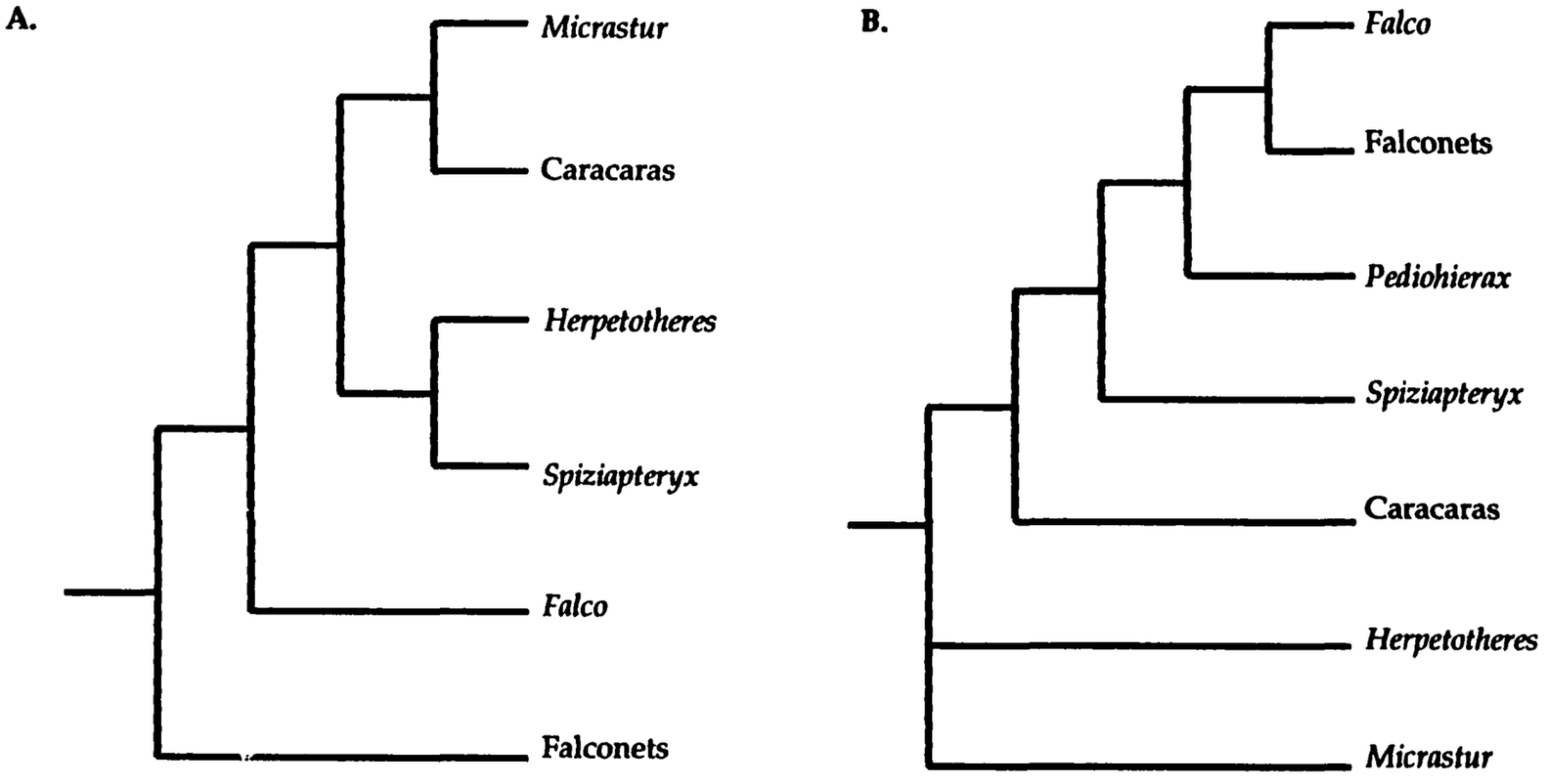

Figure 1. Alternative phylogenetic hypotheses for the Falconidae derive from (A) Allozyme data (Boyce 1989) and (B) Osteology (Becker 1987).

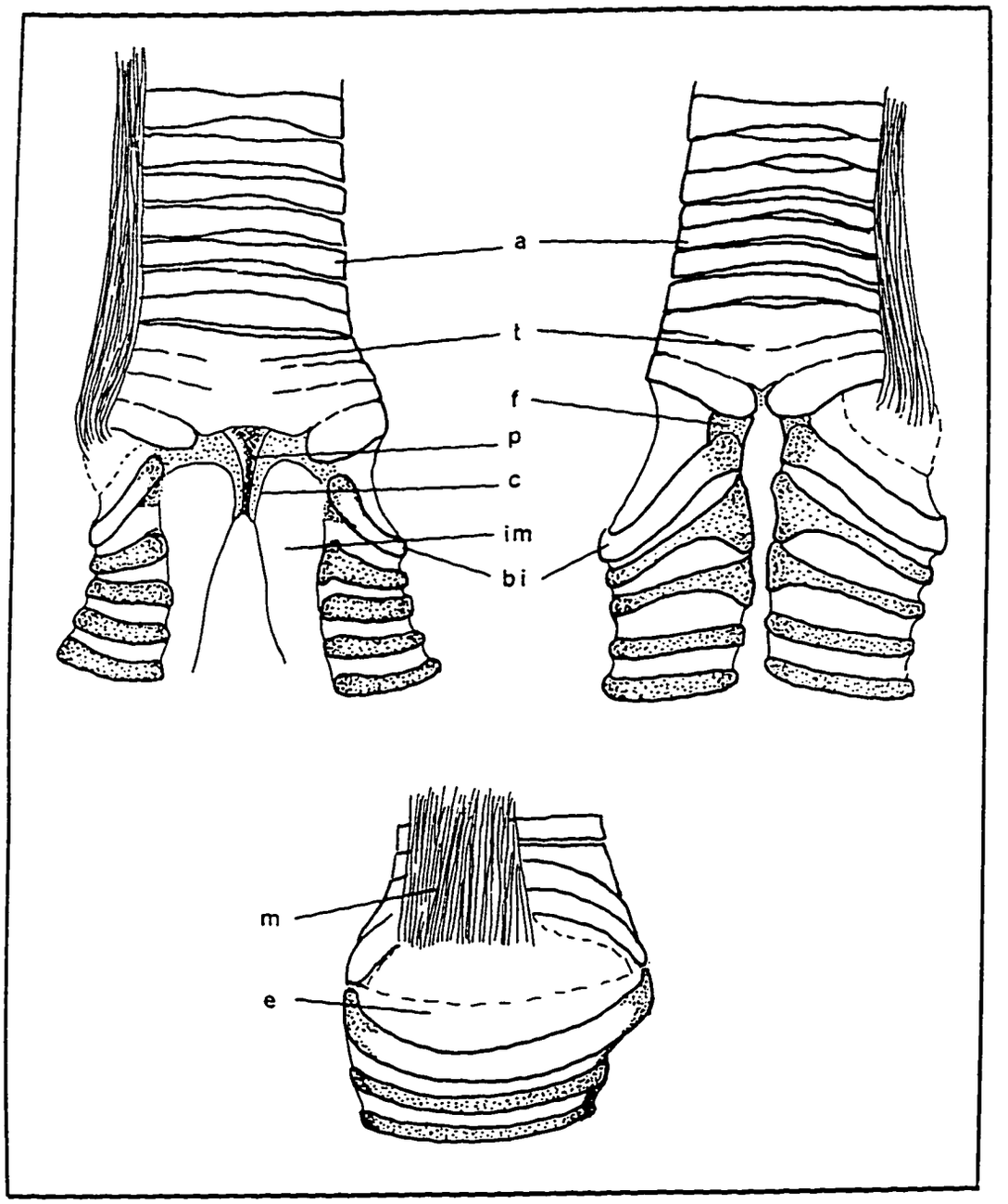

Figure 2. Syrinx of *Falco berigora* (AMNH 193358). Top: left, dorsal view; right ventral view. Bottom: lateral view. Abbreviations: a (*A* elements), bi (double B1 element), c (cartilaginous border on internal membrane), e (external membrane), f (fusion of A1 and B1 elements), im (internal membrane), m (M. tracheolateralis), p (pessulus), t (tympanum). In all drawings, stippling indicates cartilaginous tissue, cross-hatching indicates dense ossified tissue.

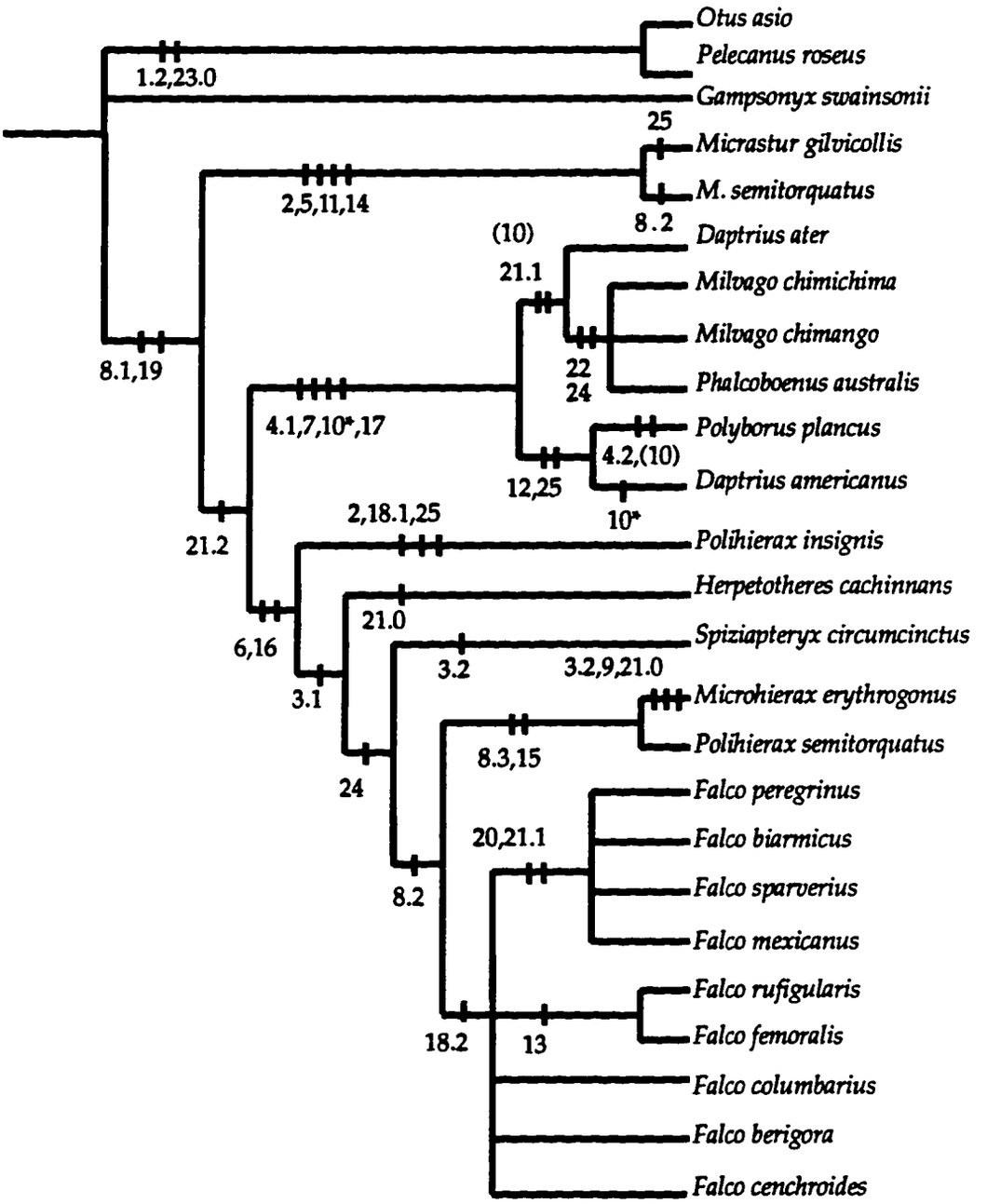

Figure 3. Strict consensus tree derived from the 27 most parsimonious cladograms. There are three polytomies: (1). *Milvago* and *Phalcoboenus*, (2). the *Falco* species: *berigora, columbarius, cenchroides*, and, (3). the Falco species: *sparverius, biarmicus, mexicanus,* and *peregrinus*. Character support for each node is indicated: states for the five multistate characters (3,4,8,18,21) are shown as decimals following the number. Optimization of character 10 is ambiguous; in the alternative DELTRAN optimization, characters states marked with asterisks are eliminated, character states in parentheses are acquired. The effect is to delay the transformations and to eliminate reversals.

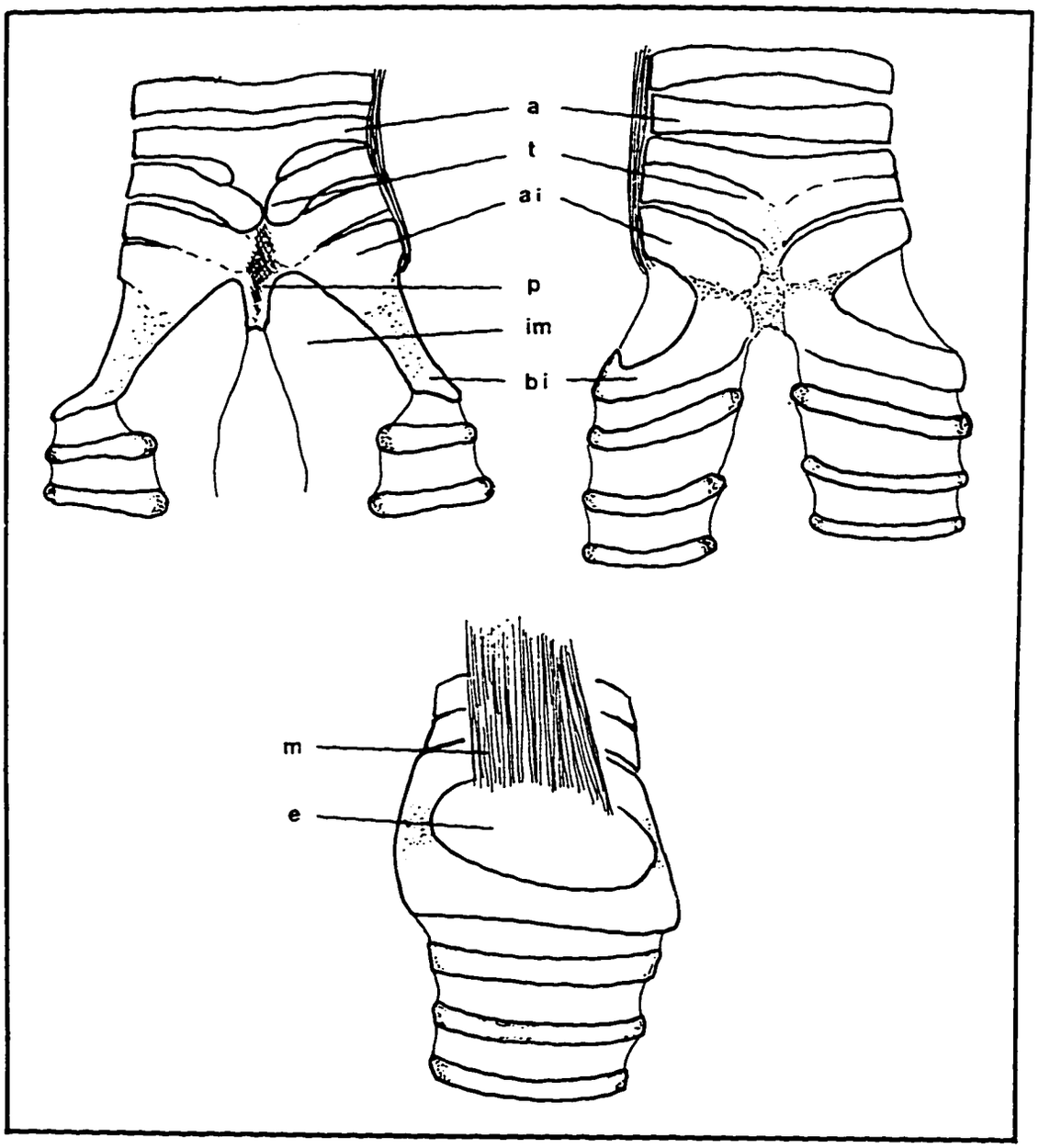

Figure 4. Syrinx of *Micrastur semitorquatus* (USNM 507797). Top: left, dorsal view; right, ventral view. Bottom: lateral view. Abbreviations: a (*A* elements), ai (double A1 elementa), bi (double B1 elements), e (external membrane), im (internal membrane), m (M. tracheolateralis), p (pessulus), t (tympanum).

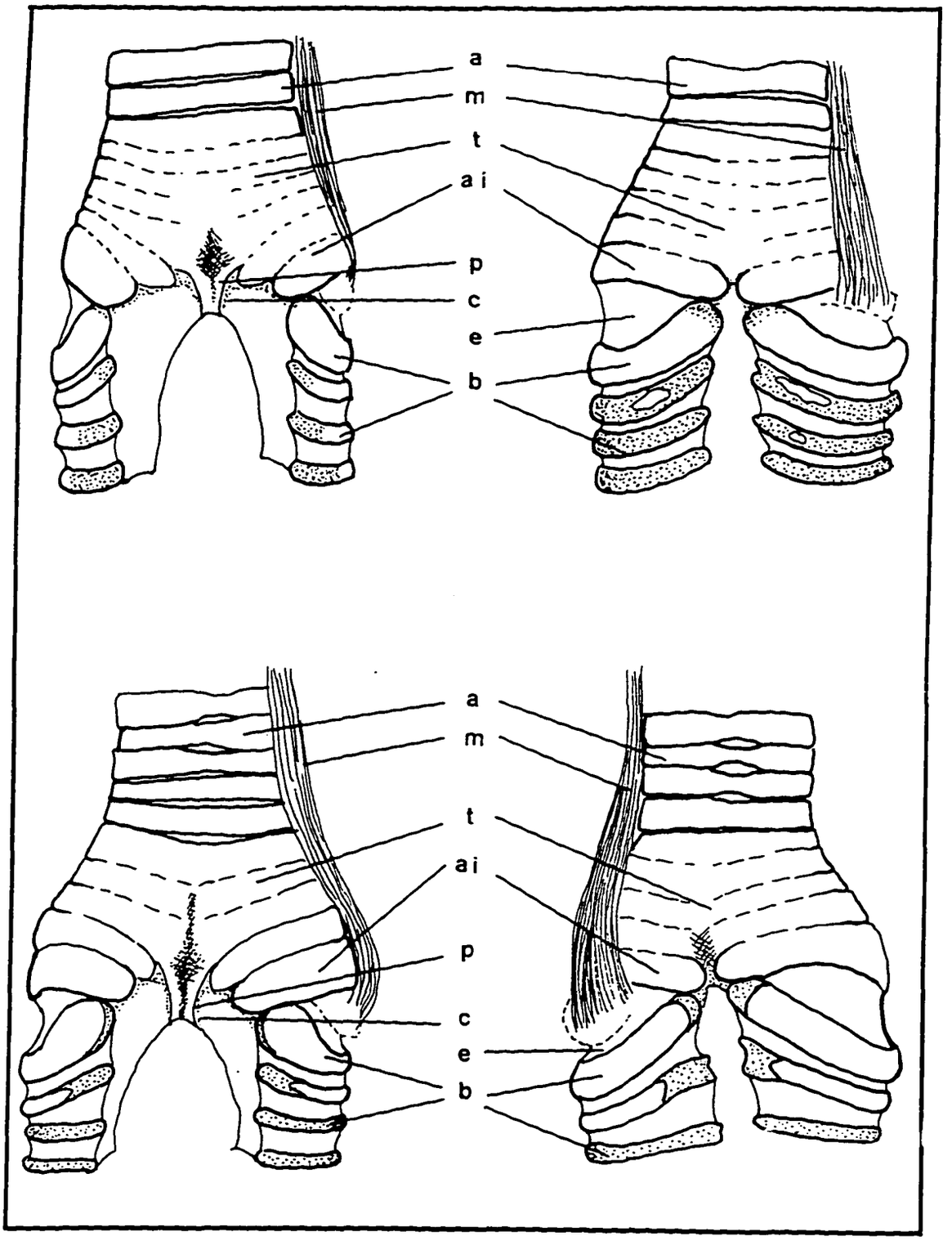

Figure 5. Syrinx of two caracaras. Top: *Milvago chimachima*(LSUMNS 120427). Bottom: *Daptrius ater* (AMNH 10128). Left, dorsal view; right, ventral view. Abbreviations: a (*A* elements), ai (double A1 elements), b (*B* elements), c (cartilaginous border on internal membrane), e (external membrane), m (M. tracheolateralis), p (pessulus), t (tympanum).

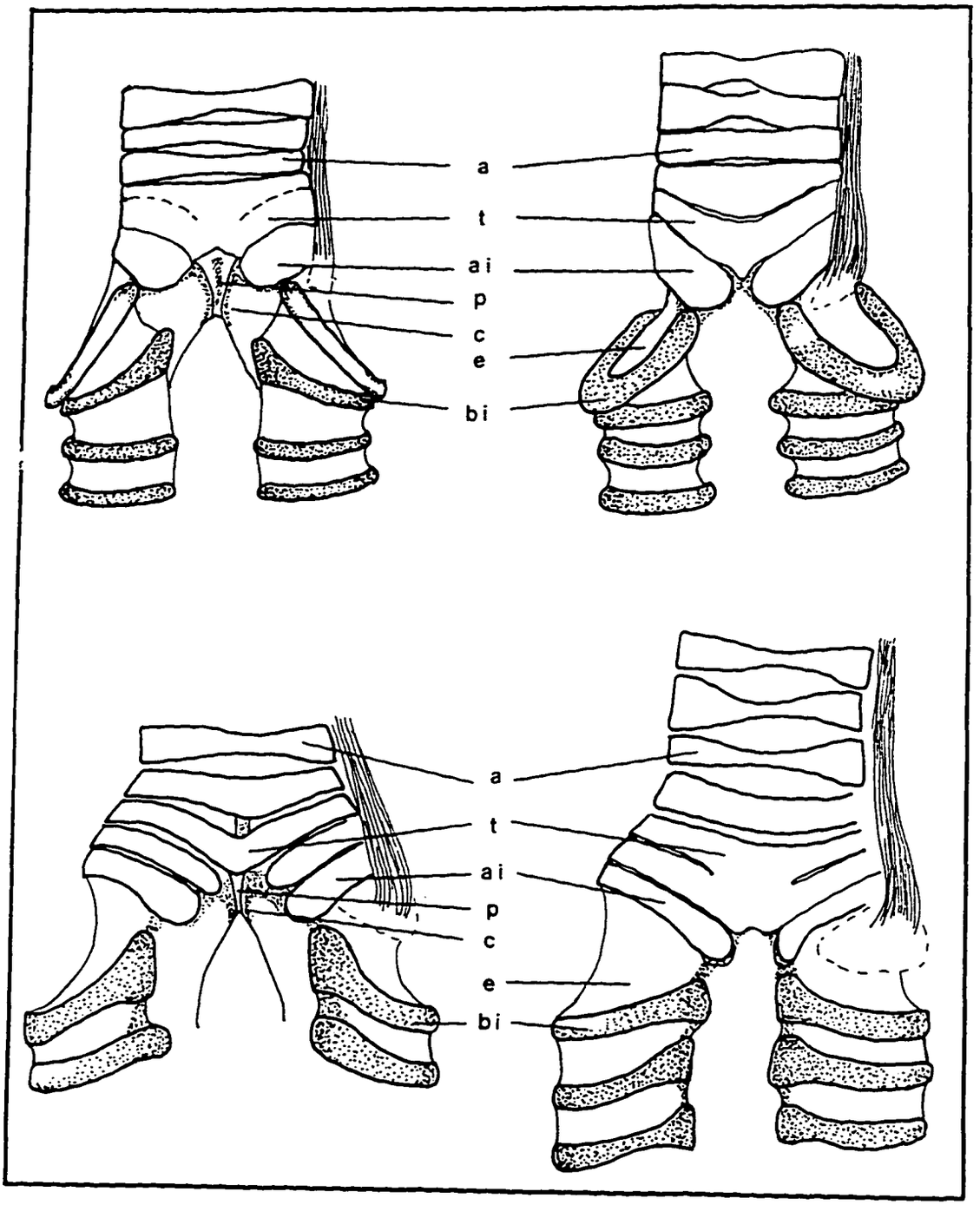

Figure 6. Syringes of the two *Polihierax* species. Top: *P. semitorquatus* (USNM 615218). Bottom: *P. insignis* (AMNH 8627). Left, dorsal view; right, ventral view. Abbreviations: a (*A* elements), ai (double A1 elements), bi (double B1 elements), c (cartilaginous border on internal membrane), e (external membrane), ıp (pessulus), t (tympanum).

CHAPTER 3

MOLECULES, MORPHOLOGY AND THE PHYLOGENY OF THE FALCONIDAE

ABSTRACT. - Molecular data and a reanalysis of variations in syringeal morphology were used to infer phylogenies for the avian family Falconidae (falcons and caracaras). In addition, three issues currently of interest in systematics were addressed; (1) *a priori* analysis and differential weighting of molecular data, (2) the reliability of molecular vs. morphological data in phylogenetic analysis, and, (3) the treatment of multiple data sets in phylogenetic analysis. Cytochrome *b* sequences were partitioned into six subsets, transitions and transversions at each codon position. Saturation of substitutions within these partitions was then assessed graphically and saturated partitions downweighted before phylogenetic analysis. The analysis of differentially weighted molecular data resulted in a phylogeny congruent with the hypothesis inferred from variations in syringeal morphology, and with a cladistic analysis of osteological data. This divides the family into three clades. The Forest Falcons and Laughing Falcon are basal to two sister clades; (1) the four caracara genera, and, (2) the Spot-winged Falconet, Pygmy Falcon and species within the genus *Falco*. Molecular and morphological data complement each other and share similar problems caused by taxa connected by long branches. The separate analysis of molecular data indicated that saturation at third position transitions may be occurring for species with an uncorrected total sequence divergence of 10%. In addition, some mutations at different codon positions may not be independent. There is evidence of correlation of change by codon position along lineages.

Problems inherent in molecular sequence data are becoming apparent (Irwin et al., 1991; Graybeal, 1993; Helm-Bychowski & Cracraft, 1993; Cummings et al., 1995; Hackett, 1996). These exist at two levels: (1) in the initial hypotheses of character homology, i.e., sequence alignment, and, (2) in the hypotheses of homology of character states. For sequences of protein-coding genes, the alignment problem may be easily solved by aligning sites based on codons, or by using alignment programs. However, the second problem, homoplasy of character states, does not have an easy solution.

This latter problem is caused by multiple substitutions at site in sequences. These might not be a problem if the taxa in a study are closely related, and time of divergence is recent. The number of sites differing among these taxa will be small, and the probability of a new substitution occurring at a site already varying will be low. However, as divergence times among taxa increase, the number of sites differing among these taxa also increases. At some point, all potentially variable sites among those sequences will have had hits and the data will be saturated (Brown, 1983). For saturated data, new mutations cannot increase sequence divergence, and the number of sequence differences between taxa underestimates the actual number of substitutions that occurred (Brown, 1983). When data are saturated, recovering phylogenetic signal can become problematic.

Methods to demonstrate saturation, and, thus, resulting problems, are relatively easy to perform. Saturation can be detected graphically, by plotting differences between sequences of pairs of taxa against time of divergence (actual or estimated) of the taxa (Brown, 1983; DeSalle et al., 1987; Moritz et al., 1987). For unsaturated data, pairwise sequence differences between taxa increase as time since divergence of taxa increases (Figure 1A). For saturated sites, pairwise

differences in sequences asymptote towards a maximum, the difference expected between two randomized sequences (Figure 1B).

Graphical assessment of saturation may be complicated by the variation in substitution rates among sites in data. For example, in the mitochondrial genome, transitions generally occur at a higher rate than transversions (Brown et al., 1979; DeSalle et al., 1987; Moritz et al., 1987; Miyamoto & Boyle, 1989). For protein coding genes, rates of substitution vary at each of the three codon positions (DeSalle et al., 1987; Arctander, 1991; Irwin et al., 1991; Kornegay, 1993; Graybeal, 1993; Hackett, 1996). This variation may cause differences in time to saturation among these sites. More detailed saturation graphs can be drawn to accommodate these differences by partitioning sequences into subsets of transitions or transversions, or subsets based on codon position. Pairwise sequence differences of these subsets of data can then be plotted.

Although saturation plots are easy to produce, the potential for saturation in data is generally not examined before phylogenetic analysis. Instead, saturation is assumed based on age or sequence divergence of the taxa (Milinkovitch et al., 1994; Janczewski et al., 1995; see Reeder, 1995). Current problems in recovering phylogenies, however, may require the explicit assessment of potential noise in a data set, and compensation for that noise (Hillis et al., 1993).

I assessed saturation in sequences of cytochrome *b*. Saturated partitions of data were then differentially weighted before phylogenetic inference. Results were compared to phylogenetic hypotheses inferred from equally weighted data. The cytochrome *b* sequences were one of two data sets collected to infer a phylogeny for species within the avian family Falconidae (falcons and caracaras). The use of two different data sets allowed me to consider two additional issues in phylogenetic inference. First, are molecular or

morphological data more reliable for phylogenetic analysis (Donoghue & Sanderson, 1992; Hillis, 1987). Second, what is the appropriate treatment of multiple data sets in phylogenetic analysis, separate or combined analysis (Kluge, 1989; Bull et al., 1993; Huelsenbeck et al., 1996)?

MATERIALS AND METHODS

TAXON SAMPLING

Fifteen species of Falconidae were examined (Table 1), including representatives from all 10 currently recognized genera (Stresemann & Amadon, 1979). Taxa were sampled in an attempt to match the taxa in the morphological analysis (Griffiths, 1994b). However, tissue from one species, the White-rumped Pygmy Falcon (*Polihierax insignis*), was not available. The Sharp-shinned Hawk (*Accipiter striatus*), a species from Accipitridae, the sister taxon to the Falconidae (Griffiths, 1994a), was used to root the tree. In addition, sequence from a distant outgroup, chicken (*Gallus* : Desjardins & Morais, 1990), was used for the saturation analysis.

Samples were obtained from tissue collections at the American Museum of Natural History (AMNH), the Louisiana State University Museum of Natural Science (LSUMNS), the Philadelphia Academy of Natural Science (ANSP), the National Museum of Natural History (USNM), the Zoological Museum, University of Copenhagen (ZMUC), and the Museum National d'Histoire Naturelle (MNDN).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

A total nucleic acid preparation was made from 0.1 gram or less of liver or muscle tissue using a 5% chelex solution (Walsh et al., 1991). Four pairs of

primers (Table 2) were used to amplify and sequence overlapping regions of both strands of the entire cytochrome *b* gene. Double stranded template was generated for asymmetric PCR in 10 ul. capillary tubes, using a 1605 Air Thermo-Cycler™ (Idaho Technology). General protocol used was 1 second at 94°, 0 seconds at 46° and 15 seconds at 72°, running for 38 cycles.

Double stranded DNA was recovered from agarose gels, diluted to 200 ul., and used to produce single stranded template through the unbalanced primer method (Gyllensten & Erlich, 1988) in a Perkin Elmer Cetus Thermocycler. Reactions were run for 38 cycles: 1 minute at 94°, 1 minute at 52° and 55° at 72°, with the limiting primer at a 1/50 dilution. Single stranded DNA was cleaned by washing three times with ultrapure water through Ultrafree-MC 30,000 NNMWL™ filters (Millipore Corp., Bedford, MA) and concentrated to a final solution of 12-15 ul. Seven ul. of this solution was sequenced using T7 DNA polymerase (Sequenase™ version 2.0, United States Biochemical, Cleveland OH).

Alternatively, double stranded DNA for automated sequencing was generated in 50 ul. solutions using an Idaho Technology Rapidcycler for 40 cycles: 8 seconds at 94°, 8 seconds at 55° and 24 seconds at 72° degrees. The double stranded DNA template was purified using Geneclean II (Bio 101 Inc., Vista, CA) and resuspended with 18 ul. of ultra pure water. Two ul. were used as template for cycle sequencing in a Perkin Elmer GeneAmp PCR System 9600, using a Prism™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit. Protocol for the 6 ul. reactions was: preheating for one minute at 95° , then 35 cycles at 95° for 15 seconds, 50° for 15 seconds and 60° for four minutes. The product was purified through Centri-Sep™ columns (Princeton Separations, Adelphia, N. J.), resuspended in 2.2 ul. of a 6 to 1 solution of formamide-

EDTA. Two ul. of the sequenced product were loaded into a 6% acrylamide and analyzed in an ABI Model 373 DNA sequencer.

DATA ANALYSIS

MORPHOLOGICAL DATA

An additional syringeal specimen of the Laughing Falcon recently became available. Because the syrinx used to code characters for the original analysis (Griffiths, 1994b) had been poorly preserved, this new specimen was examined and newly obtained information used to recode the data matrix. A phylogenetic analysis was performed with the re-analyzed data.

MOLECULAR DATA

a Priori Character Analysis -Saturation of Substitutions

Before phylogenetic analysis, sequences were assessed for potential noise caused by saturation of substitutions. Sequences were divided into three subsets corresponding to each of the codon positions. Within each codon position, the numbers of transition or transversion differences for pairs of taxa were plotted against an estimate of time of divergence of the taxa. Divergence time was estimated because actual or even approximate dates of divergence of these taxa are not available. Three time estimates were used: total uncorrected pairwise sequence distances, and two transformations of total distance: the Kimura two-parameter distance (Kimura, 1980) and the log determinant transformed distance (Lockhart et al., 1994). Since all three distances yielded the same approximate shapes, only graphs plotted against total sequence divergence are shown. A program developed by G. Barrowclough was used to calculate all pairwise divergences.

Because saturation is assessed before phylogenetic analysis, relationships within the ingroup are not known and outgroup comparisons are necessary to assess saturation. For unsaturated data, the sequence differences in comparisons to outgroup species will be greater than differences within the ingroup (the curve increases monotonically). For saturated data, outgroup comparisons stay at the same level, or decrease. This is of particular importance if species within the ingroup are clustered into several clades, and divergence within clades is large. Some comparisons within clades may overlap comparisons between clades, and points of the graph will scatter. Determining the shape of the curve fitting those points can be difficult unless outgroup comparisons are included. In this analysis, two outgroup species were included, the Sharp-shinned Hawk, a closely related species, and Chicken, a more distant outgroup.

Two additional factors may lead to empirical graphs that depart from the theoretical expectation. (1) Differential rates of silent and replacement substitutions may cause third position transitions to accumulate rapidly and appear to reach a maximum level of divergence. As transversions start accumulating at these positions, however, the proportion of third position transitions may then decrease. A graphical representation of this phenomenon would be a curve which peaks and then descends, rather then a curve which asymptotes. (2) Constraint on variation within a partition of data may limit the number of sites free to vary and, thus, the amount of possible divergence. The small number of differing sites may inflate the effect of sampling error (Swofford et al., 1996), and cause the increase in the graph to be too small to interpret.

Phylogenetic Analyses

Two sets of phylogenetic analyses were performed to assess the results of different assumptions about character weighting: (1) equal weighing and *a posteriori* differential weighting (successive weighting; Carpenter, 1988; Farris, 1989) of molecular data (alone and combined with the morphological data), and (2) *a priori* differential weighting of molecular data (alone and combined).

All phylogenetic analyses were performed employing the heuristic algorithm in PAUP 3.1.1 (Swofford, 1993). To avoid finding local optima, each analysis consisted of 100 replicates varying the order in which taxa were added.

Equal Weighting and a Posteriori Differential Weighting

The analysis using equally weighted molecular data was performed to provide an hypothesis of relationships, and a measure of character congruence, under the assumption that variation in rates of substitution among sites does not compromise phylogenetic inference. The tree obtained from this analysis was also used to provide a topology for calculating indices assessing the reliability of characters in the six different data partitions used in the saturation analysis. Transitions and transversions in each of the three codon positions were mapped onto this tree, and the consistency, retention and rescaled consistency indices were calculated using MacClade (Maddison & Maddison, 1992).

The maximum value of the rescaled consistency index (Farris, 1989) for each character was then used to reweight the characters for a second phylogenetic analysis. This was run to assess the results of *a posteriori* weighting (Carpenter, 1988), to determine if giving more weight to the most consistent characters would filter the noise in the molecular data.

Data sets were then combined and analyses repeated to determine if combining data sets, without differentially weighting molecular data, would be sufficient to allow the weaker phylogenetic signal in the separate data sets to overcome the noise (Barrett et al., 1991).

a Priori Differential Weighting

Information from the saturation analysis was used to develop two weighting protocols, implemented as stepmatrices in PAUP. In both, saturated data partitions were given a weight of zero. In the first analysis, all the remaining data partitions (those that were not clearly saturated) were given the same weight. In the second, the partitions that best fit the model of unsaturated data were given a higher weight then those partitions whose graphs were ambiguous to interpret.

A final analysis was run using the combined data sets, with the molecular data differentially weighted.

Assessment of Phylogenetic Hypotheses

Different methods exist to assess the reliability of a phylogenetic hypothesis (reviewed in Hillis, 1995). A data set can be examined for hierarchical signal through skewness of the distribution of tree lengths (Hillis & Huelsenbeck, 1992) or randomization methods (e.g., Archie, 1989; Faith & Cranston, 1991). Congruence of characters within a data set can be measured (Kluge & Farris, 1969; Farris, 1989). Support for nodes in a particular hypothesis can be assessed through resampling methods (e. g., bootstrapping; Felsenstein, 1986) or decay indices (Bremer, 1988).

Interpretation of the results of any of these methods must be done with caution; these tests are often misapplied (Hillis, 1995). For example,

bootstrapping is an attempt to generate a representation of sampling error within the data set. Done correctly, the sampling error generated should mimic the variation in the population being sampled. However, bootstrapping either requires that characters are independent and identically distributed, or that characters are randomly sampled (see Sanderson, 1995 for a thorough critique of bootstrapping). Neither assumption is met for sequence data.

Although there are tests to assess heterogeneity of multiple data sets (Farris, 1995; Huelsenbeck & Bull, 1996), there are no tests that evaluate the accuracy of hypotheses developed from different character sets. There is, however, the idea that the level of congruence of hypotheses from different data sets is a measure of the accuracy of those hypotheses (Nelson, 1979; Swofford, 1991; Miyamoto & Cracraft, 1991; Lockhart et al., 1994; Adkins & Honeycutt, 1994; Hillis, 1995; Miyamoto and Fitch, 1995). For this research, there were three different data sets from which hypotheses of relationships were inferred, osteological data (Becker, 1987), syringeal morphological data (Griffiths, 1994b), and molecular sequence data. Thus, I used congruence as the criterion to assess the hypotheses resulting from the different weighting protocols.

a Posteriori Character Analysis

Phylogenetic hypotheses inferred from the two different data sets were examined to determine relative support of nodes within each data set. This was done by examining unique synapomorphies in the morphological data set, and by determining decay indices (Bremer, 1988) for the molecular data. In addition, molecular characters were mapped onto the tree and patterns of change by codon position were examined.

RESULTS

MORPHOLOGICAL DATA

New information obtained from the examination of the Laughing Falcon syrinx allowed the recoding of two characters. Character 16, the modification of the ends of the B1 element, changed from one to zero. Character 3, dorsal fusion of the tympanum, changed from one to two. In addition, three characters (characters 5, 6 and 7), originally coded as binary representations of alternative character states, were recoded as one unordered multistate character. The data matrix of characters describing the variation in syringeal morphology (Griffiths, 1994b) was used with those modifications. The final data matrix (Appendix I) consisted of 23 characters, five of which were multistate.

Phylogenetic analysis of these data resulted in 54 most parsimonious trees, with a consistency index (C.I.) of 0.59, a rescaled consistency index (R.C.) of 0.49, and a retention index (R.I.) of 0.81. The strict consensus tree is illustrated in Figure 2. There are two major clades, the caracaras and a clade composed of the *Falco* species, the two small falconets (*Microhierax* and *Polihierax*) and the Spot-winged Falconet. The remaining two genera, the Laughing Falcon and Forest Falcons, form a dichotomy basal to these two clades. These results are consistent with the phylogeny inferred from osteology (Fig. 3). This phylogeny also demonstrates that the genus *Polihierax* is not monophyletic; the two species within that genus are not sister taxa.

MOLECULAR DATA

a Priori Character Analysis - Saturation of Substitutions

Graphical displays of saturation for the six partitions of sequence data are shown in Figure 4. For three of the six partitions, the assessment of saturation can be determined unambiguously. Third position transversions (Fig. 4F) are

unsaturated. Pairwise comparisons of these transversions show a monotonic increase, and differences between ingroup taxa are less than differences in comparisons to the outgroups.

In contrast, first and third position transitions (Figs. 4A & 4C) are saturated. Most of the pairwise differences between ingroup taxa are as great as, or greater than, differences to the outgroups. In addition, comparisons to the more distant outgroup, Chicken, are less than or equal to comparisons to the closer outgroup. Third position transition differences between falconid species and Chicken range from 12% to about 21%, and from 15% to about 23% in comparisons to the Sharp-shinned Hawk. Species within the Falconidae begin to accumulate this level of difference of third position transitions at about 9-10% total sequence divergence, a result demonstrated for other avian species (Hackett, 1996).

The interpretation of saturation for the other three partitions is not straightforward. For second position transitions and transversions (Fig. 4B and 4E), the increase in divergence over time may be too small for saturation to be interpreted unambiguously. However, differences in most of the ingroup comparisons of second position transitions are less than comparisons to the outgroups.

Finally, pairwise differences in first position transversions within the Falconidae do not show a clear monotonic increase (Fig. 4D), which may also be an effect of small sample size. However, these may be considered unsaturated because all comparisons to outgroup taxa are greater than comparisons within the ingroup.

Phylogenetic Analyses

Equal Weighting of Molecular Data

Assumptions used for the phylogenetic analyses, and results of the analyses are summarized in Table 3. Complete cytochrome *b* sequences, and Genbank accession numbers for the species included in the analysis are illustrated in Appendix II. Of the 1143 characters in the matrix, 328 were phylogenetically informative.

Two most parsimonious trees was inferred using equally weighted data, with length of 1044, C.I. of 0.46, and RI of 0.44. The strict consensus tree is illustrated in Figure 5. Two results are of interest. The Spot-winged Falconet is sister taxon to the caracaras, a result incongruent with the morphological analyses. A more surprising result is that the Forest Falcon genus is not monophyletic. Although two other falconid genera have been found to be polyphyletic (Griffiths, 1994b), the divergence of the two species within each of those genera had been recognized previously (Brown & Amadon, 1968). Similar divergence among the species within the Forest Falcons has not been indicated (e.g., Brown & Amadon, 1968).

This phylogeny was used to assess the congruence of characters within the six partitions used in the saturation analysis. Indices measuring the congruence of each subset of data are shown in Table 4. As noted in the Methods sections, comparisons of the indices for the different partitions should be done with caution. For example, the number of characters within each of the partitions differs and the C.I. may be sensitive to number of characters. Comparisons of the R.I. may be more appropriate; however, the R.I. is inflated by the presence of autapomorphic character states in multistate characters (Naylor & Kraus, 1995). For the three partitions of transversions, this bias was eliminated by recoding multistate characters as two state characters (purines or

pyrimidines), removing any autapomorphic transition character states. Thus, any bias will inflate the R.I. of transitions only.

For third position transitions, the R.I. is 0.33; for first position transitions, the R.I. is 0.50. The R.I.'s for all other partitions are greater than 0.60. This indication of greater homoplasy in third and first position transitions further corroborates the results of the saturation assessment for those two data partitions.

The combined analysis using equally weighted molecular data produced five most parsimonious trees with a length of 1238, C.I. of 0.46, R.I. of 0.45, and R.C. of 0.24. The strict consensus tree of these five (not shown) indicates support for only three clades; the *Falco* species, the caracara species and the 'falconet' clade. There is no resolution of relationships among these clades. Successive weighting of these data produced one tree with the same basic topology as the tree inferred from equally weighted molecular data (Fig. 5), differing only in the greater resolution of species within the genus *Falco*.

a Priori Differential Weighting

Based on the assessment of saturation, two step-matrices were developed to differentially weight characters. In both, the saturated partitions (first and third transitions) were given a weight of zero. Because the interpretation of saturation was equivocal in both graphs of second position changes, these were all given a weight of one, as were first position transversions. The two step-matrices differed in the weight given to third position transversions. These were weighted one in the first step-matrix. Step-matrix two assigned a weight of two to third position transversions, reflecting the assessment of highest signal to noise ratio in that partition.

The first analysis, weighting saturated partitions zero and all others one, produced six most parsimonious trees, with two basic topologies; four with topology A and two with topology B (Fig. 7). Multiple trees within those topologies reflect differences in the resolution of *Falco* species and of three of the five caracara species.

The second analysis, differing from the first in the weight given to third position transversions, produced six most parsimonious trees, all with topology A. As with the first analysis, multiple trees reflect differences in the resolution of *Falco* and caracara species.

These two topologies are congruent in the recognition of four monophyletic clades: (1) the four species of *Falco*, (2) the five caracara species, (3) a falconet clade, (*Polihierax semitorquatus* and *Microhierax erythrogonys*), and, (4) the Laughing Falcon and Forest Falcons. In addition, both analyses place clade 4 basal to all other clades. Differences arise in the connection of clade 4 to the others (Fig. 8). This basal clade connects to the branch leading to the caracaras in topology A, and to the branch leading to the falconets in topology B.

As discussed in the Methods section, congruence with existing hypotheses was the criterion used to choose between the two different weighting procotols. The analysis using step-matrix two produced trees with topology A. These trees are congruent with trees inferred from two morphological analyses (Figs. 2 & 3), differing only in resolving the polytomy with the Forest Falcons and Laughing Falcon.

Because the phylogenies inferred from morphological and differentially weighted molecular data were congruent, the data sets were combined for the final analysis. Step-matrix two was used to weight the molecular data. This produced four most parsimonious trees, all with topology A. Differences

among these four trees were in the resolution of relationships within the four *Falco* species, and within three of the five caracara species.

a POSTERIORI CHARACTER ANALYSIS

Patterns Of Divergence by Codon Position

Within this avian family, maximum divergence between pairs of species reaches 17%. In comparisons to the outgroup, divergence increases to 19% (Table 5). When pairwise divergence is examined by codon position, variation follows expected patterns. Divergence is least at second positions (0 to 4% within the falconids, and 3 to 4% in comparisons to Sharp-shinned Hawk), and increases at first positions (1 to 10% within falconids, 8 to 12 % compared to Sharp-shinned Hawk).

Third positions show the greatest divergence (8 to 40%). When this is broken down into transitions and transversions, the pattern of change is consistent with the pattern of saturation. Thus, pairwise divergence of third position transitions decreases from a maximum of 25% among falconids, to 23% in comparisons to Sharp-shinned Hawk, while transversion differences increase from a maximum of 16% within the Falconidae, to 22% in comparisons to Sharp-shinned Hawk.

However, these figures underestimate the proportion of sites that have changed. Among species within the closely related *Falco* and caracara clades, 35% of the 381 third position codon sites vary. At the familial level, 86% of the third position sites have mutations that can be observed. This observed rate of increase in variable sites (35 % to 86%) can be used to obtain a rough estimate of the amount of noise, in the form of unobserved mutations, in third position transitions. Maximum pairwise divergence of these transitions within the *Falco* species is 19%. This should increase to an expected divergence of 45% at

the familial level. The maximum observed divergence is only 22%, half that expected.

Character Support and Conflict at Nodes

Morphological data strongly support the clade of caracara species (3 unique synapomorphies) and the 'Falconet' clade (3 unique synapomorphies). No other nodes have as much support. The inclusion of *Polihierax insignis* in the morphological analysis, and its position as sister taxon to the clade including the Spot-winged Falconet, the Falco species and the Falconet clade (Fig. 2), strengthens the support for the Spot-winged Falconet within that clade.

Differentially weighted molecular data strongly support six clades on the tree (decay indices of 7, Fig. 7A). The position of the Spot-winged Falconet has weak support. It moves from the Falco clade to the caracara clade in trees one step longer, and then back to the Falco clade in trees an additional one to four steps longer. Because tissue was unavailable for that species, *Polihierax insignis* was not included in the molecular analysis. Thus, its effect on the position of the Spot-winged Falconet could not be determined.

Finally, the Laughing Falcon is also strongly supported as sister taxon to the Forest Falcons in the molecular tree. The relationships of these taxa are unresolved in the morphological tree.

Correlation of Change at Codon Positions

When molecular characters are mapped onto the tree, changes by codon position can be traced along lineages. In this analysis, there were 18 informative second position characters. For eleven of these sites, the first position of that codon changes also. For six of these sites, 14915, 14981, 15530, 15590, 15602, and 15974 these changes occur at the same node. In addition, for

positions 15601 and 15602, the first and second position change (CTC to TAC) is dependent on a previous third position change from A to C at the node leading to the Falconidae (Fig. 7). If that third position transversion had not occurred, then the CTC to TAA change would have replaced leucine with a stop codon.

DISCUSSION

A Priori Analysis and Differential Weighting of Characters

Hypotheses of phylogenetic relationships are dependent on the characters, the hypotheses of synapomorphy, used to derive those phylogenies (Neff, 1986). Systematists collecting morphological data organize and distill the variation they observe to postulate these hypotheses (Novacek & Wheeler, 1992). *A priori* character analysis, while not explicitly described, is an essential part of morphological systematics.

Molecular systematists lack this *a priori* analysis (Brower & DeSalle, 1994). However, problems in recovering phylogenies from molecular data (e.g., Cummings et al., 1995; Honeycutt et al., 1995) point to the need for some assessment, before phylogenetic inference, of the reliability of the molecular characters used as synapomorphies (Hillis et al., 1993). For molecular sequences, noise can be introduced in the form of multiple hits and subsequent saturation of sequence data (Brown, 1983). The introduction of noise is further complicated by variation in the substitution rate at sites in sequences; i.e., at the three different codon positions for protein coding genes. The graphical determination of saturation is a simple approach to assessing the reliability, or noise, in molecular characters.

When sequence data are partitioned by codon position, saturation analyses can pinpoint the potential for noise. Analysis of saturation in cytochrome *b* sequences of the Falconidae demonstrates that multiple hits are

obscuring the signal in third and first position transitions, while third position transversions appear to be unaffected. In an analysis with equally weighted characters, the saturated data partitions have distinctly lower retention indices than the other partitions. Filtering the noise in these data partitions through differential weighting produces a phylogenetic hypothesis congruent with two morphological phylogenies.

The use of *a priori* differential weighting is controversial. As thorough reviews of this topic have been published (Brower & DeSalle, 1994; Simon et al., 1994; Swofford et al., 1996), I will discuss only those issues that are directly related to this analysis. One of the strongest criticisms is that equal weighting requires fewer assumptions, whereas differential weighting requires assumptions that may be ad hoc (Brower & DeSalle, 1994). The basis for differential weighting used in this analysis was not ad hoc but based on general knowledge of mitochondrial DNA, i.e., that transitions occur more readily than transversions (Brown et al., 1982; Moritz et al., 1987; Miyamoto & Boyle, 1989), and that rates vary among codon positions (Arctander, 1991; Irwin et al., 1991; Kornegay, 1993). To use equal weights, an implicit assumption required by these data is that conditions leading to inconsistent estimates have not occurred (Swofford et al., 1996). For this study, inconsistent estimates occur because phylogenetic signal is compromised by variation in substitution rates among sites in the sequences. Analyzing data without weighting that accounts for this variation may be unjustified (Novacek and Wheeler, 1992).

A corollary to the concern about assumptions required by weighting is the criticism that the data may not adhere to those assumptions. Thus, characters may be weighted based on assumptions of saturation rather than an explicit demonstration (e.g., Milinkovitch et al., 1994; Janczewski et al., 1995).

The use of saturation plots, as in this analysis, is one method of explicitly demonstrating that the data meet the assumptions.

An additional criticism is that partitioning data to determine which sites to weight may be arbitrary or difficult to determine (Eernisse & Kluge, 1993; Brower & DeSalle, 1994; Chippindale & Wiens, 1994). This analysis partitioned data based on the three codon positions of protein coding genes, and on rate differences in transitions and transversions, and these are neither arbitrary (Hillis et al., 1993; Brower & DeSalle, 1994) nor difficult to implement.

Finally, the problems of translating observed patterns into *a priori* weighting schemes have been noted (Donoghue & Sanderson, 1992). For example, weighting has been based on ratios of observed transitions and transversions, or on the expectation of transitions and transversions for randomized data. Weights used in this analysis were conservative and within the range suggested by analytical and empirical studies (Holmquist, 1983; Adachi & Hasegawa, 1985). There is, however, an element of subjectivity to any weighting scheme. I used congruence with other hypothesis to assess and choose between the alternative weighting protocols. More research needs to be done in this area.

Total Evidence Or Separate Analysis of Different Data Sets

One of the most contentious issues in systematics is the treatment of different data sets. Should data sets be combined for analysis or analyzed separately? Alternative treatments range from (1) separate analysis initially, then combining data for a final analysis (Wiens & Chippindale, 1994), (2) separate analysis initially, then combining data if initial analyses are not significantly different (Bull et al., 1993; De Quieroz, 1993), (3) combining data initially, the 'total evidence' approach (Kluge, 1989; Brower & DeSalle, 1994).

As with the weighting issue, the 'total evidence' arguments have been reviewed recently (Miyamoto & Fitch, 1995; Huelsenbeck et al., 1996); thus, I will discuss only those issues directly related to this analysis. For these data, the analysis of DNA sequence data alone gave insight into variation in substitution rates among sites in cytochrome *b* sequences, and illustrated potential problems in recovering phylogenetic signal. Separate analysis of each data set facilitated the comparison of support for nodes in the analyses, and the incongruities between data sets. Finally, separate analysis allowed the examination of possible correlation of character change within each data set.

Separate analysis of multiple data sets and the use of congruence is an effective method to assess the hypotheses inferred from the different data sets (Miyamoto & Fitch, 1995). Given substantial congruence, the best estimate of a phylogeny is then produced by an analysis combining all the data. Because the phylogenies produced by the data sets in this study did not differ significantly, the decision to combine data for the final analysis was trivial. Given significant conflict, on the other hand, is an indication that at least one of the data sets does not reflect phylogenetic signal, and combination of data sets under those conditions is unwarranted. However, determining whether conflict is significant is not straightforward. As noted above (Fig. 7), significant differences among topologies can result from a difference in rooting. Tests of heterogeneity among data sets (Farris et al., 1996; Huelsenbeck et al., 1996) can be used to quantify conflict, but these should be used only after detailed examination of the alternative phylogenies.

Molecules vs. Morphology

In these analyses, molecular and morphological data differ in their support at two nodes. Molecular data support the sister taxa relationship of the

Laughing Falcon and Forest Falcons, which is not resolved by morphological data. On the other hand, the position of the Spot-winged Falconet within the *Falco* clade is more strongly supported by morphological data.

Both data sets have more characters supporting terminal nodes than basal nodes. Problems arise in the long branches connecting the monotypic taxa (Laughing Falcon and Spot-winged Falconet). It is possible that more sequence data would increase the signal at these nodes. Alternatively, additional sequence data may not resolve these branches, if these additional data have the same patterns of noise and signal as the present data (Brun et al., 1995). Because the Laughing Falcon and Spot-winged Falconet are monotypic taxa, increased taxa sampling within the family would probably not resolve the problem.

There is no *a priori* reason to presume that either molecular or morphological data are better for phylogenetic inference. In this analysis, both sets of data had similar problems; convergence, correlation of character change, and monotypic, divergent taxa whose relationships are not resolved with strong support. Both are valuable, and complementary, for deriving a hypothesis of relationships within the Falconidae.

APPENDIX 1. Data matrix of 23 syringeal morphological characters for 23 Falconidae and outgroup species

										1									2			
Taxa	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3
Accipiter striatus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Daptrius americanus	1	0	0	1	3	1	0	0	0	1	0	0	0	0	1	0	1	0	2	0	1	1	1
D. ater	1	0	0	1	3	1	0	1	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0
Falco berigora	1	0	2	0	2	2	1	0	0	0	0	0	0	1	0	2	1	0	2	0	1	1	0
F. biarmicus	1	0	1	0	2	2	0	0	0	0	0	0	0	1	0	2	1	1	0	0	1	1	0
F. cenchroides	1	0	1	0	2	2	1	0	0	0	0	0	0	1	0	1	1	0	2	0	1	1	0
F. columbarius	1	0	1	0	2	2	0	0	0	0	0	0	0	1	0	2	1	0	2	0	1	1	0
F. femoralis	1	0	1	0	2	2	0	0	0	0	1	0	0	1	0	2	1	0	2	0	1	1	0
F. mexicanus	1	0	1	0	2	2	0	0	0	0	0	0	0	1	0	1	1	1	1	0	1	1	0
F. peregrinus	1	0	1	0	2	2	0	0	0	0	0	0	0	1	0	2	1	1	0	0	1	1	0
F. rufigularis	1	0	1	0	2	2	0	0	0	0	1	0	0	1	0	2	1	0	2	0	1	1	0
F. sparverius	1	0	1	0	2	2	1	0	0	0	0	0	0	1	0	1	1	1	0	0	1	1	0
Herpetotheres cachinnans	1	0	2	0	2	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0
Micrastur gilvicollis	1	1	0	0	1	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0
M. semitorquatus	1	1	0	0	1	2	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1
Microhierax erythrogonys	1	0	2	0	2	3	1	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0
Milvago chimachima	1	0	0	1	3	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1	0
M. chimango	1	0	0	1	3	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1	0
Phalcoboenus australis	1	0	0	1	3	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1	0
Polihierax semitorquatus	1	0	1	0	2	3	0	0	0	0	0	0	1	0	0	0	1	0	2	0	1	1	0
P. insignis	1	1	0	0	2	1	0	0	0	0	0	0	0	1	0	1	1	0	2	0	1	0	1
Polyborus plancus	1	0	0	2	3	1	0	1	0	1	0	0	0	0	1	0	1	0	2	0	1	1	1
Spiziapteryx circumcinctus	1	0	2	0	2	1	0	0	0	0	0	0	0	1	0	0	1	0	2	0	1	1	0

APPENDIX 2. Cytochrome *b* Sequences

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                          10        20        30        40        50        60
Daptrius americanus       ATGGCCCCCAACATTCGAAAGTCACACCCCCTATTAAAAATAGTCAACAACTCCCTAATT
Daptrius ater             ..............C.....A.....................A.T..............
Falco femoralis           .....A.....T........A........T...A........A..............
Falco peregrinus          .....A.............A........TT..G........A....T...........
Falco sparverius          .....A.............A............A........A..............
Falco verpertinus         .....A...........G..A............A........A....T.....T.....C
Herpetotheres             ............C.C.....A..G........CC.........................
Micrastur semitorquatus   ........A..TC.......A...........C.........A.T.....T........C
Micrastur gilvicollis     ..............A.....A...........CC.........................
Microhierax               ..............A..G..ACAC...................A.T..............
Milvago                   ..............C.....A...........G.........A.T..............
Phalcoboenus              ..............C.....A.........................T..T...........
Polihierax                ..............C.....ACAC..T..A..............T..............C
Polyborus                 ...........T..C.....A..C.........C.........................
Spiziapteryx              ..............C..G..A...........G.........A..............C
Accipiter                 ..............C.....A..T.....A...C........A..............

                          70        80        90        100       110       120
Daptrius americanus       GATCTTCCTACCCCATCAAACATCTCTGCCTGATGAAACTTTGGATCTCTGCTAGGAATT
Daptrius ater             ..C..A..C.....C.................................G..A..G..T..C
Falco femoralis           ..C..C........CC.C.....T..CA.A...........C.....C..A........C
Falco peregrinus          ..C.....C..T...C.C........CATT.................C..A........C
Falco sparverius          ..C..C..C......C.C........CATA...........C.....CT.A......G.C
Falco verpertinus         ..C..C..C......C.C........CATA.........T..C.....C..A........C
Herpetotheres             ..C.....C..T..CC..........A.....G..G...........C..A..G..C...
Micrastur semitorquatus   ..C..C........C...........A..............C..G..A..A.....C..C
Micrastur gilvicollis     ..C..C..C.....T..C........A..............C.....A..C.....T..C
Microhierax               ..C..C..A..T..T..C........C..A.........T..C........A........C
Milvago                   ..C..A..C.....C.................................G..A..G..T...
Phalcoboenus              ..C..A..C.....C..............T..................A..A.....T...
Polihierax                ...T.A..C.....C..T........CA.A.....G..T..C..G......T....G..C
Polyborus                 ..C.....C.....C.....T.....C..............C......T.A.....T..C
Spiziapteryx              ..C..A..C.....C..C........A....................CT.......C...
Accipiter                 ..C..G..A..T..TC..........CATT........T..C.....CT.A........C

                          130       140       150       160       170       180
Daptrius americanus       TGCCTCATAACCCAAATCCTAACAGGCCTACTACTAGCCATACACTACACCGCAGACACG
Daptrius ater             ............................G.............................A
Falco femoralis           .....TGCC..T...........C.........................A........A
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Falco peregrinus          ..T..AGCC.............T......T.G.................A..G..T..A
Falco sparverius          ..T..AGCC.............T............................A........A
Falco verpertinus         ..T..AGCC.............C............................A........A
Herpetotheres             ..T..A..C..A..........................T.....................A
Micrastur semitorquatus   .....A.CC.TA.................C.....T..T........T..T........A
Micrastur gilvicollis     .....AGCC..A.................T.....................T........A
Microhierax               .....A.CC........T.....................TGC......T..A.....T..A
Milvago                   ......C.......................G.............................A
Phalcoboenus              ............................G.............................A
Polihierax                .....G.CC.................T.....T....T.....T.....A..C.....A
Polyborus                 .....TG.........................T.................T........A
Spiziapteryx              .....TGC.....................T..C.................A........A

                                   190         200         210         220         230         240
Daptrius americanus       AACCTAGCCTTCTCGTCAGTCTCCCACACATGCCGAAACGTACAATACGGCTGACTAATC
Daptrius ater             ..............A.....................G.................T....T
Falco femoralis           .C...G........A..C...G..........T........G........A.........
Falco peregrinus          .C.........T..A..C...G..........T.................A.........
Falco sparverius          .CA..G........A..T..TG.....................G..G.....A..GT.G...
Falco verpertinus         .CA...........A..C..TG..........T.................A.........
Herpetotheres             .C............A..C..AG.....................C..................
Micrastur semitorquatus   .C.T.............T...G.T............G.......G................
Micrastur gilvicollis     .C............A..C...G..........T..........................
Microhierax               .CA...........C..C..TG.................T..C.....T..T.........
Milvago                   ..............A.....................................T....T
Phalcoboenus              ..............A.....................................T.G..T
Polihierax                .CGT..........C..C..TG.T..T..............C........A........T
Polyborus                 ..............A............T..............................
Spiziapteryx              ..............A......G....T..T........T..................T...
Accipiter                 TC............A..C..AG................?.....................

                                   250         260         270         280         290         300
Daptrius americanus       CGCAATCTCCACGCCAACGGAGCCTCACTTTTCTTCATCTGCATCTACCTGCACATCGGA
Daptrius ater             .....C..A.................G..............T..T...T.A.........
Falco femoralis           .....C..A..T...........A..C..A.....T........T...A.A.........
Falco peregrinus          .....C..A..T...........A..CT.A...........T......A.A.....T..G
Falco sparverius          ..T..C..A..T...........A.....A..............T...A.A.........
Falco verpertinus         .....C..A..............A..C..A...........T......A.A........C
Herpetotheres             .....CT.A..T..............T..A...........TG.A..T..C........C
Micrastur semitorquatus   .....C..A..T..T..T........C..A..T..T..T..TG.T.....T..T......
Micrastur gilvicollis     .....C..A.....................A............G.G...T.C.........
Microhierax               ..A..C...........T.........T.A.................T..C.....T..T
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Milvago                  .....C..A.....................C..........T......T.A.........
Phalcoboenus             .....C..A.....................................T......T.A.........
Polihierax               .....C..A..T.................A.....T.............A.........C
Polyborus                .........A.....................................................A...........
Spiziapteryx             .....C..A..T..............T.C.....................A..T..T...
Accipiter                .....C..A.....T...........CT.C..T.....T..T........A.........C

                                    310        320        330        340        350        360
Daptrius americanus      CGAGGCATCTACTATGGCTCCTACCTATACAAGGAAACCTGAAACACAGGTATCATCCTT
Daptrius ater            ........T..T..C..........G.....A.................C..T.....C
Falco femoralis          ..............C.................A...........T.....C.........C
Falco peregrinus         ........T.....C.....T..TT.G..T..A........G..T.....C.........
Falco sparverius         .....A........C.....T..TT.......A.................A.........C
Falco verpertinus        .....A........C..........G.....A.................A..T.....A
Herpetotheres            ......C..................G..T..A.................C..T.....C
Micrastur semitorquatus  ......C..........T........G.....A.................C.........C
Micrastur gilvicollis    .... .C.............T.....C.......................C..T.....C
Microhierax              .....A..T........T..T........T..A.................C.........C
Milvago                  ........T..T..C.....A.....G.....A.................C..T.....C
Phalcoboenus             ........T..T..C.................A.................C..T.....C
Polihierax               .....T.....................G.....A.................AG.T.....C
Polyborus                .....T.....................C.....A.................C.........C
Spiziapteryx             ............................C..T..A.................C.........C
Accipiter                .....AC.T.....C.....A...T....T..A.................A..T..T...

                                    370        380        390        400        410        420
Daptrius americanus      CTCCTCACCCTAATAGCAACAGCCTTCGTGGGCTACGTTCTACCCTGAGGACAAATATCT
Daptrius ater            ..A.....................T...........T..C..............G.....C
Falco femoralis          T.A.......................T..A..T..T..A.....................A
Falco peregrinus         ..A..............................A.....T..A.....................A
Falco sparverius         ..A..............C...........C.....T..GT.................G..A
Falco verpertinus        ..A.....T..G.................C.....T..A.....................A
Herpetotheres            ..A..............................A..T..T..AT.....................A
Micrastur semitorquatus  ..A.....AT.............T.....A..T...........A..............A
Micrastur gilvicollis    ..A.....A.....................A........C.....G........G.....A
Microhierax              ..A.....AT.................T..A.....T..C.....A..............A
Milvago                  ..A.....................T...........T..C.....................A
Phalcoboenus             ..A..T.................T........T..T..C.....................A
Polihierax               ..T..T..TT.....................A..T.....C.....A..............C
Polyborus                ..A..............G..............T..T..C.....................C
Spiziapteryx             T.A.....................T.....A........A.................G..A
Accipiter                ..A..A..T..C........T...........T..T..A.....A.....C........C
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                          430        440        450        460        470        480
Daptrius americanus       TTCTGAGGGGCCACAGTCATCACCAACCTATTCTCAGCCATCCCCTACATCGGCCAAACC
Daptrius ater             ........C..T.........................T.............T......
Falco femoralis           ..........T.....T.............T.....A.....A........T......
Falco peregrinus          ........A..........T..................A.....A..............
Falco sparverius          ........A..........T..................A.....A..T...........
Falco verpertinus         .....G..A.............T........T.....A.....A..............
Herpetotheres             ........A..T..C..........T.....T..G........T.....T........
Micrastur semitorquatus   ..........T.........................T........T...........
Micrastur gilvicollis     ........A.............................T...........T........
Microhierax               ........A..........T......T..............A..T..T..T......
Milvago                   ........C..T.........................T....................
Phalcoboenus              ........A..T.........................T...........T........
Polihierax                ....................T..T.....................T..T...........
Polyborus                 ........A..T..............T....T........T..................
Spiziapteryx              ....................................TG.T........T........
Accipiter                 ........A........A..T..................T..............G......

                          490        500        510        520        530        540
Daptrius americanus       CTCGTCGAATGGGCCTGAGGAGGCTTCTCCGTAGACAACCCGACATTAACCCGATTCTTC
Daptrius ater             A..........A........G..T.................A...C.............
Falco femoralis           ..A.....G..............A..T..A........T..A...C.G...........
Falco peregrinus          ..A...........T.....G..A..T..A...........A...C.G...........
Falco sparverius          ..A.....................A.....A...........A...C.......C......
Falco verpertinus         ..A.....................A..T..A.....T.....A...C.............
Herpetotheres             ..A..A..G..A........C........A...........T...C.............T
Micrastur semitorquatus   T.A..A.....A..T.....T..T.....A..G.....T..A...C.............
Micrastur gilvicollis     .....A.....A..T.....C........A...........A...C.............
Microhierax               ..A...........A........A.....A...........A...................
Milvago                   ...........A........G..T.................A..GC..............
Phalcoboenus              ...........A...........A..................A...C..............
Polihierax                T.A..A.....A........G..A.....A..C........A...C....T.....T..T
Polyborus                 ..T.............................G..T.....A...................
Spiziapteryx              A.T........A........G.....T..A.....T.....C...C..............
Accipiter                 A....A........A........A..............T..T..CC.C............

                          550        560        570        580        590        600
Daptrius americanus       GCCCTACACTTCCTACTACCATTTCTAATCGCAGGCCTCACTCTAATCCACCTAACCTTC
Daptrius ater             ............T.............................C...........G.....T
Falco femoralis           ..............C........C...........G.....CT..........C......
Falco peregrinus          ................T.....C...........A.....C...........C......
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Falco sparverius          ...T.........C........C...........G..T..CT.........C......
Falco verpertinus         ..........T..C........C...........G.....C.....T.....C......
Herpetotheres             ..T..........C.....C..C........C.................T..C......
Micrastur semitorquatus   .....T.....T..T.....C..C........TA.......C...........C......
Micrastur gilvicollis     ..............C........C........CA.......C.....T..T..C......
Microhierax               ..............C........CT.........G.....C..C.....T..C......
Milvago                   .....T......T....G.....C.................C...........G......
Phalcoboenus              ...........TT.............................C...........G......
Polihierax                ..............C........C.....T...A.......CA.C.....T..C.....T
Polyborus                 .................G.....C..............T..............G......
Spiziapteryx              ...........T..C........C.........A....A..C.....T.....C......
Accipiter                 .....G...........C.....................T..C...........C......

                                    610        620        630        640        650        660
Daptrius americanus       CTGCACGAATCCGGCTCAAACAACCCCCTAGGAATCACATCAAACTGTGACAAAATCCCA
Daptrius ater             ..A........A.................G.............................
Falco femoralis           ..A..T.....A..T.................................C..T.........
Falco peregrinus          ..A..T.....A........T.........................T..C...........
Falco sparverius          ..A..T.....A..T..C..............G.............................
Falco verpertinus         ..A........A..T.................................C...........
Herpetotheres             ..T..T...........T..T........G..T..TGT.........................
Micrastur semitorquatus   ..C........T..T.....................GTG..G...................
Micrastur gilvicollis     ..T...........T.................T..T.........................
Microhierax               ..C..T.....A..A.................T..TT....................T...
Milvago                   ..A........A.................G........G.....................
Phalcoboenus              ..A........A...................................................
Polihierax                ..C........A..A........T...........TT.........CC............
Polyborus                 ..A........A.................G..................C............
Spiziapteryx              ..C........A........T..............TT.......T..C............
Accipiter                 ..T........T..T...........A.........GTC..G...G..............

                                    670        680        690        700        710        720
Daptrius americanus       TTCCACCCCTACTTCTCCTCAAAAGATATCCTAGGATTCATACTCCTATACTTCCTACTA
Daptrius ater             ........................C..............T...................
Falco femoralis           .....T.....T.A...TCTC.....CC.................A.....C...CC...
Falco peregrinus          .........A....A...TCTC.....C...........T......A.....C.G.CC...
Falco sparverius          .............A...TCTC.....CC.......T..T..G...A..CT.C.G.CC...
Falco verpertinus         .....T.......A...TCT......CC.TT.....G........A.....C...CC...
Herpetotheres             ..T.....A.....T...CT......CC........GC.GCT...A..CT..C..CC...
Micrastur semitorquatus   ..T...........T....T......C..T......AC..C...TA..CT.C...CC..G
Micrastur gilvicollis     ...................T......C..........CG......A...T..C..CC...
Microhierax               ........A.........CTC.....CC........ACG..T...A...T..C..CC...
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Milvago                    ........................C...T.........................
Phalcoboenus               ........T................C........G.....................
Polihierax                 .....T..A.........CTC.....C..T........T......A.........C....
Polyborus                  .....T.....................................................
Spiziapteryx               ........A..T..T........G..CGC.T.G..G..T..C...A.....C...C...C
Accipiter                  .....T..G.........CT...G..C........C....C...AA..CTAC...C....

                                     730        740        750        760        770        780
Daptrius americanus        ACAACCTTAGCCCTACTCTCCCCCAACCTCCTAGGGGACCCAGAAAACTTCACCCCGGCA
Daptrius ater              .....TC...................T.....G..A...................A...
Falco femoralis            .T.............T..A.T........A.....A..............T..A..A...
Falco peregrinus           .T....C........T.TA....A.....G.....A..............T..A..A...
Falco sparverius           .T.G..C........T..A....A.....G.....A.................A..A..G
Falco verpertinus          .T....C........T.TA....A...T.A.....A.................A..A...
Herpetotheres              G...T.C.....T..T..A....A...T.......A...................A...
Micrastur semitorquatus    GT.G..C........T.TA.A..A............A..............T.....A...
Micrastur gilvicollis      G.....C........T.TA....A.....T.....A...................A...
Microhierax                .T...TC........T..A.T..............A..............T..A..A...
Milvago                    ......C.........................G..A...........T........A...
Phalcoboenus               ......C.C.....G........T........G..A...................A...
Polihierax                 .T.........T...T..A....A.....T.....A..T...........T.....A...
Polyborus                  ......C........T...................A...................A...
Spiziapteryx               CT...TC..A.....T..CT...A...........A.................A..A...
Accipiter                  ......C........T.............A..T..A..T...........T.....A...

                                     790        800        810        820        830        840
Daptrius americanus        AACCCTCTAATCACACCTCCCCATATCAAGCCCGAATGATACTTCCTATTTGCCTACGCC
Daptrius ater              .....A...G...................A..T..G..G.....T..............
Falco femoralis            ..T..C...G....C..C..A..C.....A..A..................C.........
Falco peregrinus           ..T..C...G....C..C..A..C.....A..A....................T......
Falco sparverius           .....C...G....C..A..A..C.....A..A.........................T
Falco verpertinus          .....C...G.......C..A..C.....A..A.........................
Herpetotheres              .....A...G.......C..T..C.....A...................C..A......
Micrastur semitorquatus    .....A...G....T..C..T..C..T..A..T...........TT.......A......
Micrastur gilvicollis      .....A...G....T........C..T..A...................C..A......
Microhierax                .....C...G.......C.........T..A..T...........T..............A
Milvago                    .....A...G...................A..........................
Phalcoboenus               .....A...G.......C..T........A..T..........................
Polihierax                 .....C...G.T........A........A.....................T......
Polyborus                  .....C...G...................A..........................
Spiziapteryx               ..T..A...G.......C.....C.....A..T.................C.....T...
Accipiter                  .....C....A.........A...........A.................C..A......
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                          850        860        870        880        890        900
Daptrius americanus       ATTCTACGCTCAATTCCCAACAAACTAGGCGGAGTACTAGCACTTGCAGCCTCCATTCTA
Daptrius ater             ..C..........C.....................T..................C...
Falco femoralis           ..C..........C.............T.....CT.......A..C.....AG.A...
Falco peregrinus          ..C..........C.....T.....G........C........A..C.....AG.A...
Falco sparverius          ........................T..........CT.......A..C......G.AT..
Falco verpertinus         ..C..........C...........G..T.....C........A..C.....AG.A...
Herpetotheres             ..C.....T.....C...........C..................A.........G.AT..
Micrastur semitorquatus   ..C.................T........A......T.......A.........G.CT..
Micrastur gilvicollis     ..C..........C...........C.........T.......A.........G.A...
Microhierax               ..C.....................................CT.A.....A..TG.AT..
Milvago                   ..C..........C.....................................C...
Phalcoboenus              ..C..........C.....................T..................C...
Polihierax                ..CT....T.....C..T...........A..C...........C..T......G.A...
Polyborus                 ..C..........C.....................................T.........
Spiziapteryx              ..C..........C.....T.......................A.........G.A..C
Accipiter                 .............C..A...........A......T....C..A..C......G.AT..

                          910        920        930        940        950        960
Daptrius americanus       ATCCTATTCCTAAGCCCCTTCCTCCACAAATCCAAGCAACGTACAATAACCTTCCGACCC
Daptrius ater             G.......T.....T..T........T.....T........C..............G...
Falco femoralis           ..T..............AC.A...........T..A..G..C..............C..T
Falco peregrinus          ...T.......G.....AC.A..............A.....C..............C..T
Falco sparverius          .....G.....T.....GC.A..A..T........A....................C..T
Falco verpertinus         ...........G.....AC.A..............A....................C...
Herpetotheres             ..T.....................A...........A.....C..................T
Micrastur semitorquatus   .............A....C..............T..A.....C..............G...
Micrastur gilvicollis     ......C..T..............A..T.....T..A.....C..................
Microhierax               ........T....T....C..............T..A.....C..............C..A
Milvago                   G........T..........T...........T.....G..C..............G...
Phalcoboenus              ...........T........T.....T.....T........C..................
Polihierax                ...........G..............T.T...A..A......T.....G.......T...
Polyborus                 .....G...........T...................A.....C..................A
Spiziapteryx              G.....................................A.....C..............C...
Accipiter                 ..T..........TT........A........T..A.....................G...

                          970        980        990        1000       1010       1020
Daptrius americanus       CTATCCCAAGCACTATTCTGACTCCTAGTCACCAACCTGTTTATTCTAACATGAATCGGC
Daptrius ater             .....................................AC....C..............
Falco femoralis           .........CTC.........................AC.C.....G..C...G.A..A
Falco peregrinus          ........GT.............T.............AC.C..C.....C...G.A..A
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Falco sparverius          .........T.............................AC.C..C.....C...G.A..A
Falco verpertinus         .........CT............A.............AC.C..C.....C...G.A..A
Herpetotheres             ..C......CTC.........T....G..AG.......AC.C..C.....C...G.A...
Micrastur semitorquatus   ..C......CTC..C......GC......AG.....T.AC.....T.G..C...G.A..A
Micrastur gilvicollis     ..G......TTG..C..T...........AG.......AC.C..C.....C...G.A..A
Microhierax               T........TT.T....T...T................AC.C..C..G..T...G.A...
Milvago                   ....................................AC..G.C................
Phalcoboenus              ..................................T..A.....C...............
Polihierax                .....T...CT............A...........TT.A..A..CT....C...G.A...
Polyborus                 .........AT..............................AC....C...........T...
Spiziapteryx              ..........TC.....T.....................CC....CT..........T...
Accipiter                 ..C......TTC.........A.T...A..G.......C..A..C..T........T..T

                                    1030       1040       1050       1060       1070       1080
Daptrius americanus       AGCCAGCCCGTAGAACACCCATTCATCATCATCGGCCAATTAGCCTCTCTCTCCTACTTC
Daptrius ater             .....A..T..............................C...................
Falco femoralis           .....A..T..T.....................T......C.......C..T.........
Falco peregrinus          .....A.....T..G.....G...........T......C....T..A............
Falco sparverius          .....A.....T..............T............C.T.....C...........T
Falco verpertinus         .....A..T..C...........................C.......C..T.........
Herpetotheres             .....A..A.............................A...C.......CT..A.......T
Micrastur semitorquatus   ..T..A..A.................T..T..T..T...C.......CT..A........
Micrastur gilvicollis     .....A..A.................T.....T......C.......C..TA........
Microhierax               ........................T.....T..T...........T..C...A.......T
Milvago                   ........T........T.....................C.......C............
Phalcoboenus              .....A..............................T......C.......C.....T......
Polihierax                .....A...........T..............T......C.......C..AA....T...
Polyborus                 .....A.........................T.........C.......C...A........
Spiziapteryx              .....A........G............T...........C.......C...A........
Accipiter                 .....A..A........T..C..T.....T.....T...C.......CA..A.A.....T

                                    1090       1100       1110       1120       1130       1140
Daptrius americanus       ACAATCCTCCTAATCCTCCTCCCCCTCGCAGGGGCCCTAGAAAATAAAATCCTTAACTAC
Daptrius ater             .....T..T.................A.C..A...........C........C......
Falco femoralis           ........T..............T...A.C..A....................C......
Falco peregrinus          ....C...T.................TA.C..............C........A......
Falco sparverius          .....T........T...T.T........T..A...T.......C........C......
Falco verpertinus         ....C.....................A.C..............C..............
Herpetotheres             ..C.CT......G.............TA....A..T........C........C......
Micrastur semitorquatus   ..C.....T.................AA................C..G.....C......
Micrastur gilvicollis     ..C.A...T..G..............A....A...........C........C......
Microhierax               .T..C...TT....T.....G......ATC..A.................A..C......
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Milvago          .....T..T.................A.C..A.....................C......
Phalcoboenus     ........T.................TA.C..A.....................C......
Polihierax       .....T........T.....G..T..TA.C..A...........C......T.A..A...
Polyborus        .....T.....................A.C..A...........C........C......
Spiziapteryx     .....T.....................TA.T..A...........C........A......
Accipiter        CT.........GCT..TT....A..AA.T..A...........C...C........C..
```

Table 1. SPECIES INCLUDED IN PHYLOGENETIC ANALYSIS

Falconidae:	
Caracaras:	*Milvago chimachima* LSU 14804
	Polyborus plancus LSU 5328;
	Phalcoboenus australis,USNM unnum.
	Daptrius ater AMNH pep1940
	D. americanus AMNH rop266
Spot winged Falconet:	*Spiziapteryx circumcinctus* LSU18708
Forest Falcons:	*Micrastur gilvicollis* LSU10720
	M. semitorquatus LSU11298
Falconets and	
Pygmy Falcons:	*Polihierax semitorquatus* AMNH unnum.
	Microhierax erythrogonys ZMUC unnum.
Laughing Falcon:	*Herpetotheres cachinnans*ANSP unnum.
Falco species:	*F. sparverius* AMNH csg1216
	F. peregrinus AMNH csg002
	F. verspertinus MNDN5
	F. femoralis LSU103909
Accipitridae:	
Sharp-shinned Hawk	*Accipiter striatus* AMNH csg9215,

Table 2. PRIMERS USED TO AMPLIFY CYTOCHROME *b*

Primer*	Sequence (5' to 3')
L14857	GGGTCTTTCGCCCTATCAAT
H14955	GAGTCAGCCATATTGGACGTCTCGGC
L14841	CCATCCAACATCTCAGCATGATGAAA
H15149	GCCCCTCAGAATGATATTTGTCCTCA
L15162	CTACCATGAGGACAAATATC
H15561	GTAGGCGAATAGGAAGTATC
L155076	AACCTACTAGGAGACCCAGA
H15915	GGAGTCTTCAGTCTCTGGTTTACAAGAC

(Helm-Bychoswki & Cracraft 1993)

Table 3. Weighting used in Phylogenetic Analyses and Results of the Analyses

Set No.	Weighting Used	Data Sets	RESULTS: Forest Falcon Monophyly	RESULTS: Spot-winged Falconet within *Falco* Clade
1	Equal	Molecular	no	no
	a posteriori	Molecular	no	no
	Equal	Both	no	no
	a posteriori	Both	no	no
2	*a priori* - A*	Molecular	yes	no
	a priori - B*	Molecular	yes	yes
	a priori - B*	Both	yes	yes

* Weighting procotols:

A and B - 1st and 3rd position transitions weighted zero.

B - 3rd postition tranversions weighted two.

Table 4. Retention, Consistency and Rescaled Consistency Indices of Equally Weighted Characters in Six Data Partitions

Codon Position		Number of Informative Sites	RI	CI	RC
First					
	Transitions	53	0.50	0.46	0.28
	Transversions	15	0.71	0.50	0.35
Second					
	Transitions	13	0.67	0.52	0.41
	Transversions	5	0.70	0.50	0.35
Third					
	Transitions	187	0.33	0.41	0.15
	Transversions	109	0.62	0.45	0.27

Table 5. Pairwise Differences in Cytochrome *b* Sequences

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Accipiter*															
2 *Daptrius americanus*	.17														
3. *D. ater*	.17	.09													
4. *Milvago*	.16	.08	.04												
5. *Polyborus*	.16	.07	.08	.08											
6. *Phalcoboenus*	.16	.08	.05	.04	.08										
7. *Falco sparverius*	.17	.15.	.15	.15	.15	.14									
8. *F. peregrinus*	.17	.15	.14	.14	.14	.13	.10								
9. *F. femoralis*	.17	.14	.14	.13	.12	.13	.10	.09							
10. *F. vespertinus*	.16	.14	.14	.13	.12	.13	.09	.08	.07						
11. *Spiziapteryx*	.19	.13	.12	.12	.11	.12	.15	.14	.14	.13					
12. *Micrastur semitorquatus*	.18	.16	.15	.15	.16	.15	.17	.17	.15	.16	.16				
13 *M. gilvicollis*	.17	.12	.12	.12	.12	.11	.14	.14	.12	.12	.12.	.12			
14. *Polihierax*	.19	.16	.16	.16	.15	.15	.16	.16	.15	.14	.16	.17	.15		
15. *Microhierax*	.18	.16	.16	.16	.15	.16	.14	.14	.14	.14	.16	.16	.14	.16.	
16. *Herpetotheres*	.17	.15	.14	.14	.13	.14	.16	.15	.15	.14	.14	.14	.10	.17	.16

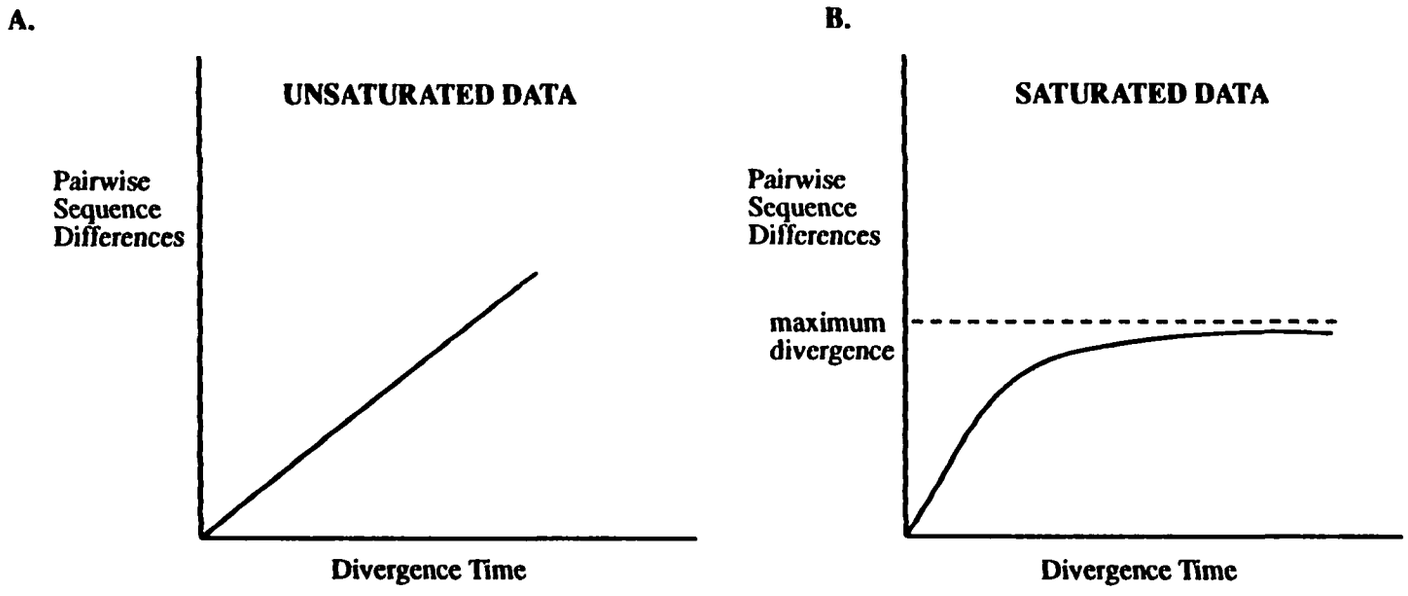

Figure 1. Graphs illustrating the theoretical expectation of saturated and unsaturated data when sequence differences between pairs of taxa are plotted against time since divergence of those taxa. (A) Substitutions between taxa are not saturated. Pairwise differences increase over time. (B) Substitutions saturate. Pairwise differences reach a maximum, and the curve asymptotes at the theoretical maximum level of divergence.

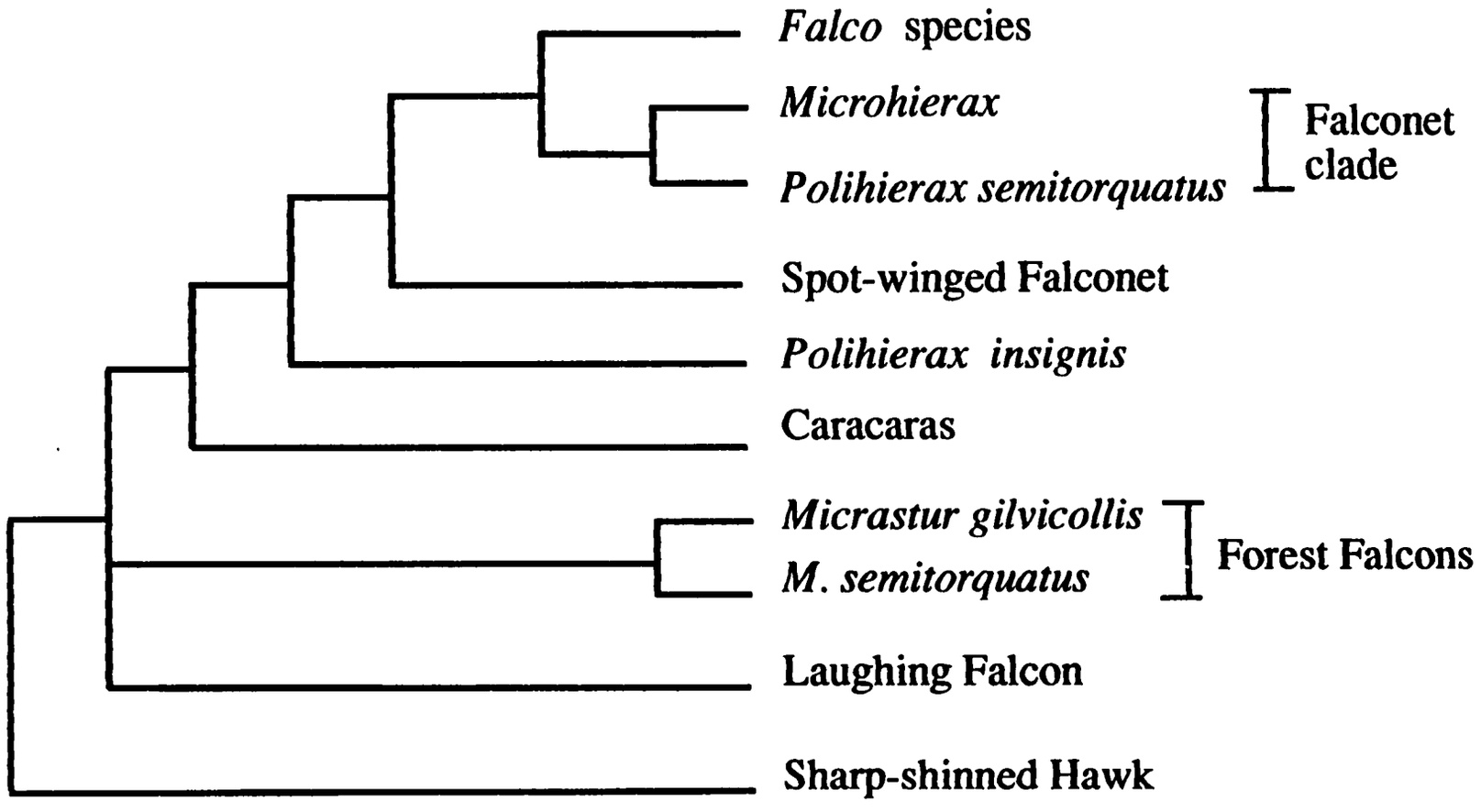

Figure 2. Phylogenetic analysis of the Falconidae inferred from syringeal morphological data. Cladogram representing the strict consensus tree of 54 most parsimonious trees of length 47, C.I. 0.59, R.I. 0.81, and R.C. 0.49.

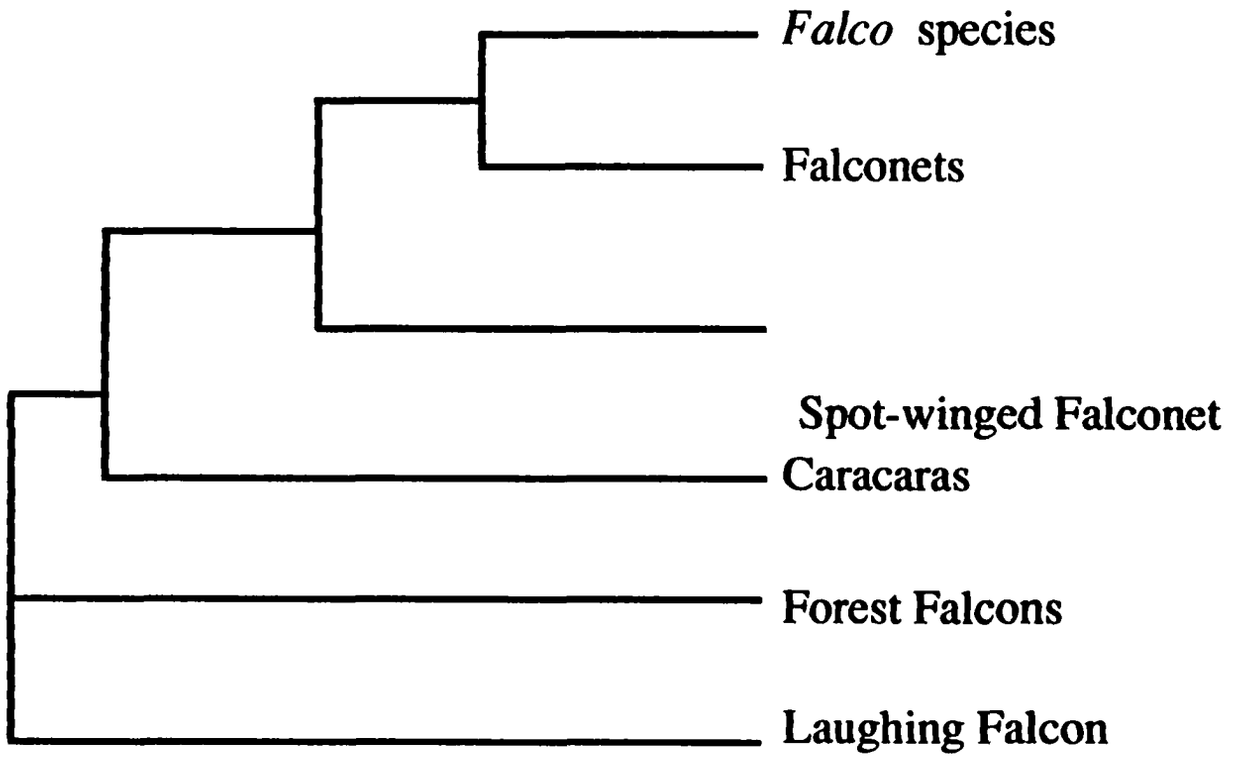

Figure 3. Phylogenetic hypothesis inferred from osteological data (Becker, 1987).

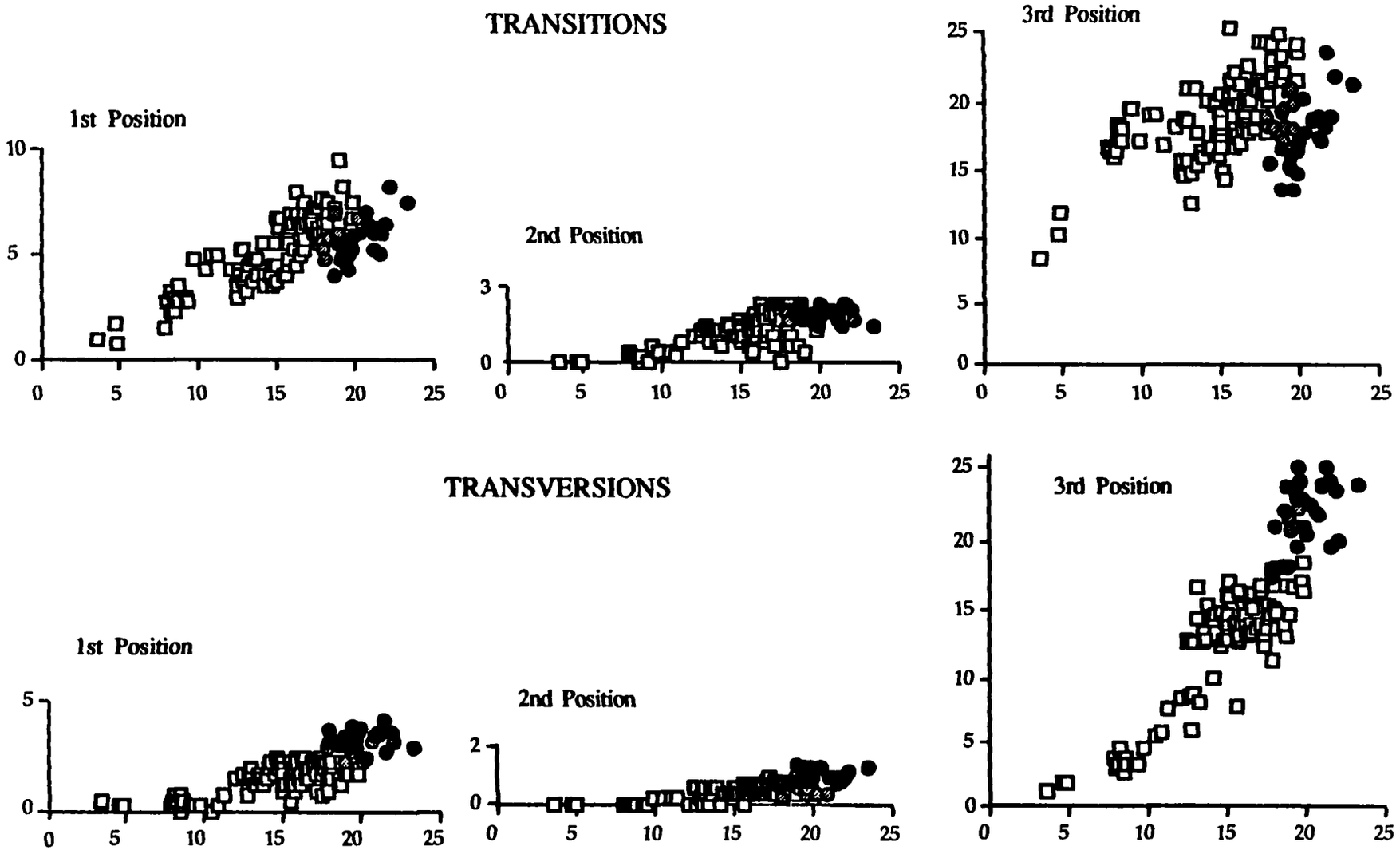

Figure 4. Graphs illustrating saturation assessments within six partitions of the 1143 base pairs of cytochrome *b*. Pairwise differences of subsets of sequences within each partition are plotted against total sequence divergence as an estimate of time of divergence. The following symbols were used in all saturation graphs - white squares: comparisons between Falconidae species; gray circles: comparisons of Falconidae species with Sharp-shinned Hawk; black circles: comparisons of Falconidae species with Chicken. Saturation is determined by a leveling off or descent of the curve in the outgroup comparisons. See text for the interpretation of saturation in each partition.

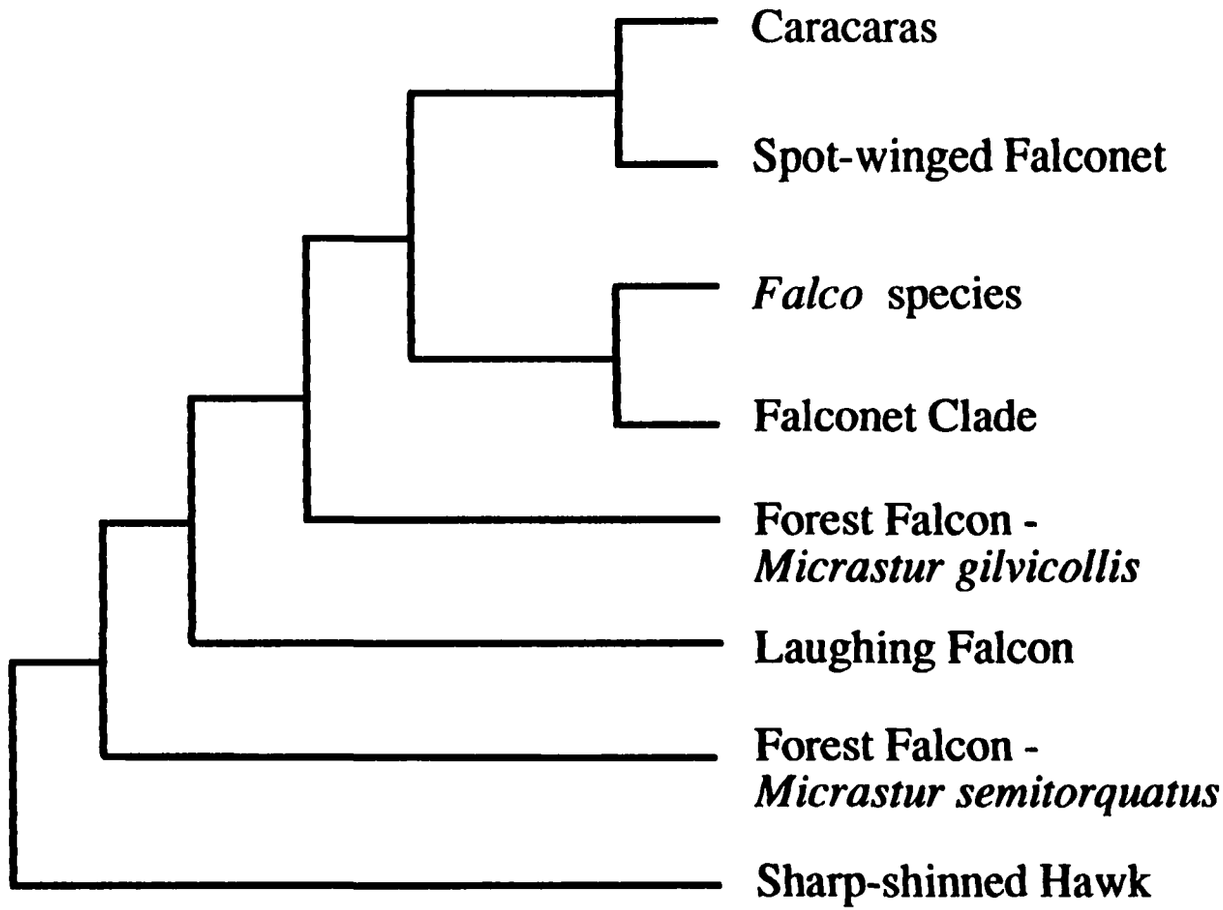

Figure 5. Strict consensus tree of two most parsimonious cladograms inferred from equally weighted data, with length 1044, C.I. 0.46, R.I. 0.44 and R.C. 0.20. Cladograms differ in the resolution of species within the Falco clade.

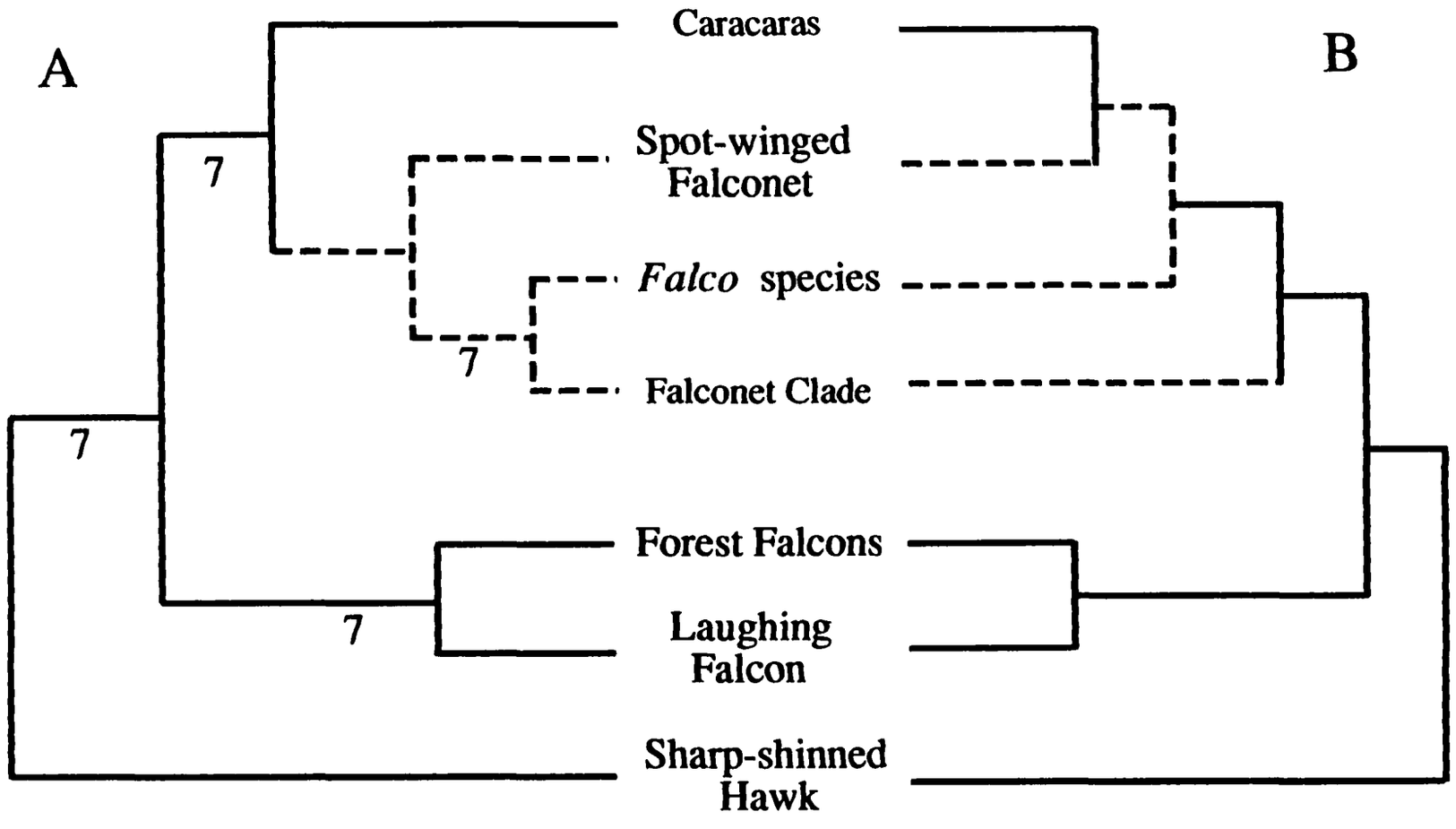

Figure 6. Cladograms representing the topologies inferred from two analyses of differentially weighted data. In both analyses, saturated partitions of data are downweighted, analyses differ in the comparative weight given to third position transversions. Branches leading to nodes differing between the two cladograms are in dashed lines. (A). Topology inferred when third position transversions are given higher weight. Numbers at nodes are decay indices (Bremer, 1988). (B). Additional topology inferred when third position transversions are given the same weight as the other unsaturated

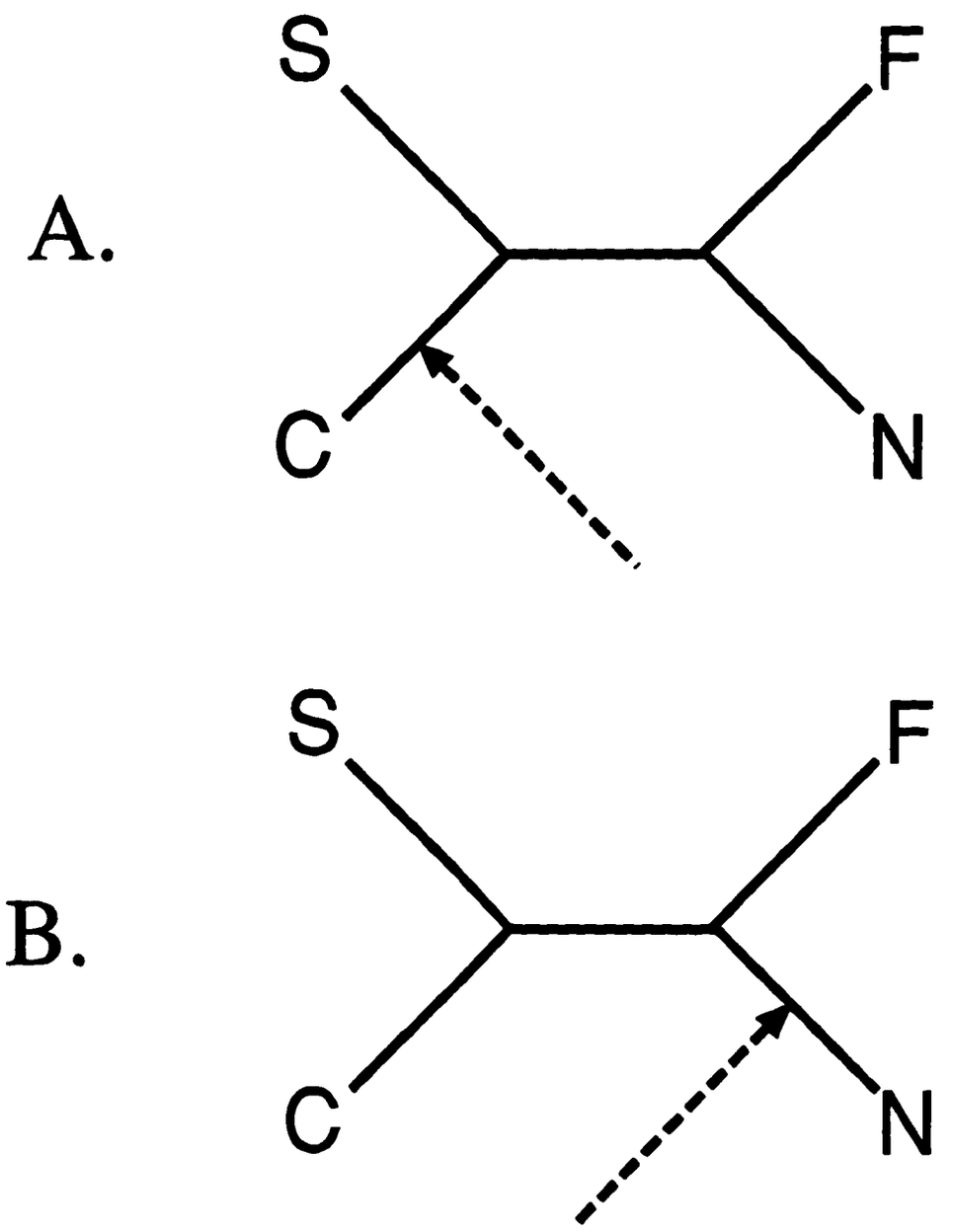

Figure 7. Networks illustrating difference between the topologies, A and B, inferred from differentially weighted data. The arrows indicate where clade 4, the Forest Falcons and Laughing Falcon, connects in the two different topologies. F; clade 1, the Falco species. C; clade 2, the caracara species. N; clade 3, the falconet clade, Polihierax and Microhierax. S; the Spot-winged Falconet.

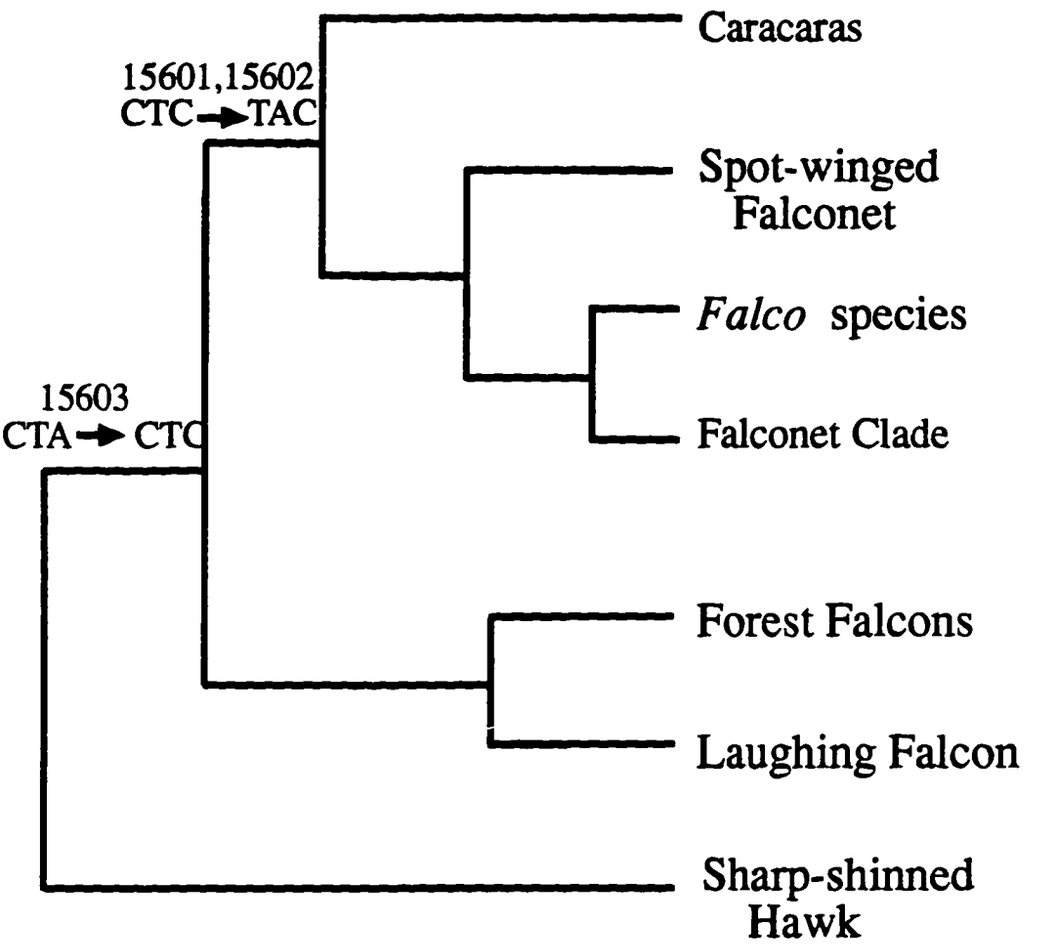

Figure 8. One example of the correlation of mutations at first, second and third codon positions. Molecular characters 15601, 15602 and 15603 are mapped onto the topology inferred from differentially weighted molecular data. The first and second position changes (CTC to TAC) at the basal node within the family could not have occurred without the third position transversion (A to C), at the node connecting the outgroup to the family.

CONCLUSION

Phylogenetic Analyses

The primary objective of this research was to infer phylogenies for the Falconiformes and the Falconidae. Details of these analyses are presented in the three chapters of this dissertation. At the ordinal level, the phylogeny inferred from variations in syringeal morphology indicates that the order Falconiformes, as currently constituted, is monophyletic. The Cathartidae (New World Vultures) are basal to the Falconidae (falcons and caracaras) and the Accipitridae (hawks and eagles).

Within the order, the phylogenies inferred from morphological and molecular data provide congruent hypotheses for the Falconidae. *Herpetotheres* (Laughing Falcon) and *Micrastur* (Forest Falcons) form a clade basal to two sister clades. The first includes the four caracara genera; the second, the genus *Falco*, and the genera *Polihierax, Microhierax and Spiziapteryx*.

These phylogenies provide the framework for tracing the evolution of the morphological and molecular structures used in the analyses.

Patterns of Evolution of the Syrinx

Within the Falconiformes, syringeal morphology generally follows three paths. In the Falconidae, *A* elements are totally ossified, *B* elements are cartilaginous and there is a fused, ossified tympanum. In all species, there is a lateral membrane between the *A1* and *B1* structural elements onto which the M. tracheolateralis inserts.

In the Accipitridae (including Sagittarius), ossification of *A* elements generally is incomplete, and elements are cartilaginous dorsally. Fusion and ossification of the tympanum vary, but there is less fusion than in the

Falconidae. The M. tracheolateralis always inserts on *B1*, which has a distinct shape.

The cathartid syrinx is quite different. Although ossification of the *A* elements is similar to the Accipitridae, there is no tympanum. Most species do not have a M. tracheolateralis, although vestigial fibers are apparent in *Coragyps atratus*.

I found no sexual dimorphism in the syringes of the falconiform species, nor for species examined within the Strigiformes and Ciconiiformes. Ossification and fusion of structural *A* elements follow an ontogenetic pathway similar to the passeriforms (Ames 1971); increasing in adults compared to juveniles. A unique pattern was discerned in the Cathartidae. Single *A* elements immediately cranial to the tracheo-bronchial junction are complete rings in juvenile; in adults, these are incomplete, with dorsal gaps.

Patterns of Evolution of Cytochrome *b*

For the Falconidae family, the pattern of substitution at the three codon positions is similar to the general patterns found. Variation is greatest at the third codon positions; 86% of the these sites have mutations that can be observed. Transition differences at that position change more rapidly than transversions. Third position transitions saturate; pairwise divergence of falconid taxa often exceeds the divergence in comparisons with the outgroup.

Systematics and the Syrinx.

There is enough variation in the morphology of syringeal structural elements to provide support for relationships of clades within the Falconiformes. The presence or location of major structural elements or membranes provide synapomorphies defining orders or families of birds. Minor structural variants provide characters that define genera and resolve some generic relationships within families. There are, however, a limited

number of structures comprising the syrinx and a limit to the variation of these structures. There is not enough variation to resolve relationships within speciose genera.

Analysis of Molecular Sequences

Saturation analysis of the sequences before phylogenetic analysis was an important first step in this research. Differential weighting of saturated sequence data allowed the phylogenetic signal to come through, and produced a phylogeny congruent to two existing hypotheses. *A priori* analysis is an essential part of morphological systematic research; it is the way characters are identified and homologies hypothesized. This study has reinforced the necessity for *a priori* analysis of molecular data.

Analysis of Different Data Sets

Advocates of taxonomic congruence promote the separate analysis of different data sets, whereas those advocating character congruence propose combining all data initially. In this study, the separate analysis of molecular data gave insight into the potential problem in recovering phylogenetic signal caused by variation in substitution rates among sites. In the final analysis, data sets were combined. However, this decision was trivial. When molecular data were differentially weighted and analyzed separately, both molecular and morphological data produced congruent phylogenies.

Both are also congruent in the level of support offered for different clades within each analysis. Thus, both give strong support to the more terminal nodes (the *Falco* species, the falconet clade and the caracaras), whereas the deeper nodes have similar problems. Few characters support these nodes, and there is ambiguity in the optimization of those characters. For this family, the similarities in morphological and molecular data outweigh the differences.

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