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Molecular Systematics of Holarctic Raptors (Order Falconiformes)

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ABSTRACT

The molecular phylogeny of holarctic falcons, vultures, eagles, buzzards, hawks and harriers was inferred from nucleotide sequences of the mitochondrial cytochrome b (cyt b) gene. The cyt b data are usually well-suited to establish the relationship within more closely related groups of raptors, i.e. within genera or families: Within the Falconiformes several systematic proposals that were based on morphological data can be confirmed by the molecular data. In some instances, however, new relationships become apparent which need further study. The molecular data imply that the closely related taxa *Falco chicquera/ F. c. horsbrughi, Falco columbarius/F. c. aesalon, Circus cyaneus / C.c. hudsoni, Aquila heliaca/ A. adalberti,*and *Hieraetus fasciatus/ H. spilogaster* which have been treated as subspecies have reached species level already. Furthermore, New World vultures, falcons, and the Secretary Bird fall outside the Accipitridae and represent independent families. However, it is difficult to ascertain these higher level classifications. The following reasons are discussed: a.) A mutational saturation of the cyt b gene of taxa whose ancestors have diverged a long time ago leads to homoplasy and ambiguity. b.) The explosive evolution of the different families of birds during a short period of 5-10 million years in the Eocene/Oligocene will produce shallow branches coming off the phyletic point of divergence which cannot be resolved with certainty.

INTRODUCTION

During recent years sequences of marker genes have been increasingly used for a more precise phylogenetic reconstruction of relationships within and between genera, subfamilies and families of birds (Avise, 1994; Sibley, 1994) because marker genes can be easily amplified by PCR and sequenced now (Hillis & Moritz, 1990; Edwards *et al.*, 1991; Cooper *et al.*, 1992; Helm-Bychowsky & Cracraft, 1993; Kocher *et al.*, 1989; Meyer, 1994; Kornegay *et al.*, 1993; Taberlet *et al.*, 1992; Hedges & Sibley, 1994).

For birds, the nucleotide sequence of the mitochondrial cytochrome b (cyt b) is a useful means to resolve phylogenetic events which took place during the last 20 million years; some recent examples for the application of this marker have been reported in Edwards *et al.*, (1991), Richman & Price, 1992, Helm-Bychowsky & Cracraft (1993), Kocher *et al.* (1989), Taberlet *et al.*, (1992), Friesen *et al.*, 1993; Birt *et al.*, 1992, Blechschmidt *et al.*, 1993; Heidrich & Wink (1994), Heidrich *et al.*, (1995), Wink (1994), Wink *et al.*(1993a,b; 1994).

The phylogenetic relationships within and between different genera and families of diurnal raptors (family Falconiformes) cannot be resolved unambiguously alone on morphological, anatomical and behavioural characters, since convergence of characters is a typical feature in this group of birds which share so many similarities in life style. The use of molecular markers, especially of nucleotide sequence data, should improve phylogenetic reconstructions, since convergence and adaptations are of minor importance and many characters are available for comparisons (Avise, 1994; Sibley, 1994).

We have chosen the mitochondrial cytochrome b gene as a molecular marker and have used it to reconstruct the phylogeny of diurnal raptors. This report is a short summary of our data, focussed on phylogenetic relationships within holarctic raptors. Although not all genera have been covered, the data provide a first overview of the underlying phylogeny. A broader account on the phylogenetic relationships within certain genera of raptors have been published already or are in preparation (for more details refer to Seibold *et al.*, 1993, 1995; Wink 1994, 1995; Wink & Seibold, 1995 and in preparation).

MATERIAL AND METHODS

Collection of blood and tissue samples

Samples consisted of blood (ca. 100 µl) collected from the brachial vein and in a few cases of muscle tissue of dead birds that had been deep-frozen. Blood was stored in EDTA-NaF-Thymol buffer (Arctander 1988) at ambient temperature during field work, transferred to Heidelberg and stored at -20°C until extraction.

DNA isolation, PCR and DNA-sequencing

Methods used for DNA isolation, PCR and DNA sequencing were performed according to Seibold (1994) and Seibold *et al.* (1995). Origins of sequence data and most sequences are documented in Seibold (1994) or Lotfikhah (in preparation). In a few cases sequence data were obtained from GeneBank.

Sequence analysis

Nucleotide sequences were aligned with the cytochrome b sequence of *Gallus gallus* (Desjardins & Morais 1990). Phylogenetic trees were reconstructed using

the maximum parsimony method (MP) with the phylogeny program PAUP 3.1.1 (Swofford 1993) and the distance method neighbour-joining (NJ) as implimented in programme package MEGA 1.0 (Kumar *et al.* 1993). In the neighbour-joining analyses genetic distances were calculated based on p-distance or the Tamura-Nei method, which takes into account the strong transition-transversion and base composition bias found in our data. With PAUP, heuristic algorithms were employed. Bootstrap analyses (Felsenstein, 1985) were performed to evaluate the robustness of the furcations found.

RESULTS AND DISCUSSION

DNA was isolated from about 20 falcon species, 16 vultures, 10 eagles, 4 sea eagles, 3 kites, 13 buzzards and hawks, and 4 harriers. The mitochondrial cyt b gene was amplified by PCR and sequenced directly. The following phylogenetic reconstructions were obtained (because of lack of space only 1 tree, i.e. usually the MP tree is illustrated for each group; however, NJ trees usually showed similar topologies).

Falcons

Falcons share a number of specialized characters in anatomy, moult, composition of eggshell, and karyotype and have been placed outside the Accipitridae as a separate subfamily Falconidae (Sibley & Monroe, 1990; Cramp & Simmons, 1980; Cade 1982) or even family (del Hoyo *et al.*, 1994).

The phylogeny of 22 taxa of falcons of the Holarctic based on 1026 base pairs of the cyt b gene was reconstructed employing *Gallus gallus* as outgroup by MP and NJ. Both phylogram are almost identical but only the MP is illustrated in Figure 1. The following results are apparent:

Falcons appear as a distinct clade that is separated from the Accipitridae, confirming the anatomical and karyological evidence and our previous molecular data (Seibold *et al.*, 1993; Wink & Seibold, 1995; Wink, 1995).

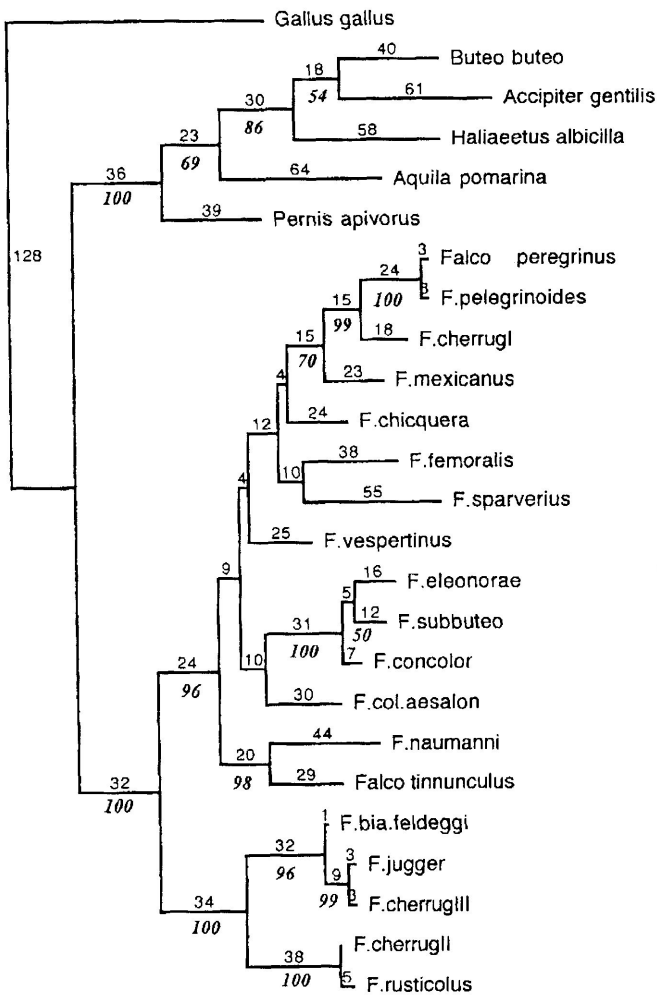
Within the true falcons, the subgroup of Hierofalco with *F. biarmicus, F., rusticolus, F. jugger* and *F. cherrug* is unambiguously recognized genetically, however, *F. mexicanus* which was considered as a member of this subgroup (Cade 1982), always clusters with the *peregrinus*-complex. The Saker appears in 3 haplotypes (I,II and III). Type I is related to the Peregrine and we have postulated that this reflects an old hybridisation between a Saker male and a female Peregrine (Seibold *et al.*, 1993). Introgression of the hybrid in the original Saker population has produced a bird with a Saker phenotype but a different mitochondrial haplotype. Type II is closely related to the Gyr Falcon and might be a hybrid between a female Gyr Falcon and a male Saker, whereas type III is most likely of the "true" Saker genotype. Of 21 birds studied, 9 belonged to type I, 7 to type III and 5 to

type II; a correlation with their geographical origins could not be established (Seibold & Wink, unpublished).

Figure 1. Phylogram of the genetic relationships within the falcon complex

Illustrated is a bootstrap analysis (100 replicates) employing the Maximum Parsimony method with heuristic search (TBR branch swapping; tree length 1164 steps [sum of minimal possible lengths 612, maximally 2003 steps]; consistency index CI= 0.526; retention index RI= 0.603).

Branch lengths are proportional to genetic distances; the number of nucleotide substitutions (for a partial sequence of 1026 bp) is given above each branch. Bootstrap values (above 50%) which indicate confidence estimates for each branch are given in italics below the branch length values.



The *Tinnunculus* group consists of *F. tinnunculus* and *F. naumanni*, but not of the North American *F. sparverius* (which might be related to the *vespertinus/ amurensis* complex; Fig. 2). Morphological data had implied a close relationship between *F. sparverius* and *F. tinnunculus* (Cade, 1982) and this species has been considered to form a supersoecies with *F. tinnunculus* (Sibley & Monroe, 1990). Alhough its true nearest relative has not been found yet with certainty, a *sparverius/ tinnunculus* relationship, however, is highly unlikely.

F. eleonorae, F. concolor and *F. subbuteo* which share many biological characters form a closely related clade which had been recognized as a the separate subgenus *Hypotriorchis*. Here, the morphological and molecular data are in agreement.

The *Peregrinus*-complex consists of *F. peregrinus* (various subspecies cannot be distinguished with the cyt b gene; Fig. 2) and the closely related *F. pelegrinoides*. Since the differences between *F. pelegrinoides* and *F. peregrinus* are so small (genestic distance 0.6% for the full cyt b gene), is needs to be studied whether *F. pelegrinoides* represents a true species. *F. mexicanus* obviously clusters with the *Peregrinus*-complex and not with the Hierofalcons as was commonly assumed (Cade, 1982).

The systematic position of *F. chicquera, F. femoralis, F. sparverius, F. columbarius* and *F. vespertinus* could not be resolved with certainty, as can be seen from the rather low bootstrap values.

For a number of taxa we have only sequenced part of the cyt b gene as yet (Fig. 2). Nevertheless, these data allow some systematic conclusions:

The North American Merlin, *F.c.columbarius* differs significantly from the European *F.c. aesalon*. Genetic distances indicate that both taxa have been separated for more than 1 million years (if we assume a molecular clock of 2% sequence divergence per 1 million years; Shields & Wilson, 1987) and might have reached species status already.

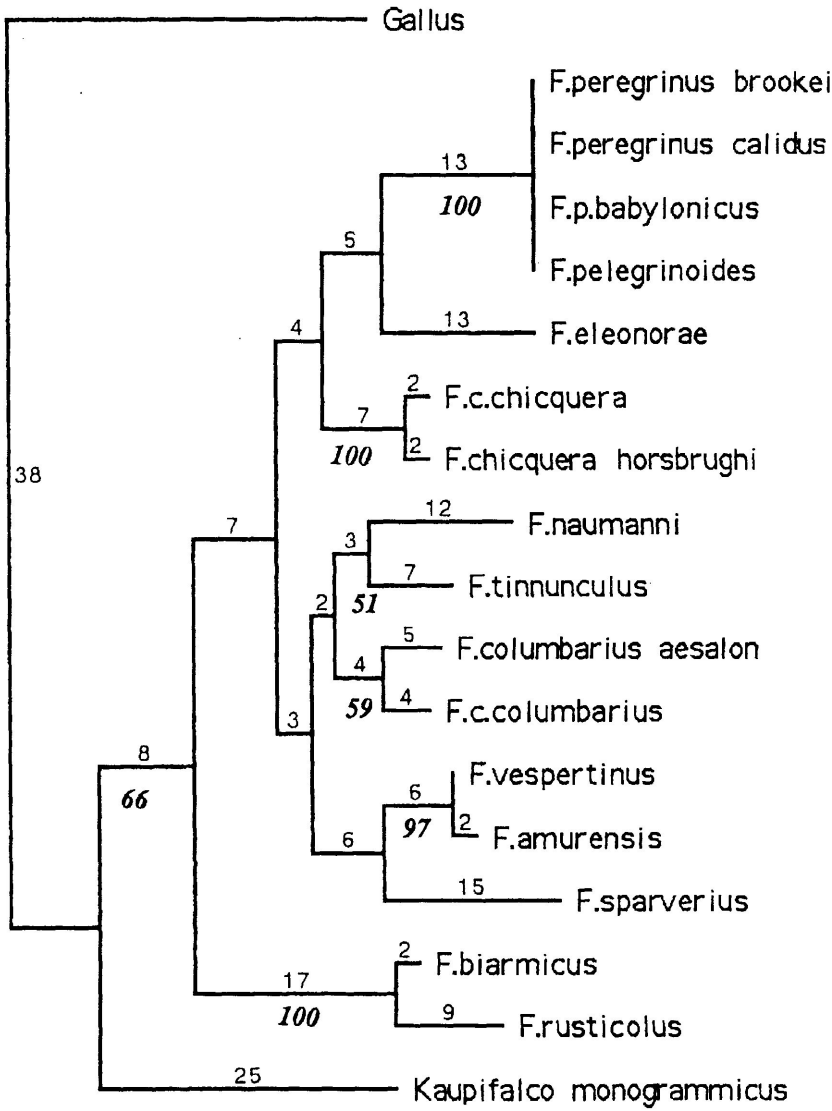
In the Red-necked Falcon, *F. chicquera* two subspecies (i.e. *F.c. chicquera* and *F.c. horsbrughi*) are recognized occurring either in India or southern Africa, which also differ genetically. The genetic distances are similar as in the Hypotriorchis complex or as in *F. vespertinus /F. amurensis*; thus, a species status for *F. chicquera* and *F. horsbrughi* would be plausible, the more since both taxa show recognizable morphological differences and their breeding ranges do not overlap.

The Red-footed Falcon *F. vespertinus* has been considered to be closely related to the Amur Falcon *F. amurensis* which substitutes *F. vespertinus* in East Asia (Sibley & Monroe, 1990). As can be seen in Figure 2 both taxa are indeed closely related and form genetically distinct sister species.

Vultures

In general, vultures do not kill their prey but have occupied a special ecological niche by feeding on carrion. A particular set of morphological and biological characters, which can be interpreted as adaptations, are evident in this

Figure 2. Phylogram of the selected genetic relationships within the falcon complex
Illustrated is a bootstrap analysis (100 replicates) employing the Maximum Parsimony method with heuristic search (TBR branch swapping; tree length 220 steps [sum of minimal possible lengths 142, maximally 355 steps]; consistency index CI= 0.645; retention index RI= 0.634).
Branch lengths are proportional to genetic distances; the number of nucleotide substitutions (for a partial sequence of 300 bp) is given above each branch. Bootstrap values (above 50%) are given in italics below the branch length values.



group of birds: Bare heads and necks avoid a pollution of feathers when feeding inside a carcass and strong hooked beaks with cutting edges are needed to tear skin apart. The broad wings enable them to ride rising air currents with little energy expenditure while searching for carcasses. The feet of vultures are more appropriate for movement on the ground than for catching prey (as in other raptors). And in addition, vultures do not show a pronounced sexual dimorphism which is typical for actively hunting raptors (Newton, 1990; Brown & Amadon, 1968; Mundy *et al.*, 1992; Glutz von Blotzheim *et al.*, 1971; Cramp & Simmons, 1980; del Hoyo *et al.*, 1994). Because of these common morphological features and the similarities of life style it has been intuitively assumed that vultures are phylogenetically related and are part of the raptor family.

On a careful examination of anatomy, morphology and biochemistry, however, it became evident that vultures must represent a phylogenetically inhomogenous, i.e. polyphyletic group, whose shared characters are based on convergence (Mundy *et al.*, 1992; Sibley & Ahlquist, 1990). New World vultures have some characters in common with storks, such as defecation on legs for cooling, composition of uropygial gland secretions, anatomy of leg and pelvis muscles, distribution of feather lanes and even karyotypes. As a consequence a close phylogenetic relationship with storks was suggested (Garrod, 1873; Ligon, 1967; König 1982; Rea, 1983). DNA-DNA-hybridization studies and first sequence analyses (Avise *et al.*, 1994) supported this assumption (review in Sibley & Ahlquist, 1990). This analysis (Fig.3) is based on a broader data set, performed with nucleotide sequences of 15 vulture species, representing the main groups of Old World vultures, but also 5 taxa of New World vultures, and for comparison 3 stork species (Sequences of *M. americana, C. burrovianus, G. californianus*, and *C. atratus* were taken from Avise *et al.*, 1994).

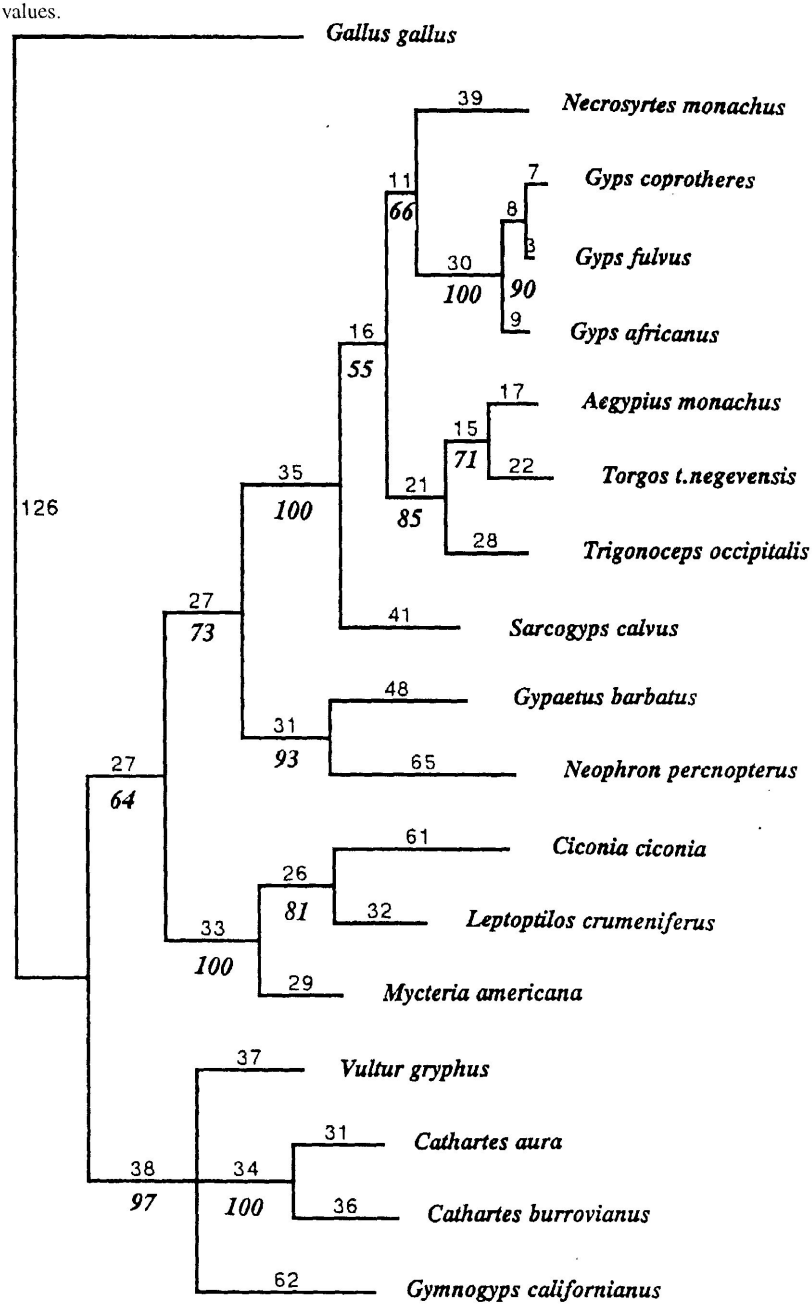
As seen in Figure 3, the vultures appear in at least three monophyletic clades, indicating a large degree of para/polyphyly which was also postulated on account of morphological, karyological and biological differences. Thus the similarities found in morphology are indeed based on convergence and not on close genetic relatedness (summaries in Mundy *et al.*, 1992; Sibley & Ahlquist, 1990; Del Hoyo *et al.*, 1994). Also data from DNA-DNA-hybridisation (Sibley & Ahlquist, 1990) and DNA sequences (Avise *et al.*, 1994) supported the divergence of Old and New World vultures, but had not resolved the dichotomy of Old World vultures. The following finer details were seen:

Aegypius and *Torgos* form a related sister clade. Within *T. tracheliotus*, birds from Sinai represent as subspecies (Bruun *et al.*, 1981), which is also recognized genetically. MP and NJ trees always combine *Aegypius, Torgos, Trigonoceps* and sometimes also *Sarcogyps* into a monophyletic group, whereas

Figure 3. Phylogram of the genetic relationships within the vulture complex

Illustrated is a bootstrap analysis (100 replicates) employing the Maximum Parsimony method with heuristic search (TBR branch swapping; tree length 1045 steps [sum of minimal possible lengths 570, maximally 1663 steps]; consistency index CI= 0.545; retention index RI= 0.565).

Branch lengths are proportional to genetic distances; the number of nucleotide substitutions (for a partial sequence of 1026 bp) is given above each branch. Bootstrap values (above 50%) are given in italics below the branch length values.



taxonomists have considered them as members of 4 monotypic genera (Sibley & Monroe, 1990; del Hoyo *et al.*, 1994).

Members of the *Gyps* (and *Pseudogyps*)-complex are closely related and monophyletic. Although some morphological differences exist (del Hoyo *et al.*, 1994), genetic distances imply that they are all members of one genus, e.g., *Gyps*. *Necrosyrtes* either clusters with the *Aegypius*-complex or more often with the *Gyps*-complex, but its position could not be resolved with certainty.

Gypaetus and *Neophron* form a common clade and are not related to the other Old World vultures. A close analysis of skeletal and muscular characteristics had already pointed out a common ancestry of both taxa (Jollie 1976, 1977). Also the postembryological development of both vultures share many similarities (Thaler *et al.*, 1986).

The New World Vultures *Vultur*, *Gymnogyps*, and *Cathartes* cluster together outside the Accipitridae and appear to be unrelated to the Old World Vultures. Morphological data and DNA-DNA hybridisation studies have implied that New World vultures and storks have shared common ancestry (Garrod, 1873; Ligon, 1967; König 1982; Rea, 1983; Sibley & Ahlquist, 1990). If storks (*Ciconia*, *Leptoptilos* and *Mycteria*) were included as an ingroup of the vulture sample (Fig. 3), they cluster as a separate clade. If we consider the genetic distances between New World vultures and Old World vultures or condors and storks or storks and Accipitridae distance values are between 14.89 to 15.89%. Summarizing these evidences, we concluded that a **close** relationship of cathartid vultures with storks appears unlikely (more details in Wink, 1995).

Eagles

Eagles comprise a heterogeneous group of large raptors from which we have analyzed 10 species of "booted eagles" (genera *Aquila* and *Hieraetus*) and 4 sea eagles (genus *Haliaeetus*) as the main holarctic representatives (Fig. 4).

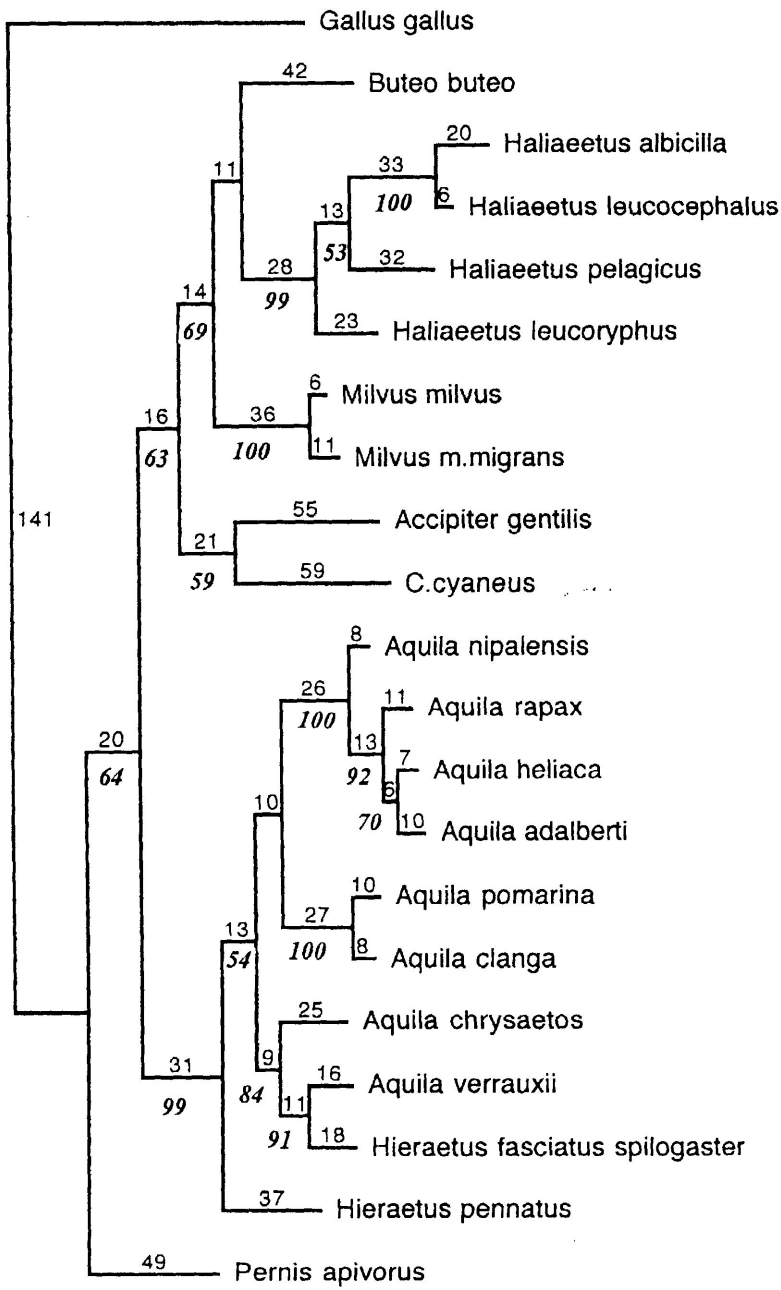
As expected from morphological and anatomical evidence (Brown & Amadon, 1968; Cramp & Simmons, 1980; del Hoyo *et al.*, 1994), birds of the *"booted-eagle"*-complex and those of the *Haliaeetus*-complex are not closely related.

Within the *Aquila*-complex *sensu strictu* 3 clades are apparent: 1.) *A. nipalensis, A. rapax, A. heliaca* and *A. adalberti*; genetically, all 4 taxa are clearly differentiated and represent distinct species (Seibold *et al.*, 1995a). 2.) *A. pomarina* and *A. clanga* represent closely related sibbling species. 3.) *A. chrysaetos, A. verrauxii* and surprisingly *H. fasciatus* fall into a single clade. The systematic position of *H. fasciatus* needs to be reconsidered. According to our sequence data, the African *H. spilogaster* which had been treated as a subspecies of *H. fasciatus*

Figure 4. Phylogram of the genetic relationships within the eagle complex

Illustrated is a bootstrap analysis (800 replicates) employing the Maximum Parsimony method with heuristic search (TBR branch swapping; tree length 932 steps [sum of minimal possible lengths 509, maximally 1592 steps]; consistency index CI= 0.546; retention index RI= 0.609).

Branch lengths are proportional to genetic distances; the number of nucleotide substitutions (for a partial sequence of 1026 bp) is given above each branch. Bootstrap values (above 50%) are given in italics below the branch length values.



(Sibley & Monroe, 1990) represents a distinct species (Wink *et al.*, in preparation). *H. pennatus* is related to the *Aquila*-complex but seems to have diverged earlier from a common ancestor.

The sea eagles represent a monophyletic group which is related to kites (*Milvus*) and buzzards (*Buteo*) but not to the booted eagles (*Aquila*-complex) (Wink *et al.*, 1996): *H. albicilla* and *H. leucocephalus* represent sister species, to which *H. pelagicus* and *H. leucoryphus* are connected. A third clade (not shown) includes *H. vocifer, H. leucogaster* and *H. sanfordi*; these species occur in Africa our South-East Asia, outside the holarctic (more details in Wink *et al.*, 1996).

Kites, Hawks, Harriers and Buzzards

The phylogenetic relationships between 18 taxa belonging to kites, hawks, harriers and buzzards are shown in Fig. 5 A+B.

Buzzards of genus *Buteo* and honey buzzards of the genus *Pernis* are of polyphyletic origin and not related (more details in Wink & Seibold; 1995, Wink, 1995), which has also implied from morphological and behavioural differences between both groups (Cramp & Simmons, 1980; del Hoyo *et al.*, 1994). *Pernis* appears as a basal taxon of the eagle/ buzzard complex and is often clustered in the *Gypaetus/Neophron* clade (Wink & Seibold, 1995; Wink, 1995). Other results are more preliminary since they are based on partial sequences (length 300 bp) only: Buzzards of the genus *Buteo* and *Parabuteo* appear in a monophyletic clade. Genetic distances within the Old World (*B.buteo, B. rufinus*) and New World (*B. regalis, B. lineatus, B. jamaicensis*) buzzards are relatively small, suggesting a more recent speciation and dispersal in this group of raptors. The African *B. oreophilus* probably does not represent a distinct species but a subspecies of *B. buteo*.

Goshawks and sparrow-hawks (genus *Accipiter*) and harriers (genus *Circus*) appear to be related to the buzzard complex. The African Lizard Buzzard (*Kaupifalco monogrammicus*) clusters with the *Buteo*-complex (Fig. 2, 5A).

Harriers of the genus *Circus* seem to be monophyletic. *Circus cyaneus hudsoni* from North America is already well separated from the Old World nominate form *C.c. cyaneus* and might represent a distinct species. *Circus pygargus* and *C. aeruginosus* are more closely related than *C. cyaneus* to *C. pygargus* (contrary to what is expected from plumage patterns).

Kites represent a polyphyletic assemblage (which had been assumed already on account of anatomical and karyological evidence (De Boer & Sinoo, 1984; del Hoyo *et al.*, 1994): According to our sequence data, members of the genus *Milvus* are related to the *Haliaeetus/Buteo*-complex (Wink *et al.*, 1996). The Black-shouldered Kite *Elanus caeruleus* appears in a group which is distinct from the

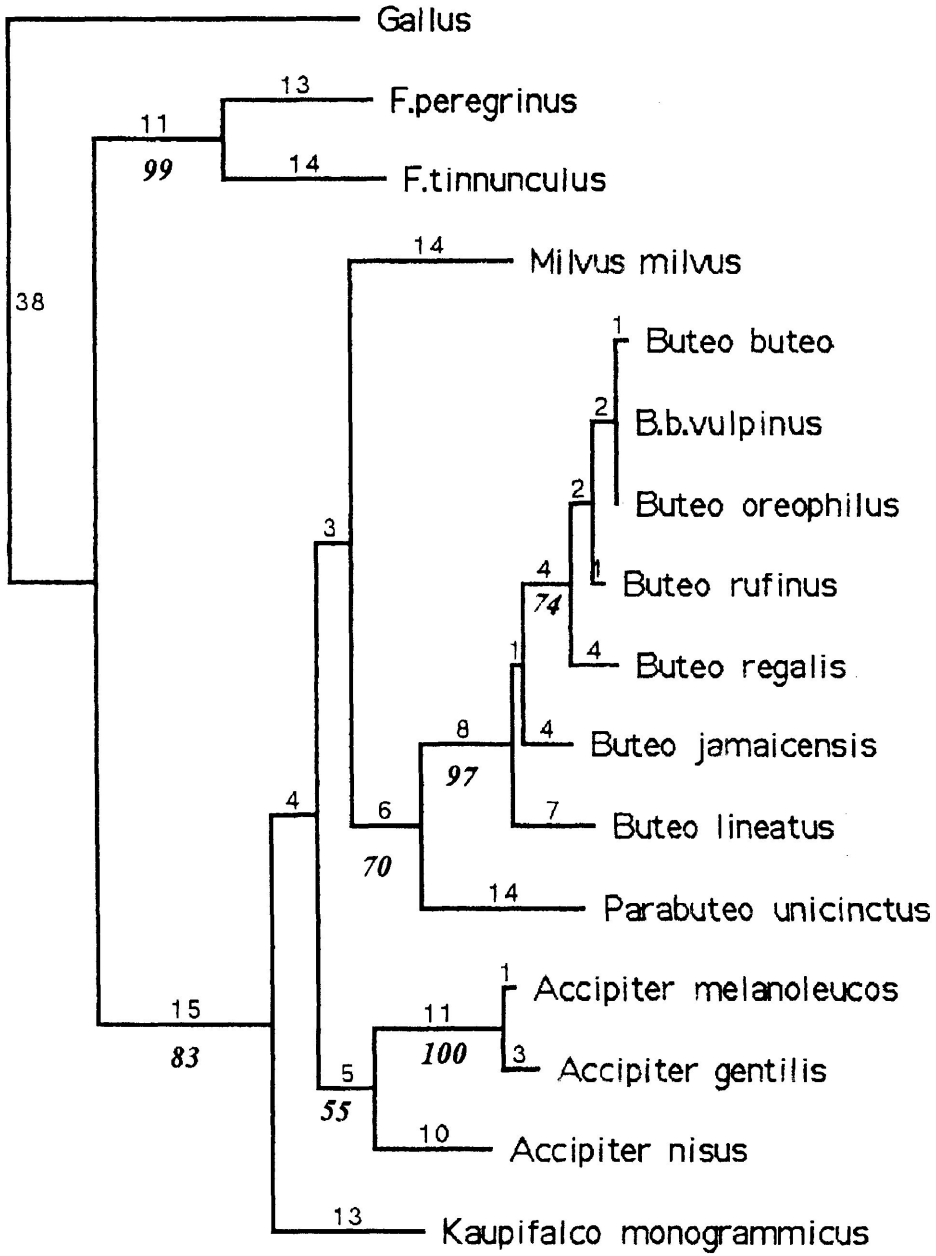
other Accipitridae and might even represent a unique family.

Relationships within the Falconiformes

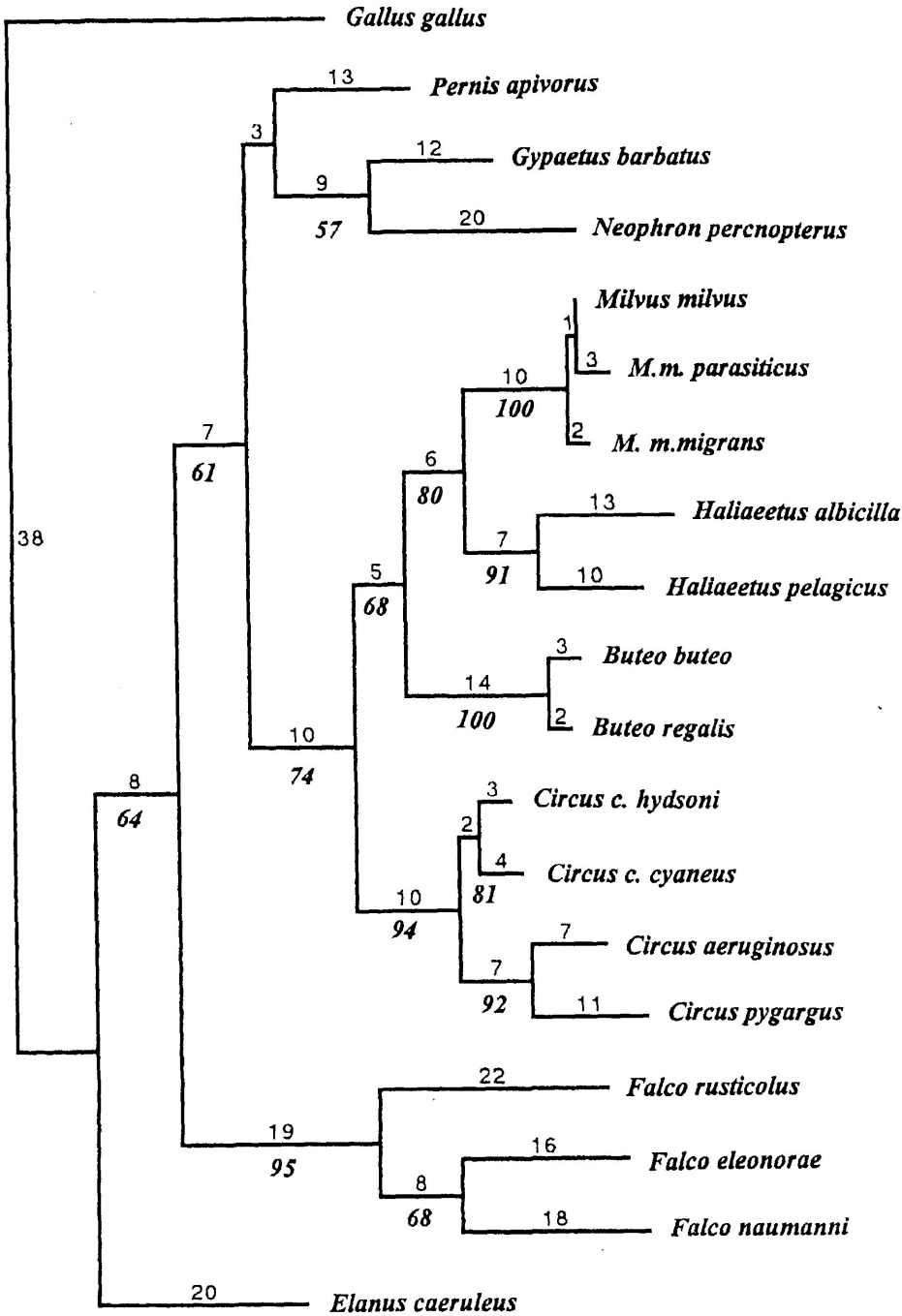
Theoretically, the polyphyly of vultures, falcons and hawks should become even more visible if the cyt b sequence data from other members of the Falconiformes and Ciconiiformes would be jointly analyzed. We have chosen representative sequences of members of the 4 families of Falconiformes (Fig. 6) and of several other members of the Ciconiiformes (*sensu* Sibley & Monroe) and Gallo-Anseriformes to assess the corresponding phylogenetic relationships. For this purpose we have added sequences (obtained from GenBank and from own sources) of the Short-toed Snake Eagle *Circaetus gallicus*, which has been considered as a close relative of the *Gyps/Aegypius*-complex and other Accipitridae, such as *Aquila, Buteo, Accipiter, Mivus, Haliaeetus* and *Pernis*. Of the family Falconidae which was found to represent a monophyletic clade in cyt b-derived trees (Seibold *et al.*, 1993; Wink & Seibold, 1995), *Polyborus* and three falcon species were included. Also comprised was the Secretary Bird, *Sagittarius serpentarius*, which had been placed in a monotypic family of the Falconiformes (Brown & Amadon, 1968). Since New World vultures share a number of characters with storks, several members of the Ciconiiformes (*sensu* Sibley and Monroe, 1990) were also evaluated, including the storks and relatives (ibises, flamingos, spoonbills), such as *Ciconia, Mycteria, Leptoptilos, Plegadis, Platelea* and *Flamingo*. Also *Calonectris* (as a representative of the Procellariidae), *Pelecanus* (for the Pelecanidae), *Larus* (for the Laridae) and *Grus* (for the Gruiformes) were included. More distantly related groups came from the Gallo-Anseriformes, such as *Cairina, Gallus, Alectoris, Coturnix, Pavo* and *Lophura*

Some results can be drawn unambiguously: Within the family Accipitridae (*sensu strictu*) whose monophyly is supported by a boostrap value of 82%, some phylogenetic relationships from previous studies were confirmed: *Milvus/Buteo* and *Haliaeetus* cluster together as sister groups (Wink *et al.*, 1996) and are always in a clade with *Accipiter* (Wink & Seibold, 1995). Interestingly, *Circaetus gallicus* can be found at the base of the *Gyps/Aegypius* clade (albeit with low bootstrap values), where this species was placed already by Jollie (1976,1977a,b) and Mundy *et al.* (1992) based on morphological evidence. The Honey Buzzard *Pernis apivorus* does not cluster with buzzards of the genus *Buteo*, but can be found either together with or as a direct neighbor to *Neophron/Gypaetus*. Since this clade is always positioned at the base of the accipitrid tree, *Pernis* must represent an old taxon. Falcons which have been considered as a family within the Falconiformes represent a monophyletic group. The polyphyly of diurnal raptors is evident. The Falconidae, Cathartidae and Sagittariidae cluster outside the Accipitridae which appear to be monophyletic. This result would support the view treating these systematic units

Figure 5. Phylogram of the genetic relationships within the buzzard, kite and harrier complex
Illustrated are a bootstrap analysis (100 replicates) employing the Maximum Parsimony method with heuristic search. Branch lengths are proportional to genetic distances; the number of nucleotide substitutions (for a partial sequence of 300 bp) is given above each branch. Bootstrap values (above 50%) are given in italics below the branch length values.
A: Buzzards: TBR branch swapping; tree length 209 steps [sum of minimal possible lengths 146, maximally 343 steps]; consistency index CI= 0.699; retention index RI= 0.68).



B: Kites and Harriers: TBR branch swapping; tree length 343 steps [sum of minimal possible lengths 188, maximally 541 steps]; consistency index CI= 0.548; retention index RI= 0.561).



as families (del Hoyo *et al.*, 1994).

Spoonbill and Puna Ibis (both of the family Threskiornithidae) cluster together with high bootstrap values, supporting the findings of Avise *et al.* (1994). The flamingos (order Phoenicopteriformes), *Grus vipio* (Gruiformes) and the Secretary Bird (*Sagittarius serpentarius*) share some morphological similarities (i.e. long legs); it is doubtful however that the common assemblage in the phylogram reflects phylogenetic relatedness (see low bootstrap values). Members of the Galliformes form a common clade at the base of the tree with phylogenetic relationships as were those found by Kornegay *et al.* (1993).

As can be seen in Figure 6 most branches originating from closely connected nodes and leading to the different bird families, are not supported by significant bootstrap values irrespective of the algorithms or weightings used for phylogeny reconstructions. How to explain this observation?

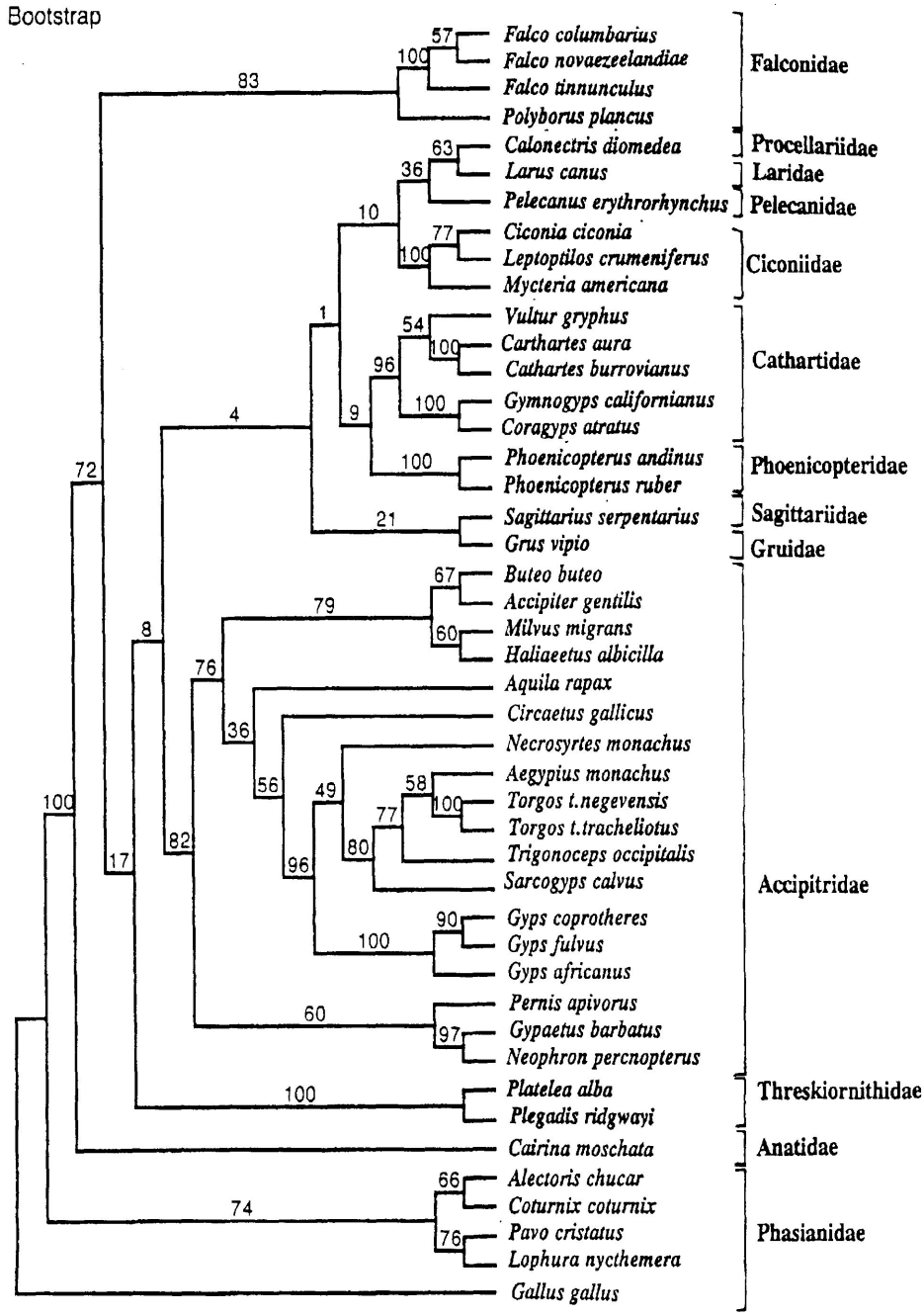
First, birds originated from the late Jurassic/early Cretaceous (150 million years ago). The fossil record indicates that birds endured massive extinctions at the late Mesozoic and at the turn of Cretaceous/Tertiary (about 65 million years ago) saw an explosive phyletic evolution (Feduccia, 1995). Massive fossil finds in deposits of the Eocene and Oligocene (35 million years ago) clearly indicate that all orders of birds (except Passerines) were already present at that time (including raptors). It has been suggested by Feduccia (1995) that this phenomenon "can only be characterized as an extraordinary explosive evolution, one that may have produced all of the living orders of birds within a time frame of some 5 to 10 million years."

As a consequence such a short time period for bird evolution would imply that branches coming off this phyletic point of divergence would necessarily be shallow. This fact has strong implications for phylogenetic reconstructions of bird phylogenies by molecular data. The difficulties of DNA-DNA hybridisation (Sibley & Ahlquist, 1990) and of sequence data to resolve these nodes and to ascertain higher level classifications of birds could thus be explained (Feduccia, 1995). Besides shallow branches another factor needs to be considered: Multiple substitutions of nucleotides must be abundant if taxa are to be compared whose ancestors diverged in the Eocene/Oligocene. The resulting homoplasy which can also be seen in our data sets of the cyt b gene (the homoplasy index accounts for 0.7 using the data illustrated in Fig. 6) will necessarily lead to a reduced resolution and to low bootstrap values. The deficiency of cyt b as a marker to resolve events which took place 20 and more million years ago has already been pointed out by Meyer (1994). A related conclusion had been reached by Avise *et al.* (1994) who stated that the "irresolution of deep branches (of the Ciconiiformes-assemblage) is most likely attributable to a close temporal clustering of nodes, rather than to ceiling effects (mutational saturation) producing an inappropriate window of re-

Figure 6. Genetic relationships within the Falconiformes

The MP bootstrap (100 replicates) consensus tree (analysis of 1000 bp) of 2987 steps in lenght (sum of possible minimal lengths 882, max. 4787 steps); CI= 0.295, RI= 0.461 is illustrated as a cladogram; bootstrap values are given at each node.

Sequences of some taxa derive from Avise *et al.* (1994) and Kornegay *et al.* (1993) (see Wink, 1995).



solution for cytochrome b sequences ".

Although the sequence data cannot resolve the descent of the different families of raptors and storks with certainty, the phylogenetic reconstructions implicate that the Cathartidae, Falconidae and Sagittariidae are not closely related to the Accipitridae. Since the fossil record also provides no evidence that the different families within the Falconiformes had a common ancestor, "raptors" could be a result of an evolutionary convergence between bird groups of polyphyletic origin. Consequently, the classification of Brown and Amadon (1968) which recognizes the order Falconiformes with the subgroups Cathartae, Accipitres, Sagittarii and Falcones is probably artificial and does not reflect phylogenetic descent (Del Hoyo *et al.*, 1994).

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