Phylogeny of Old and New World Vultures (Aves: Accipitridae and Cathartidae) Inferred from Nucleotide Sequences of the Mitochondrial Cytochrome *b* Gene

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Cytochrome *b* Gene, Nucleotide Sequences, Falconiformes, Vultures, Storks

The molecular phylogeny of 11 Old World and 5 New World vultures was inferred from nucleotide sequences of the mitochondrial cytochrome *b* (cyt b) gene. According to this analysis carrion-feeding has evolved independently at least three times during evolution: 1.) In the New World vultures, which are clearly separated from vultures of the family Accipitridae; 2.) in the *Neophron-Gypaetus* clade which is positioned at the base of the Accipitrid tree and 3.) in the *Gyps-Aegypius*-complex which encloses the largest group of Old World vultures. Thus the genetic data clearly show that the carrion-feeding lifestyles and associated morphologies shared by New and Old World vultures are rather based on convergence than on close genetic relatedness. Employing the cyt *b* sequences of 12 other members of the Falconiformes and 10 members of the Ciconiiformes (sensu Sibley and Monroe, 1990) the phylogenetic relationship between the three clades of vultures and these other taxa was assessed. New World Vultures appear to share distant ancestry with storks but a close relationship is unlikely.

Introduction

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 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. Long intestines, a stomach with strong acid are suitable for the digestion of rotten meet and bones and furthermore the latter kills microrganisms present in the putrified carrion. 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 to catch 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 and Amadon, 1968; Mundy *et al.*, 1992; Glutz von Blotzheim *et al.*, 1971; Cramp and 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 all 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 and Ahlquist, 1990). Del Hoyo *et al.*(1994) even called them a "textbook example of convergent evolution". 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 later supported this assumption (review in Sibley and Ahlquist, 1990).

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 and Moritz, 1990; Edwards

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et al., 1991; Cooper *et al.*, 1992; Helm-Bychowsky and Cracraft, 1993; Kocher *et al.*, 1989; Meyer, 1994; Kornegay *et al.*, 1993; Taberlet *et al.*, 1992; Hedges and 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 and Price (1992), Helm-Bychowsky and Cracraft (1993), Kocher *et al.* (1989), Meyer (1994), Taberlet *et al.*, (1992), Heidrich and Wink (1994), Heidrich *et al.*, (1995), Wink (1994), Wink *et al.* (1993a,b; 1994, 1996) and Seibold *et al.* (1993, 1995).

Parallel to our studies on the molecular phylogeny of raptors (Seibold, 1994; Seibold *et al.*, 1993, 1995; Wink and Seibold, 1995) Avise *et al.* (1994) have analyzed the nucleotide sequences of cyt *b* from New World vultures in relation to storks and other members of the Ciconiiformes. Avise *et al.* (1994) showed that New World vultures (4 species studied) -as suggested earlier (König, 1982; Rea, 1983; Sibley and Ahlquist, 1990)- appear to be closer related to storks than to Old World vultures (3 species studied). Our own data set of vultures comprised more taxa from Old World vultures (11 taxa) (Seibold 1994; Wink and Seibold, 1995). For this communication we have combined both sets of sequence data (which now covers 6 of 7 New World and 11 of 15 Old World vultures) and have analyzed the phylogenetic relationships within the group of Old world vultures, between Old and New World vultures and those of both groups in relation to other members of the Accipitridae and of the order Ciconiiformes (sensu Sibley and Monroe, 1990).

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): PCR and sequencing primers (modified from Kocher *et al.*, 1989): (5' – 3'; positions in the mtDNA of *Gallus gallus*; L = L-strand, H = H-strand); mt-A (L-14995): CTCCCAGCCC CATCCAACAT CTCAGCATGA TGAAACTTCG, mt-F (H-16065): CTAAGAAGGG TGGAGTCTTC AGTTTTTGGT TTACAAGAC, mt-B (H-15298): TTGTGATTAC TGTAGCACCT CAAAATGATA TTTGTCCTCA, mt-C (L-15320): TAYGTCCTAC CATGAGGACA AATATCATTC TGAGG, mt-D (L-15578): AAAATCCCAT TCCACCCCTA CTACTCCACA AAAGA, mt-G (L-15180): CWTCCTTMTT CTTCATCTGC ATCTAC, and mt-H (L-15722): CCYCCACACA TCAAACCMGA ATGATACTTC CTATT. PCR conditions: 1 µg of total DNA, 50 pmol each of primers mt-A and mt-F, 1.5 mM $MgCl_2$ and 2 units Taq-polymerase (Promega). After initial denaturation (2.5 min at 94 °C), 32 cycles of 30 sec at 93 °C, 45 sec at 45 °C and 90 sec at 72 °C were performed on a Biometra thermocycler. PCR products were separated by agarose gelelectrophoresis (1% agarose), excised and extracted using the Qiaex gel purification kit (Diagen). After elution, the amplified DNA was precipitated with isopropanol, sodium acetate and glycogen as a carrier. The pellet was redissolved in 6.5 µl H_20. Direct sequencing of the double-stranded DNA was carried out with the chain termination method at room temperature using ^{35}S-dATP as a radioactive marker and Sequenase 2.0 (USB) according to the distributor's specifications. Primer mt-B, mt-G, mt-C, mt-D and mt-H were used as sequencing primers in such a way that overlapping sequences were obtained. Products of the sequencing reactions were separated on a 6 % polyacrylamide/7 M urea gel by electrophoresis at 65 Watt. After drying, the gel was exposed to X-ray film for 3–4 days. About 300–400 nucleotides were readable per sequencing run.

Nucleotide sequences for 1026 bp of the cyt b gene (corresponding to positions 14995 to 16020 of mtDNA from *Gallus gallus*; Desjardins and Morais, 1992) are documented in Table I; although some sequences for vultures have been published

Table I. Nucleotide sequences of cytochrome *b* from Old and New World vultures. . = base identical to that in the first line; ? = base could not be determined with certainty.

	11111111112	22222222233333333334	44444444455555555556	66666666677777777778
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	GGGTCCCTACTAGGAATCTG	CCTGCTCACACAGATCCTAA	CTGGCCTCCTACTAGCTACG	CACTACACCGCAGACACAGC
Neophron percnopterus	.....T..C.....	...AA.....A.....	.....T.....C.TA	.....
Necrosyrtes monachus	..A.....C.....	...A..A.....A.....	.C.....T..A.TA	.....T.....T.
Aegypius monachus	..C.....C.....	...A..A.....A.....	.C.....A.TA	.....T.....T.
Sarcogyps calvus	..A..T..C.....	...A..A.....A...G...	.....A.TA	.....T.
Torgos t. negevensis	.....C.....	...T..A.....A.....	.C.....G.TA	.....T.....T...T.
Torgos t. tracheliotus	.....C.....	...T..A.....A.....	.C.....G.TA	.....T...T.
Trigonoceps occipitalis	..A.....C.....	...A..A.....A.....	.....A.TA	.....T...T.
Gyps coprotheres	.....T..T.....	...A..G.....A...T.G.	.C.....T..A.TA	.....T.
Gyps africanus	.....T..T.....	...A..G.....A...T...	.C.....T..A.TA	.....T.
Gyps fulvus	.....T..T.....	...A..G.....A...T...	.C.....T..A.TA	.....T.
Vultur gryphus	..A.....G.....C.....	...AA.G.....A.....	.C.....A.....G..C.TA	.....G.....A.
Cathartes aura	..A.....C.....	...AG.G..C..A.....	.C...T.A.....C.TA	..T..T..T.....A.

	1	11111111111111111111	11111111111111111111	11111111111111111111
	88888888899999999990	00000000011111111112	22222222233333333334	44444444455555555556
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	ACTAGCCTTCTCATCCGTCG	CCCATACATGCCGAAACGTA	CAGTACGGCTGACTAATCCG	CAACCTACACGCTAACGGCG
Neophron percnopterus	.....G.....T.	....C.T.....G....C	..A.....T.....	.....T.....A.
Necrosyrtes monachus	C.....T..T..G.....T.	....C.....	.....T..A.....	T.....T..C.....A.
Aegypius monachus	C.....T.....	.....	..A.....A..G.....	.....G.....C.....A.
Sarcogyps calvus	C.....T.....	....C.....	.....A.....?..	...T.....C.....
Torgos t. negevensis	C.....T..T.....	....C.....T.....	..A.....A.....	.....C.....A.
Torgos t. tracheliotus	C.....T..T.....	....C.....T.....	..A.....A.....	.....C.....A.
Trigonoceps occipitalis	C.....T.....	.....T.....	.....A..G.....	.....C..T..A.
Gyps coprotheres	CT.....T.....T.	....C.....	.....G.....	.....T..C.....A.
Gyps africanus	C.....T.....T.	.T..C.....	...?....G.....	...T.....T..C.....A.
Gyps fulvus	CT.....T.....T.	.T..C.....	...?....A.....	.....T..C.....A.
Vultur gryphus	C.....	....C.....T.....	..A.....	.....T..A.....A.
Cathartes aura	.....T..G.....	.T..C.....T.....	..A..T.....	.....T..A.....A.

Table I. Continued.

	11111111111111111111	11111111111111111112	22222222222222222222	22222222222222222222
	66666666677777777778	88888888899999999990	00000000011111111112	22222222233333333334
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	CATCATTCTTCTTCATCTGC	ATCTACCTGCACATTGGCCG	AGGACTCTACTACGGCTCCT	ACCTGTACAAAGAAACTTGA
Neophron percnopterus	.C.....	.....C.....	.....T.....T.....G.	..T.A.....C...
Necrosyrtes monachus	....T.....T..T...	.....C.....C.....	...C.....A.	....A..T.....G..A...
Aegypius monachus	....C.....T...	.....T.....	...C.....A.	.T..A..T.....G..C...
Sarcogyps calvus	....C.....T...	.....T..T..T..C.....	...T.....A.	....A.....C...
Torgos t. negevensis	....C.....T...	.....T.....	...C.....A.	.T..A..T.....G..C...
Torgos t. tracheliotus	....C.....T...	.....T.....	...C.....A.	.T..A..T.....G..C...
Trigonoceps occipitalis	....CC.....T...	.....T.....C.....	T..C.....A.	.T..A..T.....G..C...
Gyps coprotheres	....C.....T...	.....A.....C.....	...T.....A.	....A..T.....G..C...
Gyps africanus	....C.....T...	.....C.....	...C.....T.....A.	....A..T.....G..C...
Gyps fulvus	....C.....T...	.....A.....C.....	...C.....A.	....A..T.....G..C...
Vultur gryphus	.T.....	.....C.....A..	.....T.....T..T..G.	....A.....A...
Cathartes aura	.T.....T	..T.....A..T..C..A..	.....T.....A.	.....A...

	22222222222222222222	22222222222222222222	22222222222222222223	33333333333333333333
	44444444455555555556	66666666677777777778	88888888899999999990	00000000011111111112
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	AATACAGGAGTCATCCTTCT	ACTCACCCTCATAGCAACTG	CCTTCGTAGGATATGTTCTA	CCGTGA?GCCAAATATCCTT
Neophron percnopterus	.....C..	.....	.....C...	..A...G.A..G.....
Necrosyrtes monachus	..C.....A.....C..	...T..T.....	.....T.....A...	..A...G.A.....
Aegypius monachus	..C.....A..G.A.....	G.....	.....C.....C...	..A...G.G.....?.
Sarcogyps calvus	..C.....GA.....?T.	...T.....	.....C.....?	..A...G.A....?.....
Torgos t. negevensis	..C.....A..G.T.....	G.....	.....C.....C...	..A...G.G.....
Torgos t. tracheliotus	..C.....A....T.....	G.....	.....C.....C...	..A...G.G.....
Trigonoceps occipitalis	.....GA..G.....	G.....G.....	.....C.....	..A...G.A.....
Gyps coprotheres	..C.....A.T.....CT.	G.....G.....	.....T..C..C...	..A...G.A.....?.....
Gyps africanus	..C.....A.....CT.	G.....G.....	.....T..C..C...	..A...G.A.....
Gyps fulvus	..C.....A.T.....CT.	G.....G.....	.....T..C..C...	..A...G.A.....G.....
Vultur gryphus	..C.....C.....C..	.....	.....G..C..C..C...	..A...G.A.....A..
Cathartes aura	..C.....T.....C..	.....	.T.....G..C..C..C...	..A...G.A.....A..

Table I. Continued.

	3333333333333333333	3333333333333333333	3333333333333333333	3333333333333333334
	2222222233333333334	4444444445555555556	6666666667777777778	8888888889999999990
	1234567890123456789	1234567890123456789	1234567890123456789	1234567890123456789
Gypaetus barbatus	CTGAGGGGCCACAGTCATCA	CCAACCTATTCTCCGCAATC	CCATATATCGGACAGACTCT	CGTAGAATGAGCCTGAGGAG
Neophron percnopterus	.....T.....	.....G.....G..C...	.....C..T.....AT.C..	.....?.
Necrosyrtes monachus	.....A..T.....T....	.....A..T...	.....C.....A..C..	.....
Aegypius monachus	.....T.....	.....A..T...	.....C.....A..C..	T.....G.....
Sarcogyps calvus	.....?.....	.....A..C...	.....C.....A..C..	T.....G..G.....
Torgos t. negevensis	.....A.....T....	.....A..TG.A	.....C.....A..C..	T.....G..G.....G.
Torgos t. tracheliotus	.....A.....T....	.....A..TG.A	.....C.....A..C..	T.....G..G.....G.
Trigonoceps occipitalis	.....T.....	.....A..CG..	.....C.....A..C..	T.....G.....G.
Gyps coprotheres	.....T.....	.....A..C...	.....C.....A..C..	T.....
Gyps africanus	.....T.....	.....A..C...	.....C.....A..C..	T.....
Gyps fulvus	.....T.....	.....A..C...	.....C.....A..C..	T.....G.
Vultur gryphus	.....T.....G....	....TT.....A..C...	..C.....C..A..C..	T.....G..A.....
Cathartes aura	...G..T.....A..T.	.T.....A..T...	..T..C.....C..A..C..	T.....A.....G.

	44444444444444444444	44444444444444444444	44444444444444444444	44444444444444444444
	00000000011111111112	22222222233333333334	44444444455555555556	66666666677777777778
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	GCTTTTCAGTAGACAACCCA	ACCCTTACACGATTTTTCGC	CCTACACTTCCTACTCCCAT	TCCTAATTGCAAGCCTCACC
Neophron percnopterus	.A..C.....??...C	..A..C..T.....	.....	.....
Necrosyrtes monachus	....C..C.....	..T..C..C..G..C..T..	.T.G.....T....T....	..G....C..GG.T.....T
Aegypius monachus	....C..C.....C	.....C..C..G..C.....	AT.....T....T....	..GC...C...G.....
Sarcogyps calvus	.T..C..C.....T	.....C..C.....C..T..	.T....T.....T....	..GC...C...G....T...
Torgos t. negevensis	....C..C.....C	.....C..C..G..C.....	AT.....T....T....	..G....C...G.T.....
Torgos t. tracheliotus	....C..C.....C	.....C..C..G..C.....	AT.....T....T....	..G....C...G.T.....
Trigonoceps occipitalis	....C..C.....C	.....C..T..G..C..T..	AT.G.....T....T....	..G....C..GG.T..T...
Gyps coprotheres	....C..C.....T.....C	.....C..C.....C.....	...G..T...T....T....	.TG....C...G.T..T..T
Gyps africanus	....C..C.....C	.....C..C.....C.....	...G..T...T....T....	.TG....C...G....T..T
Gyps fulvus	....C..C.....T.....C	.....C..C.....C.....	...G..T...T....T....	.TG....C...G.T..T..T
Vultur gryphus	.A..C.....C	.....A..T.....C..T..	.T.....T..C.....C.	.TGC.....G.....
Cathartes aura	.A.....C	..T..G..C.....C..T..	.T.....C.....C.	..GC.....G....T...

Table I. Continued.

	66666666666666666666	66666666666666666666	66666666666666666667	77777777777777777777
	44444444455555555556	66666666677777777778	88888888899999999990	00000000011111111112
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	CCAACTTTCTAGGTGACCCA	GAAAACTTTACCCCAGCAAA	CCCTCTAGTCACACCCCCAC	ATATCAAACCTGAATGATAC
Neophron percnopterus	.A.....CT.....T	.....?.....	.....?..C..T..C.	.....G..C.....
Necrosyrtes monachus	.A...C.A.....C.....	.....C.....C.....	...A.....	.....G..A.....T
Aegypius monachus	.A...C.GT....A.....	.....C..T..C.....	...A.....G.	.....A..G.....
Sarcogyps calvus	.A...C.A..G..C.....	.....C.....C.....	...A.....	.....A..G.....T
Torgos t. negevensis	.A...C.AT....C.....	.....C.....T.....	...A.....	.....A..G..G...
Torgos t. tracheliotus	.A...C.AT....C.....	.....C.....T.....	...A.....	.....A..G..G...
Trigonoceps occipitalis	.A...C.A?....C.....	.....C..?..?.....	...A.....	..?.....A.....
Gyps coprotheres	.A...C.AT....A.....	.....C.....	...A.....	....T..G..A.....T
Gyps africanus	.A...C.?T....?.....	.....C.....	...G.....	.....G..A.....T
Gyps fulvus	.A...C.?T....?.....	.....C.....	...A.....	.....G..A.....T
Vultur gryphus	.T..?C.G.....A.....	.....C..A.....	...A.....	.C.....A.....
Cathartes aura	.T..T..AT....A.....	.....C..A..C.....	.....A..C.	.....G..A..G.....T

	77777777777777777777	77777777777777777777	77777777777777777777	77777777777777777778
	22222222233333333334	44444444455555555556	66666666677777777778	88888888899999999990
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	TTCCTGTTTGCCTACGCCAT	CCTACGCTCCATCCCAAACA	AGCTGGGAGGAGTACTGGCC	CTGGCCGCCTCCGTACTAAT
Neophron percnopterus	.....A..C..A.....T..	.....A.....C....	.AT.A.....C..A...	..A..T..T.....T....
Necrosyrtes monachus	.....A.....A..T.....	T.....A.....T.	.A..A.....	T.A.....T.....
Aegypius monachus	.....A.....	T.....A..T.....T.	.A..A.....C..A...	..A.....
Sarcogyps calvus	.....A.....	T.....A..T.....	.A..A.....C..A..T	T.A.....
Torgos t. negevensis	.....A.....T..	T.....A..T.....T.	.A.....C..A...	..A.....
Torgos t. tracheliotus	.....A.....T..	T.....A..T.....T.	.A.....C..A...	..A.....
Trigonoceps occipitalis	..T..A.....	T.....A.....T.	.A..A..G..?..T..A...	..A..T.....T.....
Gyps coprotheres	..T..A..C..A.....T..	T.....A.....T.	.A..A.....A...	T.A..T.....
Gyps africanus	.....A..C..G.....T..	T.....A.....T.	.A..A.....A...	T.A..T.....
Gyps fulvus	..T..A..C..G.....T..	T.....A.....T.	.A..A.....A...	T.A..T.....
Vultur gryphus	.....A..C..A..T.....	T.....A.....C..T.	.A..A.....A...	..A.....G.
Cathartes aura	.....C.....T.....	T..C.....A.....C....	.A..A.....A...	..A..T.....

Table I. Continued.

	6666666666666666666	6666666666666666666	6666666666666666667	7777777777777777777
	44444444455555555556	66666666677777777778	88888888899999999990	00000000011111111112
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	CCAACTTTCTAGGTGACCCA	GAAAACTTTACCCCAGCAAA	CCCTCTAGTCACACCCCCAC	ATATCAAACCTGAATGATAC
Neophron percnopterus	.A.....CT.........T	........?..........	.........?..C..T..C.	.......G..C.........
Necrosyrtes monachus	.A...C.A.....C.....	........C.....C.....	...A..............	.......G..A.....T
Aegypius monachus	.A...C.GT....A.....	........C..T..C.....	...A.............G.	..........A..G.....
Sarcogyps calvus	.A...C.A..G..C.....	........C.....C.....	...A..............	..........A..G.....T
Torgos t. negevensis	.A...C.AT....C.....	........C.....T.....	...A..............	..........A..G..G...
Torgos t. tracheliotus	.A...C.AT....C.....	........C.....T.....	...A..............	..........A..G..G...
Trigonoceps occipitalis	.A...C.A?....C.....	........C..?..?.....	...A..............	..?.......A.........
Gyps coprotheres	.A...C.AT....A.....	........C..........	...A..............	....T..G..A.....T
Gyps africanus	.A...C.?T....?.....	........C..........	...G..............	.......G..A.....T
Gyps fulvus	.A...C.?T....?.....	........C..........	...A..............	.......G..A.....T
Vultur gryphus	.T..?C.G.....A.....	........C..A.......	...A..............	.C.........A.........
Cathartes aura	.T..T..AT....A.....	........C..A..C.....	..............A..C.	.......G..A..G.....T

	77777777777777777777	77777777777777777777	77777777777777777777	77777777777777777778
	22222222233333333334	44444444455555555556	66666666677777777778	88888888899999999990
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	TTCCTGTTTGCCTACGCCAT	CCTACGCTCCATCCCAAACA	AGCTGGGAGGAGTACTGGCC	CTGGCCGCCTCCGTACTAAT
Neophron percnopterus	.....A..C..A.....T..	.........A.....C....	.AT.A........C..A....	..A..T..T......T....
Necrosyrtes monachus	.....A.....A..T.....	T........A........T.	.A..A..............	T.A........T.........
Aegypius monachus	.....A..............	T........A..T.....T.	.A..A........C..A....	..A..................
Sarcogyps calvus	.....A..............	T........A..T........	.A..A........C..A..T	T.A..................
Torgos t. negevensis	.....A..........T..	T........A..T.....T.	.A..........C..A....	..A..................
Torgos t. tracheliotus	.....A..........T..	T........A..T.....T.	.A..........C..A....	..A..................
Trigonoceps occipitalis	..T..A..............	T........A........T.	.A..A..G..?..T..A....	..A..T........T.....
Gyps coprotheres	..T..A..C..A.....T..	T........A........T.	.A..A..........A.....	T.A..T..............
Gyps africanus	.....A..C..G.....T..	T........A........T.	.A..A..........A.....	T.A..T..............
Gyps fulvus	..T..A..C..G.....T..	T........A........T.	.A..A..........A.....	T.A..T..............
Vultur gryphus	.....A..C..A..T.....	T........A.....C..T.	.A..A..........A.....	..A..............G.
Cathartes aura	........C.....T.....	T..C.....A.....C....	.A..A..........A.....	..A..T..............

Table I. Continued.

	88888888888888888888	88888888888888888888	88888888888888888888	88888888888888888888
	00000000011111111112	22222222233333333334	44444444455555555556	66666666677777777778
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	CCTATTCCTAATCCCCCTCC	TCCACAAATCCAAACAGCGC	ACAATAACCTT?CGGCCCCT	CTCCCAACTCCTATTCTGAT
Neophron percnopterus	T........T?C......T.	......T..............	..........C..?.....	G....................
Necrosyrtes monachus	..................T.T.	....T.....A.....	..........T..A.....	..............AT...A
Aegypius monachus	..................T.T.	.....	..........C..A.....	..............A....A
Sarcogyps calvus	.........?......TCT.	.T..T.T.....T	..........C..A...A.	T..T..G..T....A....A
Torgos t. negevensis	..................T.T.	..........T.....A...	..........C.....	..............A....A
Torgos t. tracheliotus	..........?....T.T.	..........T.....A.,,.	..........C.....	..............A....A
Trigonoceps occipitalis	..................T...	..............A..?.	..........T.....	T.............A....G
Gyps coprotheres	..................T.T.	.A.....,,.	..........T..A..T..	..............A....A
Gyps africanus	..................T.T.	.A.....	......G....T..A..T..	..............A....A
Gyps fulvus	..................T.T.	.A.....A...	..........T..A..T..	..............A....A
Vultur gryphus	......T.....A..TT.T.	.....A...	..........T..C..A..	...T..............A
Cathartes aura	............A..TT...	....T.....T.....A..T	..........T..C..A..	..................A

	88888888888888888889	99999999999999999999	99999999999999999999	99999999999999999999
	88888888899999999990	00000000011111111112	22222222233333333334	44444444455555555556
	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890
Gypaetus barbatus	CCCTAG?CGCAAACCTCCT?	ATCCT?ACATG?GTAGGCAG	CCAGCCAGTAGAGCACCCCT	TCA?CATCATTGGCCAACTA
Neophron percnopterus	.T....T.....C	..T..C.....G..G.....	...A..C.....	...C....C.....
Necrosyrtes monachus	.....AT...C.....T	.....C.....AA.C..T..	...A.....	.T.T......C..T.....
Aegypius monachus	.....AT...C.....T	.....C.....AA.C.....	...A.....A.....	...T......C..T.....
Sarcogyps calvus	.....AT...C.....T	.....C.....AA.C.....	...A.....A.....	...T...T.....T.....
Torgos t. negevensis	....GAT...T.....T	.....T.....AA.C..T..	...A.....A.....	...T......C..T.....
Torgos t. tracheliotus	....GAT...T.....T	.....T.....AA.C..T..	...A.....A.....	...T......C..T.....
Trigonoceps occipitalis	..T..AT...C.....T	.....T.....AA.C.....	...A.....A.....	...T......C.....
Gyps coprotheres	.....AT...C..T.....C	.....C.....AA.C.....	.....A.....	...T......?.....
Gyps africanus	..T..AT...C..T..T..C	.....C.....AA.C.....	...A.....A..G....	...T......C.....
Gyps fulvus	.....AT...C..T.....C	.....C.....AA.C.....	.....A.....	...T......C.....
Vultur gryphus	.A....T...C.....C	.....A.....G.....	...A..C.....A.....G.	...T.....?
Cathartes aura	.A....CA..C.....T..C	.....A.....A..G.....	...A.....A..T..A.	...T......C.....

Table I. Continued.

	1	1111111111111111111	111111
	99999999999999999999	99999999999999999990	00000000000000000000 000000
	66666666677777777778	88888888899999999990	00000000011111111112 222222
	12345678901234567890	12345678901234567890	12345678901234567890 123456
Gypaetus barbatus	GCCTCCCTCACCTACTTCAC	CATCCTCTTAATTCTATTTC	CCCTAGCCGGAGCCCTAGAA AATAAA
Neophron percnopterus	.....T.....TG.	..C....C.....C.?.	.....A.....A..... .....
Necrosyrtes monachus	.....A.T.....	T..T..TC..G....CC.C.	.....A.T..G..... .....
Aegypius monachus	.....A.....	.....TC..G.C..C..C.	..T..A.T..G..... .....
Sarcogyps calvus	.....TA.T.....	T.....TC..G....C..C.	.....T..G..T..... .....
Torgos t. negevensis	.....TA.....	.....TC..G.C..C....	.....A.T..G..... .....
Torgos t. tracheliotus	.....TA.....	.....TC..G.C..C....	.....A.T..G..... .....
Trigonoceps occipitalis	.....TA.....T..	.....TC..G.C..C..C.	.....A.T..G..T..... .....
Gyps coprotheres	.....A..G.....T..	T.....TC..G....C..C.	..T..A.T..G..... .....
Gyps africanus	.....A.....T..	T.....TC..G....C..C.	..T..A.T..G..... .....
Gyps fulvus	.....A.....T..	T.....TC..G....C..C.	..T..A.T..G..... .....
Vultur gryphus	.....	.....C..?.C..C..C.	..ACCCT..... .....
Cathartes aura	.....	.....C.T..C..C..C.	..A.CCT..... ..C...

already (Avise *et al.*, 1994), we have included our corresponding sequences in Table I, since our sequences derived from birds of different origins and they also differ in a few nucleotide positions from those published by Avise *et al.*(1994). Sequence data will be deposited in the EMBL data library. Other sequences were obtained from GenBank; e.g., *Cathartes burrovianus, Coragyps atratus, Gymnogyps californianus, Pelecanus erythrorhynchus, Phoenicopterus andinus, Phoenicopterus ruber, Platelea alba, Plegadis ridgwayi, Mycteria americana* derived from Avise *et al.* (1994), *Alectoris chucar, Coturnix coturnix, Pavo cristatus, Lophura nyctemera* from Kornegay *et al.*, (1993), *Grus vipio* from Krajewski and Fetzner (1994).

Sequence analysis

Nucleotide sequences were aligned with the cytochrome *b* sequence of *Gallus gallus* (Desjardins and Morais 1990). Phylogenetic trees were reconstructed using the maximum parsimony method with the phylogeny program PAUP 3.1.1 (Swofford, 1993), the distance method neighbour-joining and the unweighted pair-group method with arithmetic means (UPGMA) using the program 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 using both simple and random sample addition (results were identical). Bootstrap analyses (Felsenstein, 1985) were performed to evaluate the robustness of the furcations found.

Results and Discussion

Sequences of the cytochrome b gene

DNA was isolated from 29 individuals of 11 Old World and 5 specimens of 3 New World vultures. The mitochondrial cyt b gene was amplified by PCR and sequenced directly. In addition to our own data, cyt *b* sequences of three further vulture species (Avise *et al.*, 1994) were obtained from GenBank. Two datasets were produced: Set I consists of a 300 bp portion of the cyt b gene including all species and specimens, whereas set II is reduced to one bird per species and contains taxa only for which ≥ 1000 bp were available.

Base composition and mode of nucleotide substitutions

Theoretically, the four bases should be equally represented in a gene and occur with a frequency of 25% at each codon position. In the reality of our datasets, base composition is uneven: G (abundance 4.5%) and T (U) (13.1%) are significantly (t-test, $p<0.001$) underrepresented in the third codon position whereas C (30.7%) is overrepresented at the first and T (U) (40.3%) at the second codon position. A similar picture emerged for cyt b sequences of mammals (Anderson *et al.*, 1991; Arnason and Johnsson, 1991, 1992; Irwin *et al.*, 1991) and birds (Desjardins and Morais, 1990; Birt *et al.*, 1992; Kocher *et al.*, 1989; Kornegay *et al.*, 1993). It has been suggested that codon usage

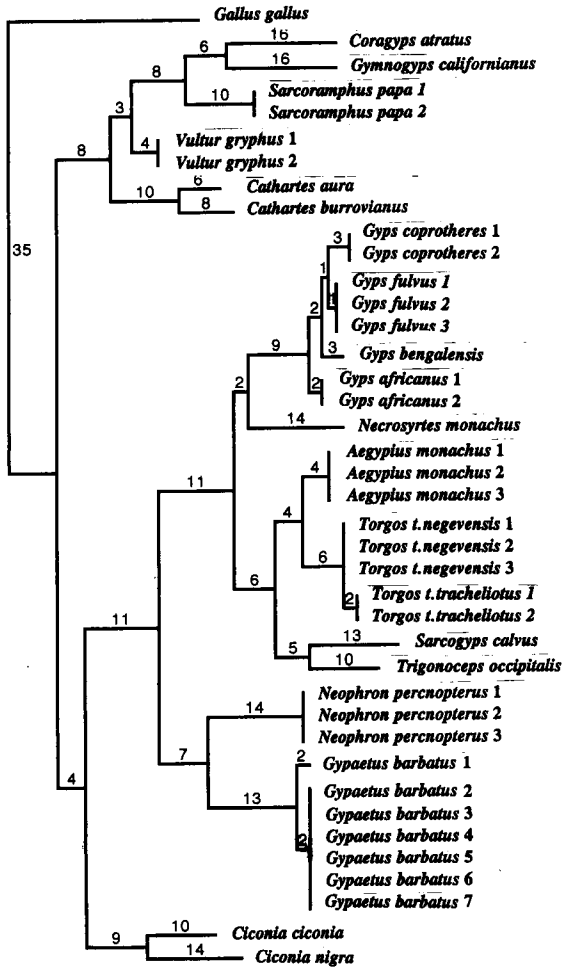

Fig. 1. Reconstruction of phylogenetic relationships between vultures by maximum parsimony (MP) based on 300 bp of the cytochrome *b* gene (corresponding to nucleotide positions 14995 to 15294 of mtDNA of *Gallus gallus* (Desjardins and Morais, 1992)). If available, several individuals per species were analyzed to assess intraspecific variation. The strict consensus tree is illustrated as a phylogram in which branch lengths are proportional to genetic distances. Number of nucleotide substitutions between taxa are given at each branch. Conditions, heuristic search with simple taxon addition and *Gallus gallus* as an outgroup; three most parsimonious trees were obtained (length 314 steps; minimally 162, maximally 992 steps; Consistency index (CI) = 0.516, retention index (RI)= 0.817, homoplasy index (HI)= 0.484) which differed only in the relative positions of the two *Torgos* subspecies to each other.

is biased thus leeding to unequal base composition (Friesen *et al.*, 1993).

Transitions (ns) (nucleotide substitutions between G and A, or T and C) are more common than transversions (nv) between closely related species. The longer the time of sequence divergence, multiple substitutions lead to an enrichment of transversions. As a consequence, the ns/nv coefficient approaches ≤ 1 if species have diverged several (> 15) million years ago (Kornegay *et al.*, 1993; Avise, 1994).

Within the vulture group (data set II) 385 positions are variable and 274 phylogenetically informative. Base substitutions are more abundant in the third codon position (n=282) than in first (n= 78) or second position (n=25). As a consequence many substitutions are "silent" and therefore less subjected to convergence than phenotypic characters. This feature is especially useful when analyzing relationships between related taxa. Due to multiple substitutions third positions may become saturated (homoplasy) which can produce difficulties when analyzing distantly related groups.

Intraspecific variation was very low (Fig.1) and only encountered in *Gypaetus barbatus* where two sequence types were found which differed by 0.6% nucleotide substitutions.

Genetic distances

The genetic distances between vultures are documented in Table II. A summary of mean distances (and their standard deviations) encountered in different hierarchic levels of birds of prey is illustrated in Table 3. Although taxonomic ranks (e.g., genera, families) are artificial, the distance criterium can be useful in some instances to discuss the taxonomic position of a species (see below).

Phylogenetic reconstructions within the vulture-complex

The aligned sequence data produce a g1 value of -0.616 (dataset II) indicating that the sequence data contain a significant ($p<0.01$) phylogenetic signal (Hillis and Huelsenbeck, 1992). When evaluated with distance matrix, phenetic and character state methods, trees produced by NJ, UPGMA or MP had almost identical topologies indicating that the trees are supported by informative sequence data.

Fig. 1 represents a phylogram for data set I (300 bp), whereas all other figures used data set II (≥1000 bp). As can be seen from Fig.1, 2 (A-C)

Table II. Genetic distances (nucleotide substitutions) between vultures (data set II).

OTUs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		0.0760	0.0908	0.0803	0.0792	0.0866	0.0771	0.0760	0.0718	0.1362	0.1468	0.1278	0.1436	0.1637	0.1521	0.1447
2			0.0707	0.0317	0.0348	0.0539	0.0792	0.0760	0.0750	0.1214	0.1373	0.1151	0.1341	0.1478	0.1436	0.1394
3				0.0834	0.0824	0.0824	0.0845	0.0803	0.0824	0.1352	0.1478	0.1299	0.1510	0.1626	0.1647	0.1552
4					0.0032	0.0623	0.0908	0.0876	0.0845	0.1309	0.1457	0.1257	0.1489	0.1616	0.1489	0.1489
5						0.0634	0.0898	0.0866	0.0834	0.1299	0.1447	0.1225	0.1468	0.1605	0.1499	0.1457
6							0.0813	0.0792	0.0750	0.1404	0.1531	0.1288	0.1468	0.1605	0.1563	0.1595
7								0.0232	0.0095	0.1309	0.1542	0.1320	0.1320	0.1521	0.1510	0.1573
8									0.0180	0.1299	0.1447	0.1331	0.1320	0.1531	0.1521	0.1542
9										0.1278	0.1478	0.1299	0.1331	0.1489	0.1468	0.1542
10											0.0887	0.1299	0.1362	0.0961	0.1035	0.0929
11												0.1394	0.1489	0.1119	0.1077	0.0634
12													0.1077	0.1510	0.1510	0.1436
13														0.1563	0.1626	0.1510
14															0.0866	0.1162
15																0.1204
16																

Taxa: *1. Necrosyrtes monachus, 2. Aegypius monachus, 3. Sarcogyps calvus, 4. Torgos t. negevensis, 5. Torgos t. tracheliotus, 6. Trigonoceps occipitalis, 7. Gyps coprotheres, 8. Gyps africanus, 9. Gyps fulvus, 10. Vultur gryphus, 11. Cathartes aura, 12. Gypaetus barbatus, 13. Neophron percnopterus, 14. Gymnogyps californianus, 15. Coragyps atratus, 16. Cathartes burrovianus.*

and 3 (A,B), the vultures cluster in three major clades: Clade A represents the New World Vultures, clade B the *Gypaetus/Neophron* group and clade C the *Aegypius/Gyps*-complex. These major groupings are supported by high bootstrap values, irrespective of the methods used for phylogenetic reconstructions.

Aegypius/Gyps-complex

The four monotypic genera of the *Aegypius* complex (*Aegypius, Torgos, Trigonoceps* and *Sarcogyps*) are separated by 3.8 to 9.1% nucleotide substitutions (Table II) suggesting that speciation within this complex occurred during the last 2 to 5 million years, if the equation of Shields and Wilson (1987) is applied (2% sequence divergence is equivalent to 1 mio years). But this calibration must be treated with caution and derived divergence times can only be crude estimates.

Aegypius monachus and *Torgos tracheliotus* always cluster as sister species, which differ by 3.8% nucleotide substitutions. *Sarcogyps calvus* is usually included in the *Aegypius* complex (Fig.1, 2B,C) albeit with bootstrap values between 50 and 65% and even clusters outside in the MP tree shown in Fig. 2A. Since the genetic distances in the *Aegypius*-complex fall into the range which is typical for intrageneric differences (i.e. 7–8%; Table III) the view of Amadon and Bull (1988) should be recalled: Based on similarities in mor-

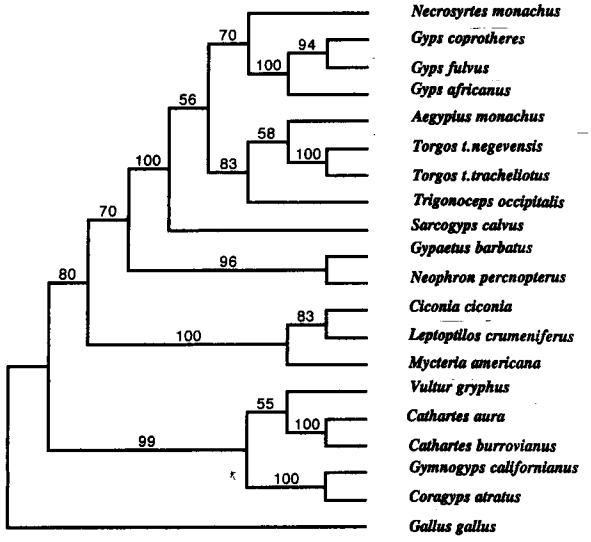

Fig. 2 A. Reconstruction of phylogenetic relationships between vultures and storks by maximum parsimony (MP), UPGMA and neighbour-joining (NJ) based on 1026 bp of the cytochrome b gene. *Gallus gallus* was used as an outgroup.
MP, Bootstrap analysis (150 replicates) with heuristic search and simple taxon addition. The bootstrap consensus tree (length 1100 steps; min. 585, max. 1871 steps; CI= 0.532, RI= 0.600, HI= 0.468) is illustrated as a **cladogram** (in contrast to the phylogram in Figures 1 and 2C branch lengths are **not** proportional to genetic distances). The "bootstrap" tree was identical with those produced by ordinary heuristic search.

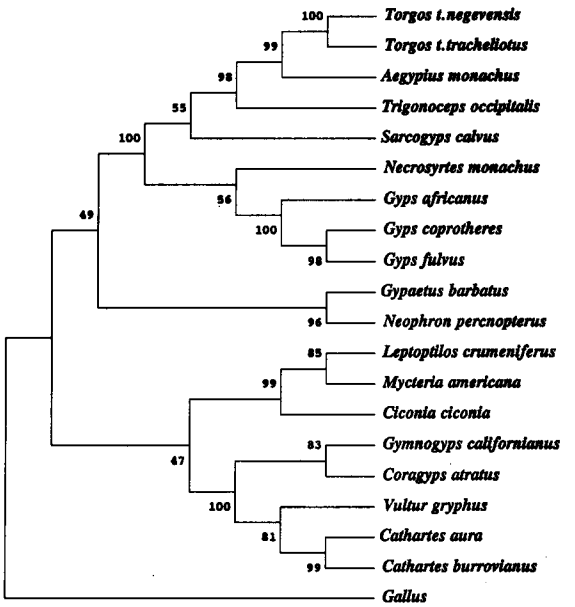

Fig. 2B. UPGMA, The phenetic relationships are shown as a cladogram; bootstrap values (500 replicates) are given at each nodes. (Note, UPGMA trees do not represent phylogenetic trees).

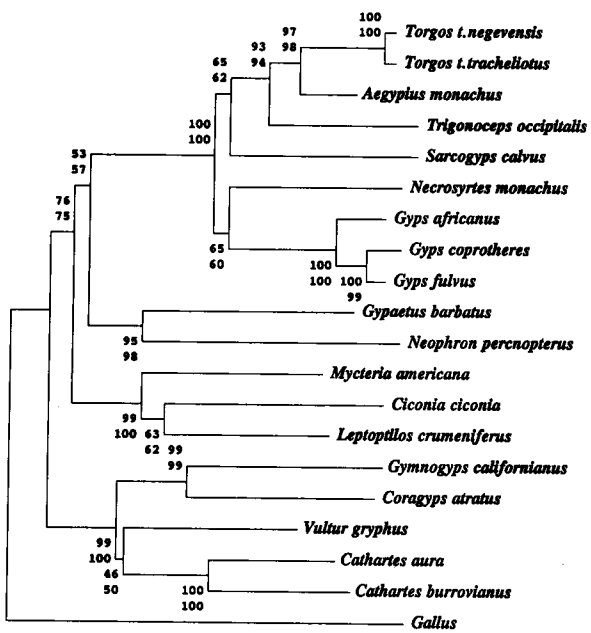

Fig. 2C. NJ, Percent bootstrap frequencies (500 replicates) are indicated above each furcation; upper values are based on Kimura two-parameter, lower value on p-distance method. Illustration as a phylogram; branch lengths are proportional to genetic distances. The distance algorithm Tamura-Nei produced a tree of almost identical topology. Abbreviations as in Fig. 1.

phology and behaviour these authors suggested to combine the 4 monotypic genera into the polytypic genus *Aegypius*.

Torgos tracheliotus negevensis which had been recognized as a subspecies recently (Bruun *et al.*, 1981) lives on the Arabian Peninsula (including the Negev) and can be distinguished from the nominate *T.t.tracheliotus* by a number of small but distinctive morphological characters (Bruun *et al.*, 1981; Mundy *et al.*, 1992; del Hoya *et al.*, 1994). *T.t.negevensis* can be separated from *T.t.tracheliotus* also on account of genetic differences, albeit with small distances (i.e., 0.3%). It has been speculated that *T.t. negevensis* is the result of a hybridisation (several million years ago) between *T.tracheliotus* and *Aegypius monachus*. Since mitochondrial genes are inherited maternally, our data rule out an ancient hybridisation between a female of *Aegypius* and a male *Torgos*, because of the small differences seen between *T.t.negevensis* and *T.t.tracheliotus* and comparably high ones between *T.t.negevensis* and *A. monachus* (Table II). Although we cannot rule out a hybridisation between a male *Aegypius* and a female *Torgos tracheliotus*, we consider this possibility rather unlikely; instead, we assume a divergence of *T.t.negevensis* from *T.t.tracheliotus* about 350000 years ago (using the "2% per million years" rule; Shields and Wilson, 1987; Avise 1994).

The genera *Gyps* and *Necrosyrtes* appear as a sister clade to the *Aegypius*-group in both MP and NJ analyses. This divergence is supported by significant bootstrap values (Figs.1–3). Nucleotide substitutions between *Necrosyrtes* and *Gyps* account for 7.4 to 7.8% and 8.6 to 9.1 to members of the *Aegypius* complex, indicating that *Necrosyrtes* was isolated from both groups 3 to 5 million years ago. Although the position of *Necrosyrtes* within the *Gyps*- or *Aegypius*-clade cannot be resolved with certainty (see low bootstrap values), a close relationship with *Neophron* which was suggested on account of morphological similarities (summarized in del Hoya *et al.*, 1994) is highly unlikely.

Distances within the *Gyps*-complex are very small, ranging from 1 to 2.3 (Table II) and sequence comparisons between *Gyps* species are characterized by a ns/nv coefficient of 21. *G. coprotheres* is closely related to *G. fulvus*; the degree of nucleotide substitution (i.e., 1%) (Table II)

Table III. Genetic distances (nucleotide substitutions) within and between genera of raptors and storks.

Comparison	Distance x	± s.d.	(n)
I. Within genera			
Haliaeetus	7.95	2.39	21
Aquila	5.86	2.07	28
Buteo	3.87	1.95	35
Circus	6.17	2.05	6
Gyps	1.75	0.64	3
Aegypius-complex	7.49	1.73	8
II. Between genera			
Gypaetus: other vultures	13.70	1.08	12
Neophron: other vultures	14.62	1.35	12
Neophron: Gypaetus	11.4		
Gyps: Aegypius-complex	8.54	0.65	18
within the New World vulture group	10.51	1.87	10
New World: Old World vultures	15.89	1.07	49
New World vultures; storks	14.89	1.01	24
Accipitridae: storks	15.17	0.60	18
Milvus: Haliaaetus/Pernis/Circus	10.30	0.85	18
Aquila: Pernis/Haliaeetus	12.61	0.85	23
Buteo: Accipiter/Circus/Pernis	11.62	1.07	63

and the high level of transitions suggests that both taxa have split from a common ancestor about 500000 years ago. *Gyps africanus* and *G. bengalensis* which differ by maximally 2.3% from other members of *Gyps* had been placed in a separate genus *Pseudogyps*, because both species have 12 tail feathers instead of 14 in *Gyps* and breed in trees instead in cliffs. Since genetic distances between genera usually fall in the range of 12–13% (Table III), we suggest that the name *Pseudogyps* should be omitted and the respective taxa included in the genus *Gyps*. *G. africanus* clearly differs from *G. bengalensis*, indicating that both vultures represent distinct species (Dowsett and Dowsett-Lemaire, 1980).

Neophron/Gypaetus-complex

Clade B includes *Neophron percnopterus* and *Gypaetus barbatus*, which is positioned at the base of the Accipitrid tree indicating that both vultures are related and represent an ancient group. This finding is in agreement with karyological (DeBoer and Sinoo, 1984) morphological, anatomical (Jollie 1976,1997a,b) and embryological data (Thaler *et al.*, 1986). Nucleotide substitutions between *Gypaetus* and *Neophron* account for 11%, which is typical for differences between genera. Although both species always cluster together, it seems to be fully justified to treat them as members of two monotypic genera. Long divergence times between *Neophron* and *Gypaetus* are implied by the ns/nv coefficient of 1.95 and long branches in Fig. 2. According to all phylogenetic reconstruction (Figs.1–3) the *Gypaetus/Neophron* group is neither closely related to the other Old World nor the New World Vultures. It thus represents an independent subgroup, supporting the view that vultures are polyphyletic.

New World vultures

As seen in Figs.1 and 2, the New World vultures are found in another independent clade A. Although the huge size of the Californian and Andean condor gives them superficial similarities, the Californian condor appears more closely related to *Coragyps atratus* than to the Andean condor (*Vultur gryphus*) (Figs.1–3). As expected from morphological appearance, *Catharthes aura* clusters together with *C. burrovianus*. Genetic distances between these cathartid vultures account for 10.0 to 12.8% and an ns/nv value of 3.7 is produced, suggesting long divergence times. According to the fossil record, the cathartid vultures have evolved much earlier than many other groups of birds with two fossil species *Palaeogyps* and *Phasmagyps* which originate from the early Oligocene about 35 million years ago. Fossils which appear closely related to modern condors have been detected in Pliocene (5 million years) or Pleistocene deposits (2 million years), indicating that the long branches in the phylogram (Fig. 2c) do indeed reflect long divergence times. According to Table III and the low ns/nv coefficients the classification of carthartid vultures into several monotypic genera (except *Cathartes* with 3 species) seems to be justified.

Phylogenetic position of Old and New World vulture in relation to other bird taxa

As seen in Figs.1 and 2, the vultures appear in at least three monophyletic clades, indicating a large degree of 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 and Ahlquist, 1990; Del Hoyo *et al.*, 1994). Also data from DNA-DNA-hybridisation (Sibley and 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.

Theoretically, the polyphyly of vultures and raptors should become even more visible if the cyt b sequence data from other members of the Falconiformes and Ciconiiformes would be jointly analyzed. 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, 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 and 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 and 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*.

The data set was analyzed by MP and NJ (Fig. 3). Since a saturation of substitutions can occur when cyt b sequences of distantly related taxa are to be compared, NJ analyses were also performed with transversions only in order to reduce "noise" (Fig.3B). MP and NJ trees share many similar clusters; other attributions (usually not supported by high bootstrap values) are more variable.

Within the family Accipitridae (sensu strictu), which are recognized as a monophyletic group in Figs. 2 and 3, some phylogenetic relationships from previous studies were confirmed: *Milvus* and *Haliaeetus* cluster together as sister groups (Wink *et al.*, 1996) and are always in a clade with *Buteo* and *Accipiter* (Wink and 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 bussard (*Pernis apivorus*) does not cluster with bussards of the genus *Buteo*, but can be found either together with or as a direct neighbor to *Neophron/Gypaetus*. Since this clade was 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. Although their nearest neighbour could not be determined unequivocally using cyt *b* sequences the phylogenetic reconstructions imply that falcons are not closely related to the Accipitridae.

Spoonbill and Puna ibis (both of the family Threskiornithidae) cluster together with high bootstrap values, as already reported by Avise *et al.* (1994). *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 MP tree reflects phylogenetic relatedness (see low bootstrap values and compare NJ tree). Members of the Galliformes form a common clade at the base of the reconstructions which was supported by a bootstrap value of 74% in MP trees (Fig. 3); phylogenetic relationships within this group were those found by Kornegay *et al.* (1993). *Cairina* (a representative for the Anseriformes) follows, but it only distantly related to the Galliformes (Fig. 3 A,B).

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 and Ahlquist, 1990). If storks (*Ciconia, Leptoptilos* and *Mycteria*) were included as an ingroup of the vulture sample (Fig. 2), they cluster as a separate clade (Fig.2 A,C). Only in UPGMA trees the storks occur as a direct sister clade to the cathartid vultures (although with a low bootstrap value of 47%; Fig. 2 B). In the phylogenetic reconstructions of Fig. 3, storks (together with *Phoenicopterus, Pelecanus* and *Calonectris*) are within the New World vulture assemblage; but the underlying

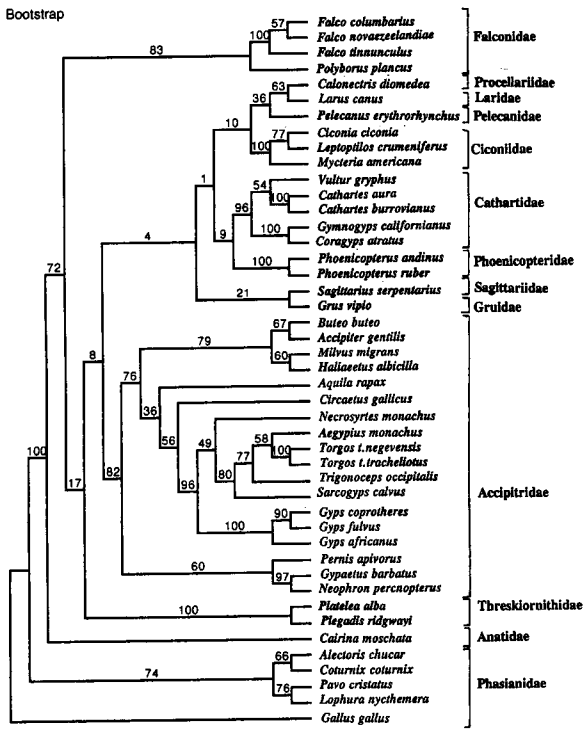

Fig. 3A. Reconstruction of phylogenetic relationships between vultures and members of the Ciconiiformes (sensu Sibley and Monroe, 1990) by maximum parsimony and neighbour-joining based on 1026 bp of the cytochrome b gene. *Gallus gallus* was used as an outgroup.
MP, Bootstrap analysis (100 replicates) with heuristic search and simple taxon addition. The bootstrap consensus tree (length 2987 steps; min. 882, max. 4787 steps; CI= 0.295, RI= 0.461, HI= 0.705) is illustrated as a cladogram.

furcations are not supported by high bootstrap values indicating that cyt b sequences cannot resolve the underlying phylogenetic events with certainty. 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% (Table III), i.e. in the same range. Summarizing these evidences, we conclude that a close relationship of cathartid vultures with storks appears unlikely. Avise *et al.* (1994) had included *Jabiru mycteria* in their analysis which clustered closely with the Black vulture, suggesting a close bond between storks and New World Vultures. It appears however, that this result might be an artifact and that the sample analyzed was not *Jabiru mycteria* (Avise and Nelson, 1995). Also another stork, *Mycteria ibis* was found to be closely associated with the New World vulture assemblage (Avise *et al.*, 1994). We had included the sequence of *M. ibis* also in a preliminary analysis; but since *M. ibis* always clustered as a close relative within the cathartid vulture clade (data not shown) and never with the true stork group which included another member of the genus *Mycteria*, we suspect a second artifact and have therefore omitted *M. ibis* from our further analyses. Since we cannot rule out a *M.ibis*/cathartid vulture relationship with certainty, this problem needs to be reevaluated with independent samples.

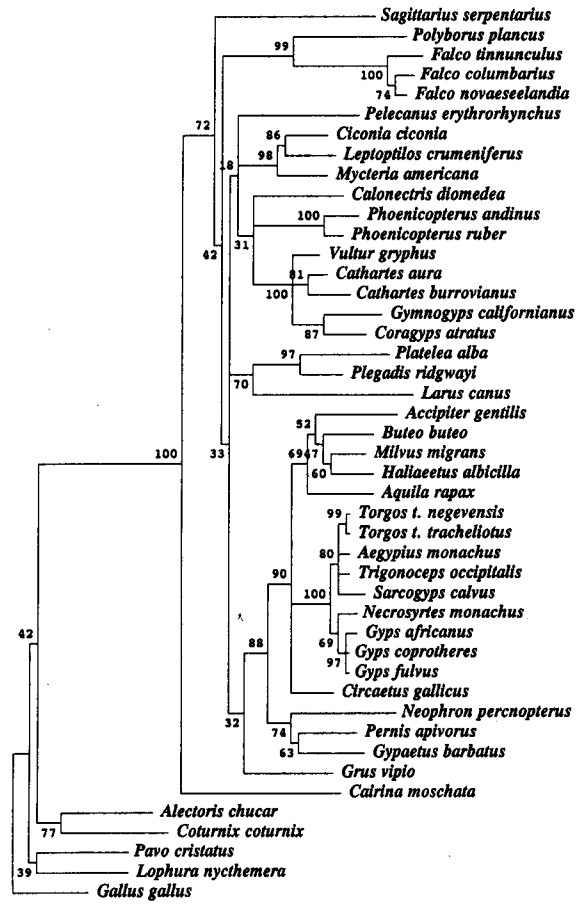

Fig. 3B. NJ, only transversions were evaluated to reduce "noise". Percent bootstrap frequencies (100 replicates; with p-distance method) are indicated at each furcation. Illustration as a phylogram; branch lengths are proportional to genetic distances. Abbreviations as in Fig. 1.

Conclusions

As can be seen in Figs. 2 and 3 most branches which originate 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 year 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 and 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. 3) 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 resolution 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 also the fossil record provides no evidence that the different families within the Falconiformes had a common ancestor, "raptors" should 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).

Albeit the shortcomings of the cyt *b* gene to ascertain higher level classifications of birds, our data and those of Avise *et al.* (1994) show that the carrion-feeding lifestyles and associated morphologies shared by New and Old World vultures are rather based on convergence than on close genetic relatedness. This lends support for previous data which came from a careful examination of anatomy, morphology and biochemistry that vultures must represent a phylogenetically inhomogenous, i.e. polyphyletic group (reviews in Mundy *et al.*, 1992; Sibley and Ahlquist, 1990; del Hoyo *et al.*, 1994). Whereas Avise *et al.* (1994) had assumed that "carrion-feeding had evolved independently at least twice in vulture lineages", our data clearly show that it had happened at least three times during evolution.

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