



A mtDNA Phylogeny of Sea Eagles (genus *Haliaeetus*) Based on Nucleotide Sequences of the Cytochrome *b*-gene

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Abstract—The mitochondrial cytochrome *b* gene of seven species of sea eagles *H. albicilla*, *H. leucocephalus*, *H. leucoryphus*, *H. leucogaster*, *H. sanfordi*, *H. pelagicus* and *H. vocifer* was amplified by PCR and sequenced (1026 bp). Phylogeny reconstructions by the Maximum Parsimony and Neighbour-Joining methods produced similar trees in which sea eagles represent a monophyletic group. In addition, the clade *H. albicilla*/*H. leucocephalus* groups with the clade *H. pelagicus*/*H. leucoryphus* in a monophyletic boreal group while *H. vocifer* clusters with the *H. leucogaster*/*H. sanfordi* clade in a monophyletic tropical clade. The nearest relatives of sea eagles are the kites (genus *Milvus*) and buzzards (genus *Buteo*), whereas 'booted' eagles (genus *Aquila*) and vultures (genera *Gyps* and *Aegypius*) have diverged earlier from the accipitrid branch. Honey buzzards (genus *Pernis*) and vultures of the genera *Gypaetus* and *Neophron* represent basal taxa of the accipitrid lineage. Falcons, New World vultures and the secretary bird (*Sagittarius*) appear in separate clades outside the Accipitridae. Copyright © 1996 Elsevier Science Ltd

Introduction

Brown and Amadon (1968) have suggested that kites (*Milvus*, *Elanus*) and honey buzzards (*Pernis*) represent an ancestral group of the Accipitridae from which two main branches have originated. One leading to hawks (*Accipiter*), buzzards (*Buteo*) and 'booted' eagles (*Aquila*, *Hieraetus*) whereas a second branch includes vultures and sea eagles. Within the sea eagles (genus *Haliaeetus*), 8 species have been recognized (Cramp and Simmons, 1980; Voous, 1977; Sibley and Monroe, 1990; Del Hoyo *et al.*, 1994) and were considered to be monophyletic (Zimbelmann, 1992; Schreiber and Weitzel, 1995). Although raptors represent a well-studied group of birds (Newton, 1990; Brown and Amadon, 1968; Glutz von Blotzheim *et al.*, 1971; Cramp and Simmons, 1980; Del Hoyo *et al.*, 1994), many aspects of their taxonomy and evolution have remained unclear or ambiguous. The evolution of adaptive traits towards bird of prey specialisations have resulted in morphological and anatomical similarities, which might be convergent.

Molecular techniques (Avice, 1994) (especially nucleotide sequences) provide new tools to study phylogenetic relationships and evolutionary history. Marker genes can be easily amplified by PCR, sequenced and employed in phylogenetic reconstructions (e.g. Kocher *et al.*, 1989; Hillis and Moritz, 1990; Edwards *et al.*, 1991; Cooper *et al.*, 1992; Cunningham *et al.*, 1992; Helm-Bychowsky and Cracraft, 1993; Milinkovitch *et al.*, 1993; Kornegay *et al.*, 1993, but see Meyer, 1994). For birds, the mitochondrial cytochrome *b* has frequently been applied as a marker to reconstruct phylogenetic events which took place during the last 20

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million years: Edwards *et al.* (1991), Richman and Price (1992), Helm-Bychowsky and Cracraft (1993), Kocher *et al.* (1989), Taberlet *et al.* (1992), Heidrich and Wink (1994), Wink *et al.* (1993a,b, 1994, 1996), Helbig *et al.* (1995); Heidrich *et al.* (1995); Heidrich and Wink (1997), Wink (1994, 1995); Wink and Seibold (1996), Seibold *et al.* (1993, 1996) and Wittmann *et al.* (1995).

In this communication we report on the phylogenetic relationships (based on nucleotide sequences of the cytochrome *b* gene) of the *Haliaeetus* species complex (only *H. vociferoides* from Madagascar was not available for this analysis) with 'booted' eagles, kites, vultures, bussards, harriers, secretary bird, New World vultures and falcons to test the phylogenetic suggestions of Brown and Amadon (1968). We also evaluate the relationships within the *Haliaeetus* complex.

Materials and Methods

Collection of blood and tissue samples. Samples consisted of blood (ca. 100 μ l) collected from the brachial vein, muscle tissue of dead birds, or feather tips 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 DNA extractions which were performed according to standard protocols (Seibold, 1994; Wink, 1994).

PCR and DNA-sequencing. Using the cytochrome *b*-specific PCR primers of Seibold *et al.* (1996) 1100 base pairs were amplified. A reaction mixture consisted of: 1 μ g total DNA, 75 μ M dNTPs, 50 pmol primers, 1.5 mM MgCl₂, 10 μ l 10x-Taq-buffer, 2 units Taq-Polymerase in a total volume of 100 μ l. After an initial DNA denaturation, 32 cycles were performed with the following conditions: 30 s at 93°C , 45 s at 45°C and 90 s at 70°C using a thermocycler (Biometra, Göttingen). After purification, the PCR products were directly sequenced employing the chain termination method (USB direct sequencing kit; Amersham, Braunschweig) and run on polyacrylamide gels as described in Seibold *et al.* (1996). Sequences were manually read from autoradiograms. Methods used for DNA isolation, PCR and DNA sequencing have been described before in more detail (Seibold, 1994; Seibold *et al.*, 1996; Heidrich *et al.*, 1995 and Wink, 1994, 1995).

Sequence analysis. Nucleotide sequences were aligned manually 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) and the neighbour-joining method (Saitou and Nei, 1987) using the program package MEGA 1.0 (Kumar *et al.*, 1993). In the neighbour-joining analyses, genetic distances were calculated based on 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 (swapping algorithm; tree bisection-reconnection, TBR). A bootstrap analysis (Felsenstein, 1985) was performed in both the maximum parsimony and the neighbour-joining searches. Sequence data (of all taxa shown in this analysis) have been documented in Seibold (1994) and will be deposited in the EMBL data library.

Results

Sequences, modes of substitution and genetic distances

The cytochrome *b* of seven out of eight sea eagle species (two specimens each of *H. albicilla* (L.) 1758, *H. leucocephalus* (Gmelin) 1788, *H. leucoryphus* (Pallas) 1771, *H. leucogaster* (L.) 1766, *H. sanfordi* Mayr 1935, *H. pelagicus* (Pallas) 1811 and *H. vocifer* (Daudin) 1800) was amplified and sequenced. Intraspecific variation was negligible: a single base substitution between two unrelated individuals of *H. albicilla* was observed. Genetic distances, i.e. nucleotide substitutions between two taxa, ranged from 0.3 (*H. sanfordi*: *H. leucogaster*) to 9.8% (*H. albicilla*: *H. vocifer*) (Table 1).

Of 1026 base pairs analysed, 192 are variable (19%) and 106 phylogenetically informative. Nucleotide substitutions are most abundant in the third codon position ($n=161$); only 28 and 0 occur in the first and second positions, respectively. Given that third position substitutions are silent (they do not lead to amino acid substitutions), they are selectively neutral, but might be subjected to homoplasy because of multiple substitutions. Only 13 of a total of 192 substitutions resulted in an amino acid exchange.

TABLE 1. PAIRWISE GENETIC DISTANCES (nucleotide substitutions) BETWEEN THE SEVEN SEA EAGLE SPECIES. Above the diagonal are mean distances (1 = 100%), below: absolute number of nucleotide substitutions

		1	2	3	4	5	6	7
1	<i>H. albicilla</i>	—	0.025	0.096	0.094	0.078	0.081	0.098
2	<i>H. leucocephalus</i>	26	—	0.089	0.087	0.071	0.069	0.090
3	<i>H. leucogaster</i>	98	91	—	0.003	0.093	0.095	0.088
4	<i>H. sanfordi</i>	96	89	3	—	0.089	0.091	0.085
5	<i>H. leucoryphus</i>	80	73	95	91	—	0.064	0.094
6	<i>H. pelagicus</i>	83	71	97	93	66	—	0.091
7	<i>H. vocifer</i>	101	92	90	87	96	93	—

Phylogenetic reconstructions

Using PAUP 3.1.1. 1000 random trees were produced whose distribution was skewed to the left, indicating that the data set contains a significant phylogenetic signal ($g1 = -0.724$; $P < 0.01$) (Hillis and Huelsenbeck, 1992). Phylogenetic relationships were investigated employing both Maximum parsimony (MP) (Fig. 1A) and Neighbour-Joining (NJ) analyses (Fig. 1B). As can be seen from Fig. 1, members of the genus *Haliaeetus* cluster in a monophyletic clade (supported by bootstrap values of 71 and 98%, respectively) in both MP and NJ reconstructions. *H. albicilla*/*H. leucocephalus* and *H. leucogaster*/*H. sanfordi* represent sibling species (bootstrap values 100%, respectively). *H. leucoryphus* and *H. pelagicus* cluster together in NJ trees and share their closest ancestry with *albicilla/leucocephalus* (bootstrap value 95%), whereas *H. vocifer* always grouped with the *leucogaster/sanfordi* clade.

In order to evaluate the relationships of sea eagles with other raptors and to test the phylogenetic suggestions of Brown and Amadon (1968), we have included sequences of *Buteo*, *Accipiter*, *Aquila*, *Aegypius*, *Circus Gyps*, *Pernis*, *Neophron*, *Gypaetus*, *Falco*, *Vultur*, *Cathartes*, *Sagittarius* and *Gallus gallus* (as an outgroup) in the phylogeny reconstruction (Fig. 1A and B). The Accipitridae represent a monophyletic group (bootstrap values 95 or 99%, respectively) which does not include falcons, New World vultures and the secretary bird, independent from algorithms methods or outgroups used.

The two species of kites, *Milvus milvus* and *M. migrans*, are closely related on account of our genetic (Table 1) and allozyme data (Schreiber and Weitzel, 1995); they represent a sister group to the sea eagles, but buzzards (*Buteo*) also appear to belong to this clade. The Honey buzzard (*Pernis apivorus*) is not supported as a true buzzard by any of our analyses. Also at the base of the Accipitridae are Old World vultures of the genera *Gypaetus* and *Neophron* which differ substantially from the other genera of Old World vultures, i.e. *Aegypius* and *Gyps* (Wink, 1995). Vultures and booted eagles (*Aquila*) diverge from the accipitrid branch but do not appear in the *Haliaeetus/Milvus/Buteo* clade.

Discussion

Since the phylogenies shown in Fig. 1A and B were inferred from a single mitochondrial gene, it cannot be ruled out that the underlying organismic evolution may differ in some parts. The use of additional nuclear sequences, such as ITS or rDNA will be the next step to corroborate the trees obtained. As can also be seen from the trees, several basal nodes are not supported by high bootstrap values indicating the limitations of cytochrome *b* sequences to elucidate phylogenetic events which took place more than 10 mya ago (Meyer, 1994). Since the sequence of furcations was very similar in a number of different analyses involving more than 100 raptor taxa, different algorithms and outgroups (Seibold *et al.*,

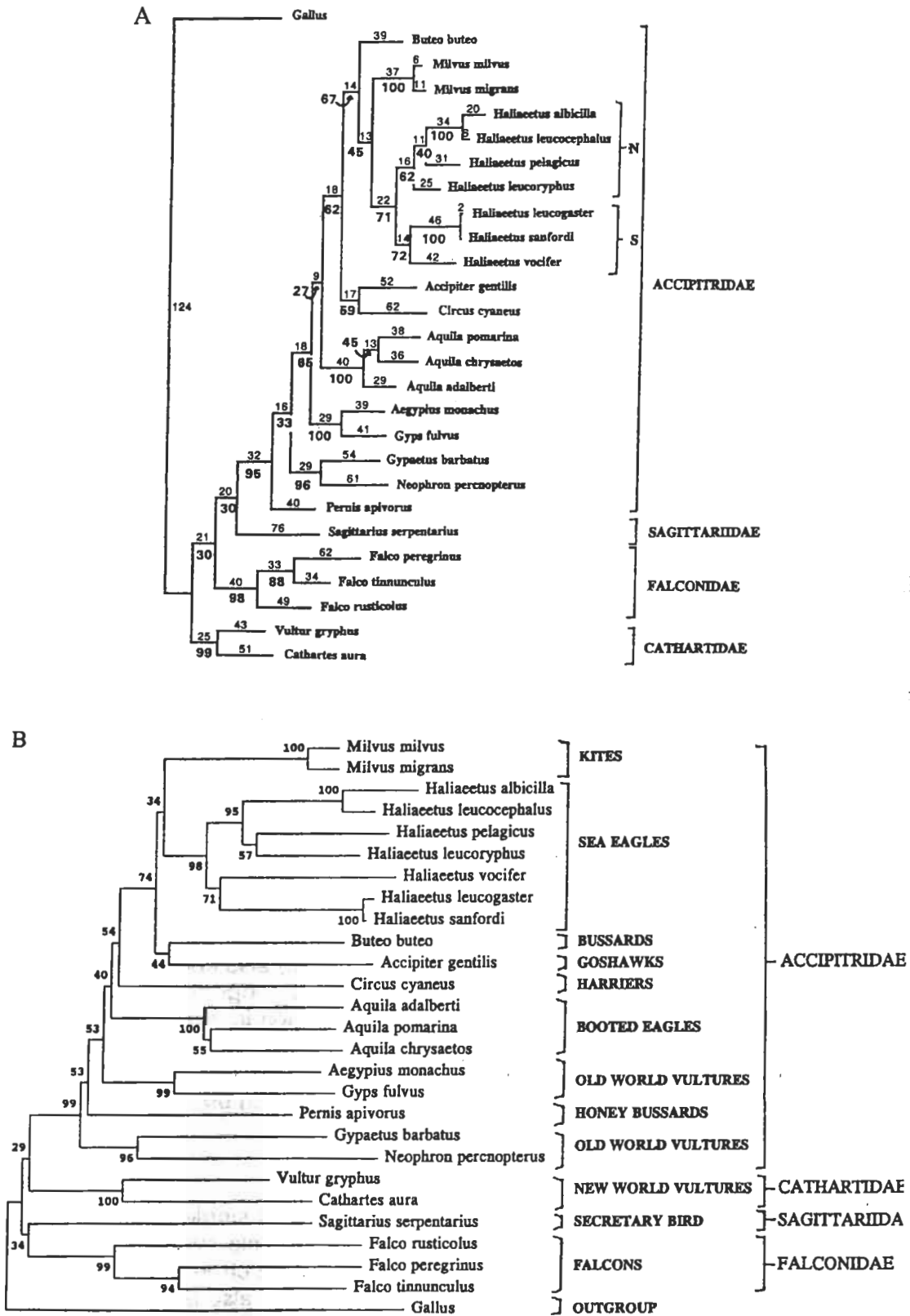


FIG. 1. MOLECULAR PHYLOGENY OF *HALIAEETUS* IN RELATION TO OTHER RAPTORS. A. Maximum parsimony (unweighted analysis with 'simple' sample addition) MP produced a single tree of 1641 steps (sum of minimal possible lengths 687, maximally 2480 steps) CI=0.419; RI=0.468. Number of nucleotide substitutions are given above each branch; B. Neighbour-joining (Tamura-Nei distance algorithm Bootstrap values (200 or 500 replicates, respectively) are given at each node. Branch lengths are proportional to the number of nucleotide substitutions).

1993, 1996; Wink *et al.*, 1997; Wink and Seibold, 1996; Wink, 1995, 1996), we believe that albeit the limitations of the cytochrome *b* data, some conclusions are apparent.

Phylogenetic relationships within the sea eagle-complex

Sea eagles share a number of common traits: Feeding on fish, water fowl or carrion, these raptors live, as their name implies, at the sea or inland at larger lakes and rivers. Only *H. sanfordi* visits tropical rain forests more than other sea eagles (Coates, 1985). Since sea eagles have many anatomical and morphological characters in common, they have been considered to represent a monophyletic group (Zimbelmann, 1992). A recent allozyme analysis (Schreiber and Weitzel, 1995) and our genetic analysis (Fig. 1) unequivocally support this assumption.

If we assume a molecular clock in which 2% sequence divergence is equivalent to approximately 1 million years (mya) (this crude equation is based on mitochondrial genes of geese and primates; Shields and Wilson, 1987; Brown *et al.*, 1982; Quinn *et al.*, 1991), overall genetic distances (Table 1) within the *Haliaeetus* group imply that sea eagles have diverged from a common ancestor approximately 5 mya ago. Fossil records of sea eagles are 2–3 times older, dating back to the middle Miocene (Lambrecht, 1933). This implies that either the modern forms derived from a later ancestor or that the calibration of the molecular clock (which should be interpreted with much caution anyway) differs in *Haliaeetus*; i.e. 2% sequence divergence is rather equivalent to 3–4 and not 1 mya. Since we cannot decide between these possibilities, the more conservative 2% = 1 mya equation is applied in the following.

Within the *Haliaeetus* cluster, *H. albicilla* and *H. leucocephalus* represent sister species which also share a number of morphological and behavioural characters (Table 2). These taxa have diverged about 1 mya ago. Since sea eagles are considered Old World raptors, we must assume that *H. leucocephalus* has invaded North America, either via Siberia or via Greenland. This finding would be in agreement with the fossil record: In La Bre (California) remains of sea eagles (possible *H. leucocephalus*) were found which date back to the Pleistocene, i.e. max. 2 mya (Lambrecht, 1933; Brodcorb, 1964).

H. pelagicus and *H. leucoryphus* have diverged earlier, i.e. 3–4 mya. As can be seen from Table 2, *H. pelagicus* shares a few characters, such as yellow beak, eyes and talons with *albicilla* and *leucocephalus*. Depending on the methods used to reconstruct the phylogeny, *H. pelagicus* does not figure as a sister taxon with *H. leucoryphus* in MP trees, but is positioned in the same clade as *albicilla/leucocephalus*, implicating that the morphological characters which they have in common, are apomorphic. The number of tail feathers is 12 in most sea eagles, except for *H. pelagicus* which has 14 (Table 2); this character cannot be plesiomorphic, but is certainly apomorphic.

Four *Haliaeetus* species occur in the southern hemisphere, e.g. *H. vocifer* and *H. vociferoides* (not studied but certainly related to *H. vocifer*) in Africa, *H. leucogaster* in SE Asia, the Malayan and Australian region and *H. sanfordi* restricted on the Solomon islands. In addition, these species share dark beaks, dark brown eyes and dark yellow talons. As can be seen from Fig. 1, these three species appear in a common clade. *H. vocifer* is closer to *leucogaster/sanfordi* (8.5–8.8%) than to the sea eagles of the Northern hemisphere (9.0–9.8%) (Table 1). A surprisingly small genetic distance was found between *H. sanfordi* and *H. leucogaster*, which differ substantially in morphology and in behaviour (Table 2). The genetic distance of 0.3% would indicate a divergence time of 150,000 years. The view of Zimbelmann (1992) that *H. sanfordi* represents an original and primitive taxon thus appears to be unlikely. Since *H. sanfordi* occurs in a comparatively small area within the

TABLE 2. MORPHOLOGICAL AND BEHAVIOURAL CHARACTERISTICS OF SEA EAGLES

	Colouration		Eyes	Head	Talons	Tail	Colouration		Tail	Tail feather Number	Angle of head when calling
	Adults Beak	Immatures Beak					Head	Tail			
<i>H. albicilla</i>	yellow	dark grey	yellow	light	yellow	white	dark grey	dark	12	120°	
<i>H. leucocephalus</i>	yellow	dark grey	yellow	white	yellow	white	dark grey	dark	12	110-120°	
<i>H. pelagicus</i>	yellow	yellow	yellow	dark	yellow	white	yellow	?	14	90°	
<i>H. leucorhophos</i>	blue grey	brownish	brownish	rusty	grey	white*	dark	?	12	90°	
<i>H. leucogaster</i>	blue black	white	brown	white	dark yellow	white*	dark	light*	12	60°	
<i>H. sanfordi</i>	blue black	dark	brown	dark	dark yellow	light*	dark	?	12	90°	
<i>H. vocifer</i>	black	white	brown	white	dark yellow	white	dark	light	12	180°	

* = with dark bands.

overall and larger distribution range of *H. leucogaster*, we suggest that *H. sanfordi* recently diverged from *H. leucogaster*. Although the genetic distance is small and rather in the range that is typical for subspecies, we consider *H. sanfordi* a distinct species, since it can also be differentiated on account of morphological and behavioural characteristics.

Phylogenetic position of sea eagles in the accipitrid tree

Sea eagles share a number of behavioural and biological characteristics (e.g. display and imposing behaviour; moulting sequence and general anatomy) with kites of the genus *Milvus* (Fischer, 1970; Brown and Amadon, 1968). Similar to some sea eagles, the Black kite, *Milvus migrans*, also feeds on fish and carrion. Although some of these biological characters might represent convergent traits, nucleotide sequence and allozyme data also imply that sea eagles and kites are closely related.

Our molecular data do not support the phylogeny hypothesis of Brown and Amadon (1968) who had suggested that 'kites' (including *Milvus* and *Pernis*) represent the basal groups of the Accipitridae. Whereas this assumption could be corroborated for the Honey bussard (*Pernis apivorus*), kites of the genus *Milvus* are the sister taxon to the sea eagles. Also karyological studies (DeBoer and Sinoo, 1984) had pointed out that the 'kites' of Brown and Amadon (1968) represent a heterogenous group.

'Booted' eagles appear to have similar ectoparasites (e.g. Mallophagae) as sea eagles (Zimbelmann, 1992), but from an anatomical and morphological point of view, both groups of eagles are not closely related (Brown and Amadon, 1968). The sequence data corroborate that *Haliaeetus* and *Aquila* are only distantly related. Brown and Amadon (1968) (who had postulated two main phylogenetic branches in the Accipitridae) had placed buzzards of the genus *Buteo* on the '*Aquila*'-branch and vultures of the genera *Gyps* and *Aegypius* on the '*Haliaeetus*'-branch. Our genetic data clearly show that these two branches do not exist; instead we find one main branch, from which the different groups of accipitrid raptors, such as vultures, booted eagles, hawks, harriers, buzzards, kites and sea eagles diverged.

Old world vultures are paraphyletic (Wink, 1995): *Gypaetus* and *Neophron* diverged from the base of the accipitrid tree, close to the genus *Pernis*. The *Gyps* and the *Aegypius* complex (including *Aegypius*, *Torgos*, *Trigonoceps* and *Sarcogyps*) evolved later and represent a genetically distinct assembly (Wink and Seibold, 1996; Wink, 1997). White shouldered kites (*Elanus*), falcons (Falconidae), New World vultures (Cathartidae) and the secretary bird (Sagittariidae) cluster outside the accipitrid tree (Wink, 1995, 1996; Wink and Seibold, 1996; Wink *et al.*, 1997) indicating that they diverged from common ancestors much earlier; they represent distantly related families of their own right. Since they share the same ecological niche with members of the Accipitridae several similarities in morphology and lifestyle appear to have evolved independently.

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References

- Arctander, P. (1988) Comparative studies of avian DNA by restriction fragment length polymorphism analysis. *J. Orn.* **129**, 205–216.
- Avise, J. C. (1994) *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York, London.

- Brodcorb, P. (1964) Catalogue of fossil birds: Part 2 (Anseriformes through Galliformes). *Bull. Florida State Mus.* **83**, 195–335.
- Brown, W. M., Prager, E. M. and Wilson, A. C. (1982) Mitochondrial DNA sequences of primates: tempo and mode of evolution. *J. Mol. Evol.* **18**, 225–239.
- Brown, L. and Amadon, D. (1968) Eagles, hawks and falcons of the world. Vol. 1. Country Life Books.
- Coates, B. J. (1985) The Birds of Papua New Guinea Including the Bismarck Archipelago and Bougainville. Vol. 1: Non-Passerines. Dove Publications, Alderley, Queensland.
- Cooper, A., Mourer-Chauvire, C., Chambers, G. K., von Haeseler, A., Wilson, A. C. and Pääbo, S. (1992) Independent origins of New Zealand moas and kiwis. *Proc. Natl. Acad. Sci., USA* **89**, 8741–8744.
- Cramp, S. and Simmons, K. E. L. (1980) Handbook of the birds of Europe, the Middle East and North Africa. Oxford University Press, Oxford, London, New York.
- Cunningham, C. W., Blackstone, N. W. and Buss, L. W. (1992) Evolution of king crabs from hermit crab ancestors. *Nature* **355**, 539–542.
- DeBoer, L. E. M. and Sinoo, R. P. (1984) A karyological study of Accipitridae (Aves: Falconiformes) with karyotypic descriptions of 16 species new to cytology. *Genetica* **65**, 89–107.
- Desjardins, P. and Morais, R. (1990) Sequence and gene organisation of chicken mitochondrial genome. *J. Mol. Biol.* **211**, 599–634.
- Edwards, S. V., Arctander, P. and Wilson, A. C. (1991) Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. *Proc. Royal Soc. London B* **243**, 99–107.
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fischer, W. (1970) Die Seeadler. Die Neue Brehm-Bücherei 221. Ziemsen Verlag, Wittenberg.
- Glutz von Blotzheim, U. N., Bauer, K. M. and Bezzel, E. (1971) In *Handbuch der Vögel Mitteleuropas*. Vol. 4: *Falconiformes*, pp. 620–637. Akadem. Verlagsgesellschaft, Frankfurt am Main.
- Heidrich, P. and Wink, M. (1994) Tawny owl (*Strix aluco*) and Hume's tawny owl (*Strix butleri*) are distinct species: Evidence from nucleotide sequences of the cytochrome b gene. *Z. Naturforsch.* **49c**, 230–234.
- Heidrich, P., Koenig, C. and Wink, M. (1995) Molecular phylogeny of the South American Screech Owls of the *Otus atricapillus* complex (Aves: Strigidae) inferred from nucleotide sequences of the mitochondrial cytochrome b gene. *Z. Naturforsch.* **50**, 294–302.
- Heidrich, P. and Wink, M. (1997) Phylogenetic relationships in holarctic owls (Order Strigiformes): Evidence from nucleotide sequences of the mitochondrial cytochrome b gene. *Proc. Intl. Conf. Holarctic Raptors* (in press).
- Helbig, A., Seibold, I., Martens, J. and Wink, M. (1995) Genetic differentiation and phylogenetic relationships of Bonelli's warbler *Phylloscopus bonelli* and Green warbler *P. nitidus*. *J. Avian Biol.* **26**, 139–153.
- Helm-Bychowsky, K. and Cracraft, J. (1993) Recovering a phylogenetic signal from DNA sequences: Relationships within the Corvine assemblage (Class Aves) as inferred from complete sequence of the mitochondrial DNA cytochrome-b-Gene. *Mol. Biol. Evol.* **10**, 1196–1214.
- Hillis, D. M. and Huelsenbeck, J. P. (1992) Signal, noise and reliability in molecular phylogenetic analyses. *J. Heredity* **83**, 189–195.
- Hillis, D. M. and Moritz, C. (1990) *Molecular Systematics*. Sinauer Press.
- Hoyo, del J., Elliott, E. and Sargatal, J. eds (1994) *Handbook of the Birds of the World*. Vol. 2. Lynx Edicions, Barcelona.
- Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. and Wilson, A. C. (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci., USA* **86**, 6196–6200.
- Kornegay, J. R., Kocher, T. H., Williams, L. A. and Wilson, A. C. (1993) Pathways of lysozyme evolution inferred from the sequences of cytochrome b in birds. *J. Mol. Evol.* **37**, 367–379.
- Kumar, S., Tamura, K. and Nei, M. (1993) MEGA - Molecular Evolutionary Genetics Analysis. Version 1.0. Pennsylvania State University.
- Lambrecht, K. (1933) *Handbuch der Palaeomithologie*. Berlin.
- Meyer, A. (1994) Shortcomings of the cytochrome b gene as a molecular marker. *Trends Ecol. Evol.* **9**, 278–280.
- Milinkovitch, M. C., Orti, G. and Meyer, A. (1993) Revised phylogeny of whales suggested by mitochondrial ribosomal DNA sequences. *Nature* **361**, 346–348.
- Newton, I. (1990) *Birds of Prey*. Merehurst, London.
- Quinn, T. W., Shields, G. F. and Wilson, A. C. (1991) Affinities of the Hawaiian goose on two types of mitochondrial DNA data. *Auk* **108**, 585–593.
- Richman, A. D. and Price, T. (1992) Evolution of ecological differences in the Old World leaf warblers. *Nature* **355**, 817–821.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

- Schreiber, A. and Weitzel, T. (1995) Biochemical systematics of sea eagles (Genus *Haliaeetus* Savigny 1809) with a note on allozyme differentiation between black and red kites (Genus *Milvus* L. 1758). *Biochem. Syst. Ecol.* **23**, 235–244.
- Seibold, I. (1994) Untersuchungen zur molekularen Phylogenie der Greifvögel anhand von DNA-Sequenzen des mitochondrialen Cytochrom b-Gens. PhD-Dissertation, Univ. Heidelberg. (Hartung-Gorre Verlag, Konstanz).
- Seibold, I., Helbig, A. J. and Wink, M. (1993) Molecular systematics of falcons (family Falconidae). *Naturwissenschaften* **80**, 87–90.
- Seibold, I., Meyburg, B.-U., Helbig, A., Negro, J. and Wink, M. (1996) Genetic differentiation and molecular phylogeny of *Aquila* eagles (Aves: Falconiformes) according to cytochrome b nucleotide sequences. In *Eagle Studies* (Meyburg, B.-U. and Chancellor, R. eds) pp. 1–15, WWGBP., Berlin, London.
- Shields, G. F. and Wilson, A. C. (1987) Calibration of mitochondrial DNA evolution in geese. *J. Mol. Evol.* **24**, 212–217.
- Sibley, C. G. and Monroe, B. L. (1990) *Distribution and Taxonomy of Birds of the World*. Yale University Press, New Haven, London.
- Swofford, D. L. (1993) PAUP: Phylogenetic analysis using parsimony. Version 3. 1.1 Illinois.
- Taberlet, P., Meyer, A. and Bouvet, J. (1992) Unusual mitochondrial polymorphism in two local populations of blue tit *Parus caeruleus*. *Molec. Ecol.* **1**, 27–36.
- Voous, K. H. (1977) List of recent holarctic bird species. Academic Press.
- Wink, M. (1994) PCR in der Evolutionsforschung. In *PCR im Medizinischen und Biologischen Labor* (Wink, M. and Wehrle, H., eds), pp. 166–184, GIT-Verlag, Darmstadt.
- Wink, M. (1995) Phylogeny of Old and New World vultures (Aves: Accipitridae and Cathartidae) inferred from nucleotide sequences of the mitochondrial cytochrome b gene. *Z. Naturforsch.* **50c**, 868–882.
- Wink, M. (1996) Molecular phylogeny of the Saker (*Falco cherrug*) and desert falcons. *Proc. Intl. Middle East Falcon Research Group* pp. 178–203, NARC, Abu Dhabi.
- Wink, M., Heidrich, P., Kahl, U., Witt, H. H. and Ristow, D. (1993a) Inter- and intraspecific variation of the nucleotide sequence for cytochrome b in Cory's shearwater (*Calonectris diomedea*), Manx Shearwater (*Puffinus puffinus*) and Fulmar (*Fulmarus glacialis*). *Z. Naturforsch.* **48c**, 504–508.
- Wink, M., Heidrich, P. and Ristow, D. (1993b). Genetic evidence for speciation of the Manx shearwater (*Puffinus puffinus*) and the Mediterranean Shearwater (*P. yelkouan*). *Vogelwelt* **114**, 226–232.
- Wink, M. and Seibold, I. (1996) Molecular phylogeny of Mediterranean raptors. (Families Accipitridae and Falconidae). In *Biología y Conservación de las Rapaces Mediterráneas* (J. Muntaner and J. Mayol, eds) pp. 1–10, SEO, Madrid.
- Wink, M. I., Seibold, F., Lotfikhah and W. Bednarek (1996) Molecular systematics of holarctic raptors (Order Falconiformes). *Proc. Intl. Conf. Holarctic Raptors* (in press).
- Wittmann, U., Heidrich, P., Wink, M. and Gwinner, E. (1995) Speciation in the stonechat (*Saxicola torquata*) inferred from nucleotide sequences of the cytochrome b gene. *J. Zool. Syst. Evol. Res.* **33**, 116–122.
- Zimbelmann, F. (1992) Ein vergleichend morphologischer Beitrag zur Phylogenetik der Seeadler (*Haliaeetus* Savigny 1809). *Mitt. Zool. Mus. Berlin. Ann. Orn.* **68(16)**, 61–114.