

Phylogenetic Relationships in Diurnal Raptors based on nucleotide sequences of mitochondrial and nuclear marker genes

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ABSTRACT

The mitochondrial cytochrome b gene was amplified and sequenced from approximately 62% of all species and 58% of genera of diurnal raptors. Nucleotide sequence data were used to reconstruct their molecular phylogeny. A number of new phylogenetic relationships were discovered in members of the Falconidae, Pandionidae, and Accipitridae (vultures, Bateleur, hawk eagles, harriers, sea eagles and booted eagles). In a number of instances DNA support the view that certain subspecies, which differ in morphology, size and distribution, can be regarded as distinct species: Such pairs are: *Falco chicquera* and *F. (c.) horsbrughii*, *F. columbarius* and *F. (c.) aesalon*, *Circus maillardi* and *Circus (m.) macrosceles*, *Circus cyaneus* and *Circus (c.) hudsonius*, *Aquila (Hieraetus) fasciatus* and *A. (f.) spilogaster*, *Pandion haliaetus* and *P. (h.) carolinensis* and *P.(h.) cristatus*. *Aquila*, *Hieraetus* and *Lophaetus* are paraphyletic; therefore *Hieraetus* and *Lophaetus* could be merged in *Aquila*.

INTRODUCTION

Diurnal raptors have been grouped into five families, Accipitridae, Pandionidae, Sagittariidae, Falconidae and Cathartidae, and placed in a common order Falconiformes (del Hoyo *et al.* 1994) or the infraorders Falconides and Ciconiides respectively (Sibley & Monroe 1990). Morphological and molecular data provide evidence that at least Cathartidae, Falconidae and Sagittariidae do not share direct ancestry with Accipitridae and Pandionidae, indicating that the order Falconiformes or infraorder Falconides are apparently artificial units (Wink 1995; Wink *et al.* 1998) which combine birds that share a common life style, especially behaviour and ecology. Because convergent traits are abundant in raptors, molecular data, such as

DNA sequences of marker genes, offer an opportunity to elucidate evolutionary relationships as DNA data provide many characters for comparison which are less biased by parallel evolution than morphological, ecological or behavioural traits (Avice 1994; Mindell 1997).

The analysis of nuclear or mitochondrial marker genes has become a widely applied tool during the last 15 years in all fields of zoology, including ornithology to reconstruct phylogenies and phylogeographic relationships (overviews in Avice 1994; Mindell 1999; Wink 2000). Molecular data have the great advantage that convergence does not impair an analysis to the same degree as morphological data do. Molecular data allow both a phylogenetic and phylogeographic analysis of the unknown past of a particular group of organisms. DNA sequences have therefore become an important tool for taxonomy and evolutionary studies including raptors (Griffiths 1997; Groombridge *et al.* 2002; Kruckenhauser *et al.*, 2003, Riesing *et al.* 2003; Seibold *et al.* 1996; Storch *et al.* 2001; Wink 1995, 1998, 2000; Wink *et al.* 1996, 2000; Wink & Sauer-Gürth 2000; Wink & Ristow 2000).

Among diurnal raptors, more than 230 species and 79 genera have been described. The molecular data published so far are based on one or two mitochondrial genes and cover approximately 58% of the genera and 62% of species (the species of this study included) and provide a first idea of their evolutionary past. Because these conclusions rely on incomplete data sets (and may thus suffer of "long branch attractions" and insufficient resolution) much more work is needed before we shall be able to understand the evolution of diurnal raptors in more precision.

MATERIAL AND METHODS

We have isolated total DNA from feather, blood or tissue samples (see Wink 2000) which had been kindly supplied by several colleagues (V. Bretagnolle, R. Simmons, W. Bednarek, C. Fentzloff, B. Clark, H. Brünning, C. Fulquhar, M. Heidenreich, G. Ehlers, W. Scharlau, C. Kaatz, M. Pomarol, H. Prehn, P. Gaucher, R. Pfeffer, D. Schmidl, J. Thibault, O. Hatzofe, D. Bird, A. Kemp, C. Jones, M. Stubbe, B.-U. Meyburg, W. Grummt, C. König, J.J. Negro, D. Ristow, D. Pepler, B. Arroyo, B. Etheridge, H.H. Witt, B. Bed'hom, S. Ostrowski, D. Ellis, U. Höfle, R. Kenward, N. Fox, T. Osborne, A. Stephenson, N. Hyde and D. Schodde). The cytochrome b gene was amplified by PCR (primer sequences in Wink & Sauer-Gürth, 2000) and sequenced by using AlfExpress (Amersham Pharmacia Biotech) or a capillary sequencer ABI 3100 (Applied Biosystems) instruments. Sequences of 1000 and more base pairs were aligned manually and analysed with the software packages PAUP*4.0b10 (Swofford, 2002) and MEGA2 (Kumar *et al.*, 2001) (see Wink 2000; Wink & Sauer-Gürth, 2000; Wink *et al.* 2002; Broders *et al.* 2003 for further details).

RESULTS AND DISCUSSION

For the present analysis a data set was selected in which most species are represented by a single sequence (The complete data set established in our laboratory usually contains several individuals for each taxon). ML, MP and NJ

produced identical or similar trees. For sake of space only MP trees are shown. We have chosen *Struthio* as a distant outgroup and *Gallus* as a distant ingroup; both groups of birds have evolved earlier than raptors (Feduccia 1996). The choice of other outgroups did not influence the tree topology significantly. Results shown in Figure 1. have partly been published and discussed in Seibold *et al.*, (1996), Griffiths (1997); Wink (1995, 1998, 2000); Wink & Sauer-Gürth, (2000); Wink *et al.* (1996, 2000).

Phylogenetic relationships between and within raptor families

As can be seen from Figure 1 the Falconidae, Cathartidae and probably the Accipitridae form monophyletic assemblages, i.e. each of these families derived from a common ancestor. The monotypic genera *Sagittarius*, *Pandion*, which are the sole representatives of their families and the genera *Elanus*, *Polyboroides*, *Hamirostra* and *Lophoictinia* cluster at the base of the furcations which lead to the Accipitridae. Especially *Elanus* appears to be a rather unique group of raptors (see below). Whether they share direct ancestry with the Accipitridae (as suggested by DNA-DNA hybridisation; Sibley & Ahlquist 1990) cannot be settled with the present data set, since bootstrap values indicate a small support for these bifurcations. However, the Falconiformes as a group apparently represent a polyphyletic assemblage, indicating that at least the families Falconidae and Cathartidae evolved independently from the Accipitridae.

Pandionidae

Ospreys form a monospecific family (bootstrap support 100%) with worldwide distribution. Four subspecies have been recognized, of which three are included in the present analysis (Figure 1, Figure 2). (see the article of M. Wink, H. H. Witt & H. Sauer-Gürth in this volume for a more detailed study). Because substantial genetic distances and differences in distribution and morphology in the three subspecies, it would be plausible to treat the New World, Eurasian and Australian Ospreys as distinct species.

Sagittariidae

Sagittarius serpentarius represents a monospecific family, which has been placed near Accipitridae and storks (del Hoyo *et al.* 1994). Cytochrome b sequences always place this taxon outside the Accipitridae (which would agree with karyotype data) but could not find an unequivocal sister so far; storks sometimes appear as a direct sister group to the Secretary Bird (Figure 1), as suggested by Sibley & Ahlquist (1990).

Cathartidae

New World vultures always cluster as a monophylum outside the Accipitridae, confirming the view that they form an independent group of diurnal raptors. A close relationship with storks, as discussed by Sibley & Ahlquist (1990), is not evident from the cyt b analyses (Figure 1).

Accipitridae

Genera *Haliaeetus*, *Milvus*, *Haliaastur* and *Buteo*.

According to Figure 3 buzzards form a monophyletic group with kites and sea-eagles. Phylogenetic relationships within the sea-eagle clade have already been published (Wink *et al.* 1996; Seibold & Helbig 1996), except for the position of the Madagascar Fish-eagle (*H. vociferoides*). Because the plumage pattern of *H. vociferoides* differs substantially from that of the African Fish-eagle (*H. vocifer*) but shows similarities to Sanford's Sea-eagle (*H. sanfordi*), a relationship between both taxa appeared possible. As can be seen from Figure 3, *H. vociferoides* is clearly a sibling species of *H. vocifer*. Thus Madagascar was probably colonized by an ancestor of *H. vocifer* about 2.5 million years ago which became isolated and developed into *H. vociferoides*. Morphological similarities between *H. sanfordi* and *H. vociferoides* are probably due to the fact that these taxa shared a distant common ancestor (Figure 3). The genetic distance between *H. leucogaster* and *H. sanfordi* is extremely small, providing us with a lower species limit for genetic distances in diurnal raptors.

Kites of the genus *Milvus* are a closely related monophyletic group. Phylogeographic relationships within the genus *Milvus* have been studied in more detail in our laboratory (see Scheider, Wink, Stubbe, Hille & Wiltschko in this volume for details). The Brahminy Kite (*Haliaastur indus*) and the Whistling Kite (*H. sphenurus*) which had been placed in the genus *Milvus*, are indeed a closely related sister group of *Milvus* (distance 7%) (100% bootstrap support). Because of the genetic distances, it would be plausible to maintain a genus *Haliaastur* (del Hoyo *et al.* 1994).

Buzzards of the New world (genera *Buteo*, *Buteogallus*, *Busarellus*, *Geranoetus*), which cluster basal to the Old World buzzards, show interspecific distances that are typical for "good" species. Old World members of the genus *Buteo* form a closely related monophyletic group in all reconstructions (Figs. 1, 3). Branch lengths leading to the recognized species are very short, indicating that these taxa should either represent a young group of raptors or that hybridisation between all of them should have occurred frequently (Kruckenhauser *et al.* 2003). *Buteo oreophilus* from South Africa is almost identical to the Eurasian Buzzard (*Buteo buteo*). Also *B. b. rothschildi* from the Azores cannot be distinguished from *B. buteo* of the mainland. In conclusion, species borders in *Buteo* cannot be confirmed by cytochrome b. Other molecular markers and a detailed morphological study are necessary to review the systematics of this genus (Riesing *et al.* 2003; Kruckenhauser *et al.* 2003) which was always difficult because of strong plumage polymorphisms in this group.

Figure 1. Molecular phylogeny of diurnal raptors (with storks as an ingroup) inferred from nucleotide sequences of the cytochrome b gene. Reconstruction with maximum parsimony; representation as a strict consensus cladogram; numbers at branches are bootstrap values (in %); arrows indicate branches that lead to the different families of the Falconiformes

Strict

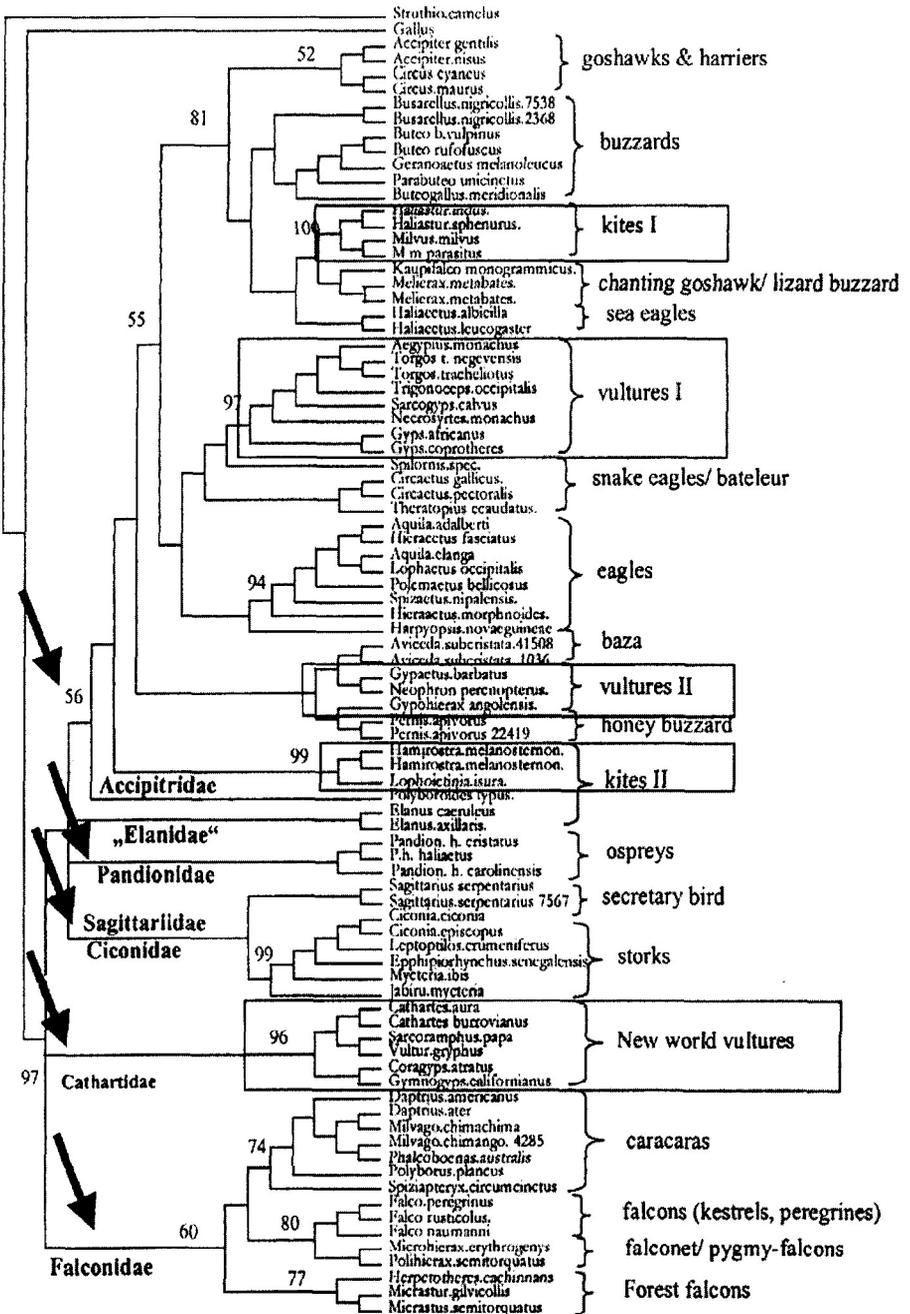
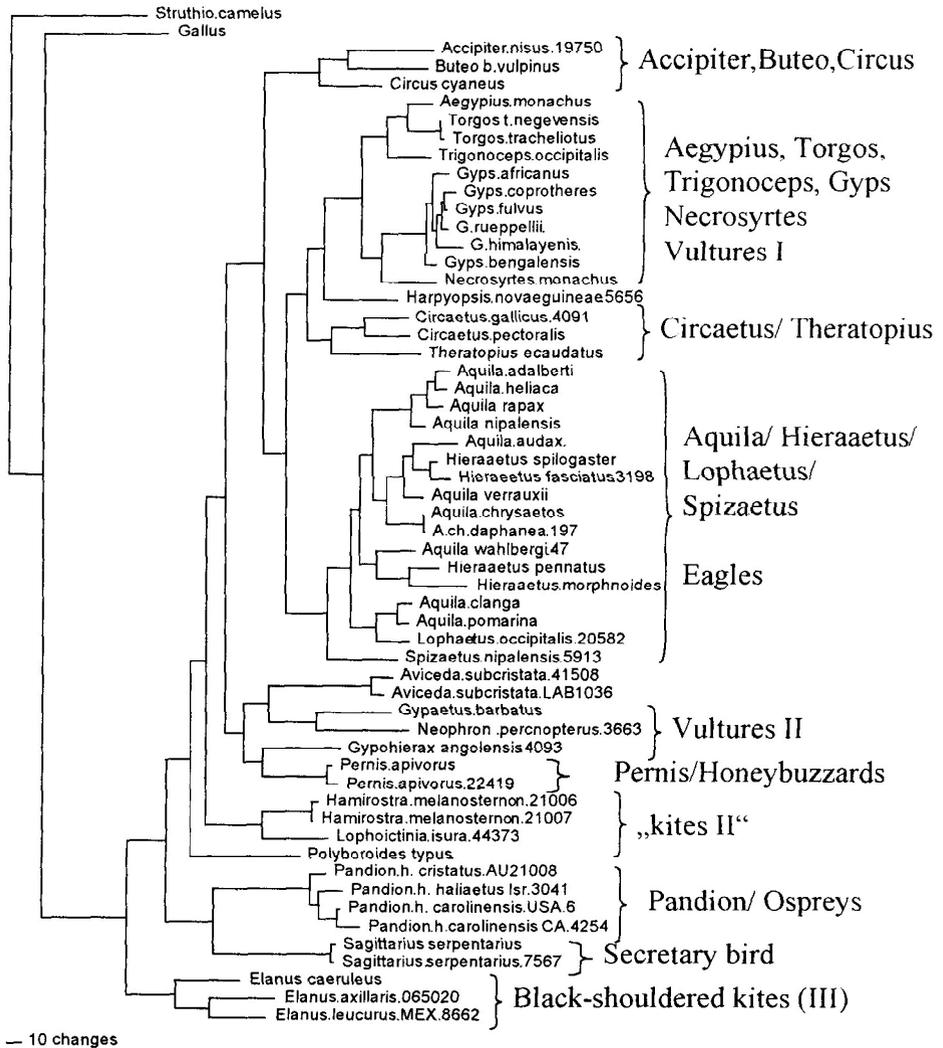


Figure 2. Molecular phylogeny of Pandionidae and basal taxa of the Accipitridae inferred from nucleotide sequences of the cytochrome b gene
 Reconstruction with maximum parsimony; representation as a phylogram



Harriers and Goshawks

Goshawks (*Accipiter*) usually cluster as a sister group to the monophyletic genus *Circus* (Figure 1, Figure 3). The Australian goshawks *A. cirrhocephalus* and *A. fasciatus* form a clade of their own. As this genus has many members, it is too early to speculate on the structure within the genus *Accipiter*.

Species of the Marsh Harrier group *C. aeruginosus*, *C. approximans*, *C. spilonotus*, *C. maillardi*, and *C. ranivorus* from South Africa form a monophyletic group (Figure 3), indicating the evolution from a common ancestor. *C. maillardi macrosceles* breeds on Madagascar and *C. m. maillardi* on Réunion. Because of geographic, morphological and genetic distances (3% divergence) both taxa have been considered to be distinct species (Simmons

2000). The Hen harrier (*C. cyaneus*), Pallid Harrier (*C. macrourus*), *C. maurus* and *C. cinereus* share close ancestry (Figure 3). *C. c. cyaneus* of the Old World differs significantly (divergence 1.7%) from *C.c. hudsonius* of North America, suggesting that both taxa might be considered as distinct species (Wink *et al.* 1998; Simmons 2000). *C. pygargus* and *C. buffoni* are not in the *cyaneus* clade, as could have been expected on account of similar plumage patterns. The Australian Spotted Harrier (*C. assimilis*) clusters at the base of the monophyletic genus *Circus*.

Old World Vultures

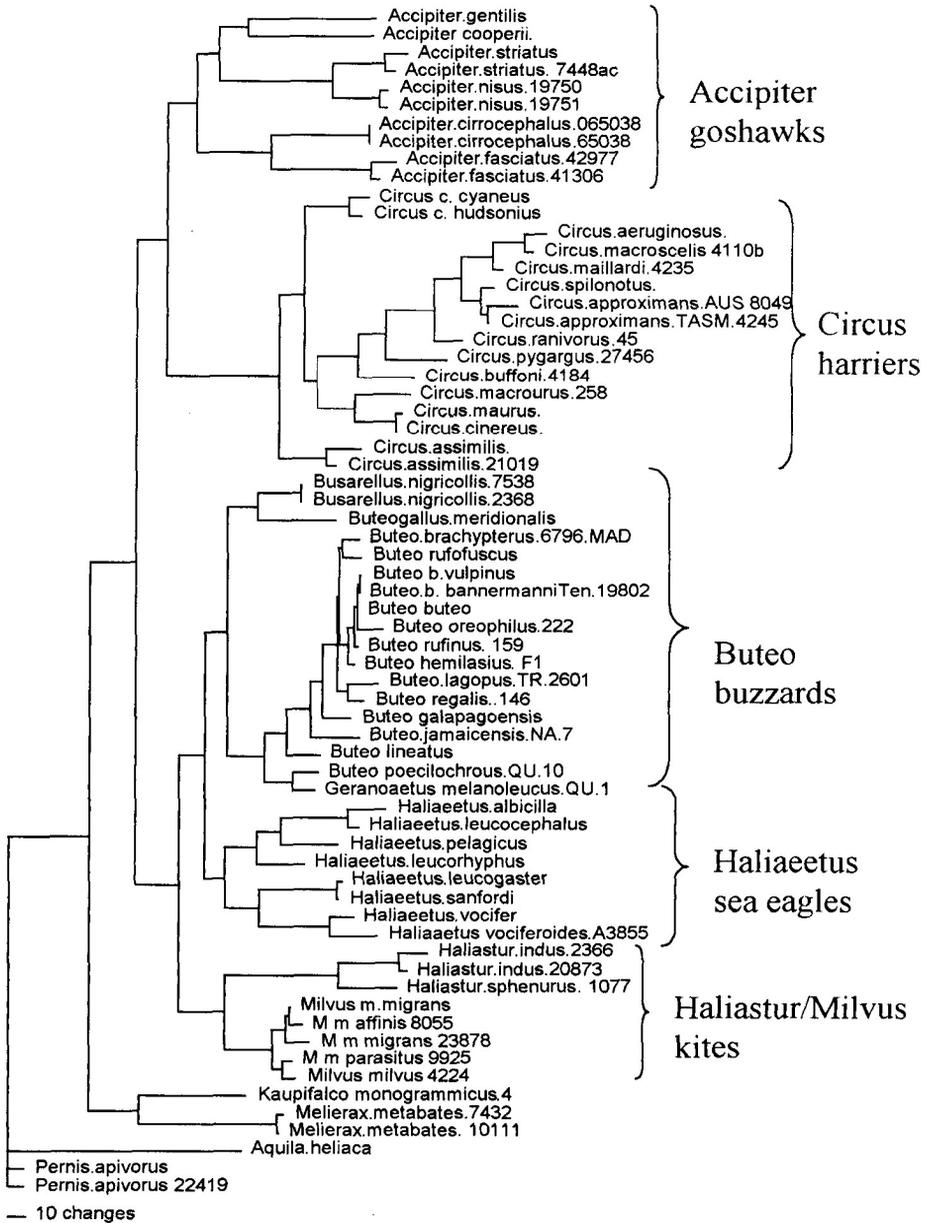
Within the group of Old World vultures two main evolutionary lineages (Figure 1, 2) are evident (Wink 1995; Wink & Seibold 1996; Wink *et al.* 1998; Seibold & Helbig 1995):

One assemblage (Vulture II) includes the Bearded Vulture (*Gypaetus barbatus*) and the Egyptian Vulture (*Neophron percnopterus*). This clade shares many biological characters and is always positioned near the base of the Accipitridae cluster, indicating an evolutionary old lineage of vultures. The Palm-nut Vulture of Africa has not been included in the molecular studies yet, but was always placed in the neighbourhood of *Gypaetus* and *Neophron*. Molecular data unambiguously confirm that *Gypohierax angolensis* is indeed a member of the *Gypaetus/Neophron* lineage (Figs. 1, 2). The Honey Buzzard (*Pernis apivorus*) never clusters with buzzards but always near the base of the Accipitridae tree, close to or in the *Gypaetus/Neophron/ Gypohierax* clade.

A second lineage (Vulture I) includes the genera *Necrosyrtes* and *Gyps* which form a monophyletic clade (bootstrap support 100%), with *Aegypius/Torgos/Trigonoceps*, and *Sarcogyps* as a sister group (Figs. 1, 2). The second lineage contains monotypic genera. Because it constitutes a monophyletic clade (69% bootstrap support) of species which share many morphological and behavioural characters, it would be plausible to place them in a single genus: The name *Aegypius* has already been proposed for this assemblage (del Hoyo *et al.* 1994; Mundy *et al.* 1992).

Gyps rueppellii and *G. himalayensis* were not included in previous molecular analysis. As can be seen from Figure 2, *G. coprotheres*, *G. fulvus*, *G. africanus*, *G. rueppellii* and *G. himalayensis* form a closely related monophyletic group (100% bootstrap support). Its members have been considered as a superspecies (del Hoyo *et al.* 1994; Sibley & Monroe 1990). Distances in this group are as small as observed in the *Buteo* complex, suggesting that they either represent young species or a species complex which shows some past and present hybridisations. *G. africanus* and *G. bengalensis* had been placed in the genus *Pseudogyps* (having 12 and not 14 rectrices) but genetic data imply a close relatedness to *Gyps* and consideration of them as part of a common genus *Gyps* (as proposed by del Hoyo *et al.* 1994; Sibley & Monroe 1990).

Figure 3. Molecular phylogeny of the more advanced taxa of Accipitridae inferred from nucleotide sequences of the cytochrome b gene. Reconstruction with maximum parsimony; representation as a phylogram



The example of Old and New World vultures clearly shows how convergent traits evolved in raptors; ecologically they are scavengers and evolved this "profession" from different evolutionary origins. Therefore, vulture is an ecologically but not a systematically meaningful term (Wink 1995; Storch *et al.*, 2001).

Booted Eagles and allies

Members of the genera *Aquila*, *Hieraaetus*, *Lophaetus* and *Polemaetus* share common ancestry (Figs. 1, 2) (100% bootstrap support). Also nuclear LDHb suggests paraphyly of *Aquila* and *Hieraaetus* (Figure 5) This clade is apparently paraphyletic, indicating that the allocation of taxa to the genera *Aquila*, *Hieraaetus* and monotypic *Lophaetus* does not reflect a phylogenetic sorting. Because *Hieraaetus* has been classified as a member of the genus *Aquila* before, the molecular data would support merging *Hieraaetus* with *Aquila*. The same applies for *Lophaetus occipitalis*, which shares a clade with *A. pomarina*/*A. clanga*. *H. pennatus* is a sister species of the Australian Little Eagle (*H. morphnoides*) which share ancestry with *A. wahlbergi* (Figure 2). A relationship between *H. pennatus* and *A. wahlbergi* has already been discussed, since the dark morph of *H. pennatus* is quite similar to *A. wahlbergi* (del Hoyo *et al.* 1994).

H. fasciatus (breeding in the Mediterranean and Asia) and *H. spilogaster* (breeding south of the Sahara) form sibling species (divergence 1.7%) (Figure 2). Morphological, geographical and genetical distances support the view to treat both taxa as distinct species (del Hoyo *et al.* 1994; Sibley & Monroe 1990). Within *H. fasciatus* a low degree of haplotype differentiation can be seen (Cardia *et al.* 2000).

The relationships between *A. clanga*, *A. pomarina*, *A. rapax* and *A. nipalensis* and the recognition of *A. adalberti* as a distinct species have been reported before (Seibold *et al.* 1996). In *A. chrysaetos* we had described two haplotypes; a new analysis clearly shows that both types can be allocated to the subspecies *A.c. chrysaetos* and *A.c. daphanea* (which breeds in East Asia) (Figure 2).

Only a single taxon of hawk eagles of the genus *Spizaetus* has been included so far; According to Figures 1 & 2 it clusters at the base to the booted eagle clade. We need more taxa to corroborate this finding.

The Short-toed Snake Eagle (*Circaetus gallicus*), the Black-breasted Snake Eagle (*C. pectoralis*) and the Bateleur (*Terathopius ecaudatus*) form a monophyletic clade in all reconstructions (Figs. 1, 2); this clade is not obvious as far as plumage patterns are concerned, but food and feeding show some similarities in these taxa and a relationship has been proposed (Sibley & Monroe 1990). We need to include all members of the genus *Circaetus* to test whether the sister group relationship remains stable.

Kites

Kites also represent a polyphyletic assemblage (Amadon & Bull 1988), similar to the situation of vultures (Figs. 1-3). The Black-shouldered Kites (*Elanus axillaris* from Australia, *E. leucurus* from Central America, and *E. caeruleus* from Europe/Africa) cluster outside the main Accipitridae (Figs. 1, 2; Kites III). We need more genetic markers to decide whether they form a family of their own similar to the Ospreys (Bed'hom & Wink, unpublished).

The Square-tailed Kite (*Lophoictinia isura*) and the Black-breasted Buzzard (*Hamirostra melanosternon*) (both are monotypic genera) both occur in Australia and form a stable sister group relationship (Figure 2); Both taxa do not belong to members of the genus *Milvus*/*Haliastur* (as suggested in del Hoyo *et al.* 1994)

but cluster at the base of the Accipitridae (Kites II). Apparently they constitute an old lineage of diurnal raptors.

The Cuckoo-hawks, such as the Australian Pacific Baza (*Aviceda subcristata*) also constitute an independent lineage of raptors which clusters near the base of the Accipitridae in the *Gypaetus/Neophron* assemblage. By morphology these raptors appear very different.

The African Harrier-hawk (*Polyboroides typus*) is another raptor whose affinities are difficult to establish. According to the present analysis (Figure 1, 2) it clusters near the base of the Accipitridae, close to the Kite II clade.

Summarising, kites form a heterogenic assemblage of raptors; *Milvus* and *Haliaeetus* cluster as a sister group to buzzards, far away from members of the genera *Elanus*, *Lophoictinia*, *Hamirostra*, and *Aviceda* which represent different and independent lineages. We need to sequence other members of the genera *Leptodon*, *Chondrohierax*, *Elanoides*, *Gampsonyx*, *Chelictinia*, *Rostrhamus*, *Harpagus*, and *Ictinia*, which all carry the name "kite" in order to find out whether an evolutionary unit of "basal kites" exists.

Falconidae

Members of the Falconidae are divided into the subfamilies Polyborinae and Falconinae. Also according to cytochrome b data both groups represent monophyletic clades (Griffiths, 1997) which share common ancestry (Figure 1, 4). The African Pygmy

Falcon (*Polihierax semitorquatus*) and the Philippine Falconet (*Microhierax erythrogenys*) represent sibling genera which form a sister group to the large monophyletic *Falco* assemblage (100% bootstrap support).

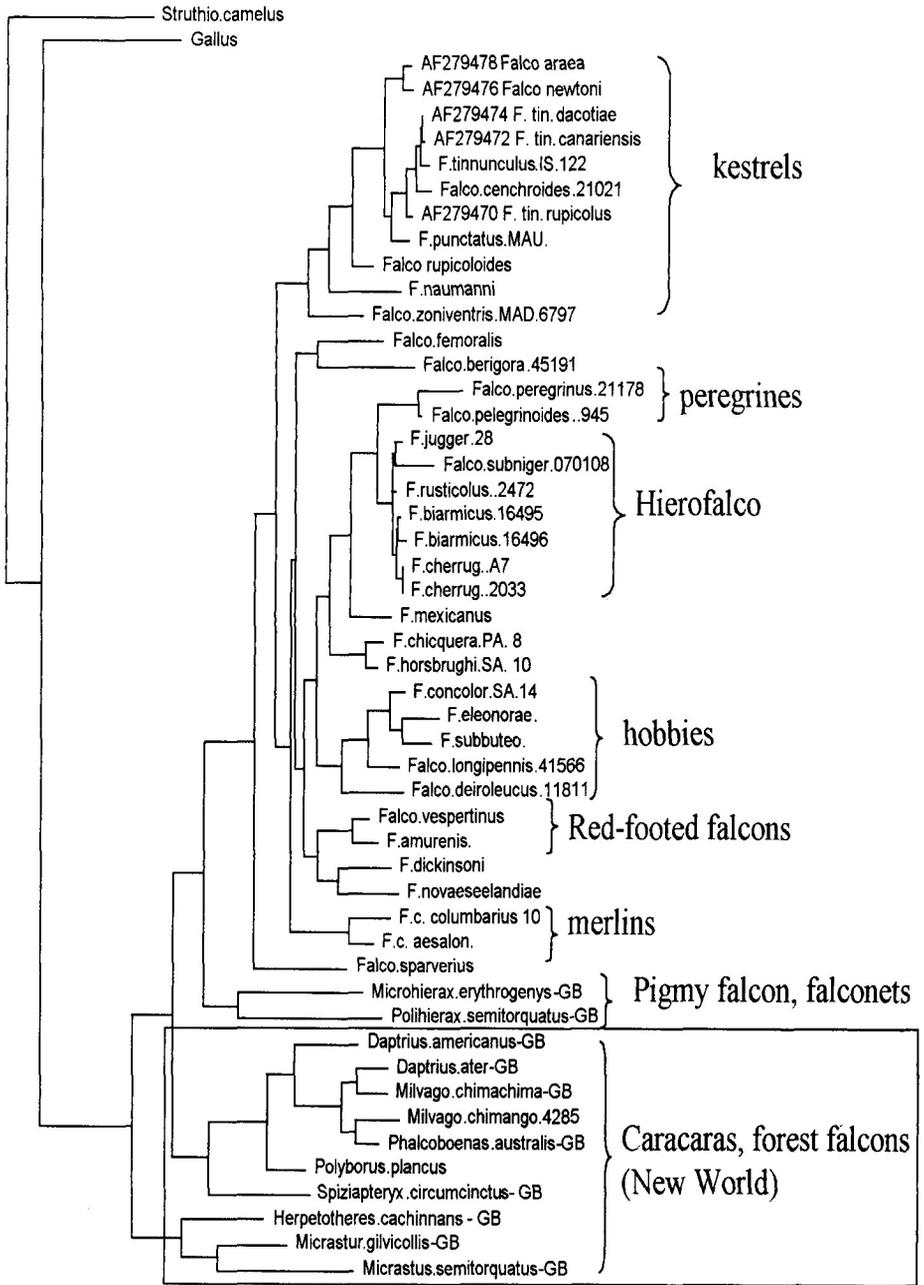
Within the genus *Falco* the following monophyletic clades can be distinguished: a) the Kestrels, b) Merlins, c) the Hobby group, d) Hierofalco (Saker, Gyr, Laggar, Lanner, Black Falcon) and Peregrines (Figure 4, 5).

a). Kestrels

Kestrels diverge near the base of the monophyletic *Falco* clade (Figs. 1,4). The Eurasian Kestrel (*F. tinnunculus*) has been subdivided in several subspecies, and some island forms have already been considered as distinct species, such as *F. newtoni*, *F. punctatus*, and *F. araea* (del Hoyo *et al.*, 1994; Sibley & Monroe 1990). Our data set contains *F.t. tinnunculus* from Europe, *F. cenchroides* from Australia and *F. punctatus* from Mauritius. Sequences from *F. newtoni* and *F. araea* were obtained from Groombridge *et al.* (2002). Differences in size, plumage patterns and distribution are also reflected at the cytochrome b level. DNA data support the view that these taxa derive from a common ancestor with the Greater Kestrel from South Africa as a sister group (Figure 4). The Lesser Kestrel, which forms a sister group to the Eurasian Kestrel (Figure 1), breeds in the Mediterranean and in parts of Eastern Europe and Asia (see Wink, Sauer-Gürth & Pepler in this volume for a detailed analysis). The Banded Kestrel (*F. zoniventris*) from Madagascar does not cluster with *F. dickinsoni*, as suggested by del Hoyo *et al.* (1994) but shows affinities to the kestrel complex (Figure 4).

Figure 4. Molecular phylogeny of the Falconidae inferred from nucleotide sequences of the cytochrome b gene. Reconstruction with maximum parsimony; representation as a phylogram

MP Falconidae



— 10 changes

b). *Merlins*

Several subspecies are recognized in *F. columbarius*. Our analysis included *F. c. columbarius* from North America and *F.c. aesalon* from northern Eurasia. The cytochrome b gene shows substantial sequence divergence (2% distance) which is in the range of distinct species. Because of geographic, size and plumage differences, it would be plausible to treat both subspecies as distinct species (Wink & Seibold 1996; Wink *et al.* 1998).

c). *Hobbies and Red-footed Falcons*

Sooty and Eleonora's falcons and Hobby share many similarities in ecology and behaviour (food: birds and insects; breeding distribution in the Mediterranean and Europe but wintering quarters in Africa) which is reflected at the cytochrome b level (Seibold *et al.* 1993; Wink & Ristow 2000); these species form an unambiguous monophyletic clade (Figure 4). Also the Australian Hobby (*F. longipennis*) and the Orange-breasted Falcon (*F. deiroleucos*) are part of this assemblage and cluster at its base. *F. deiroleucos* has sometimes been associated with the peregrines but shows affinities with hobbies (del Hoyo *et al.* 1994). Red-footed and Amur falcons (*F. vespertinus*, *F. amurensis*) are sister taxa and sometimes show affinities with the Hobby falcon complex (Wink & Ristow 2000) but cluster independently in this study (Figure 4)

d). *Hierofalcons and Peregrines*

The Prairie Falcon, *F. mexicanus*, which has been viewed as a member of the Hierofalco complex, always clusters at the base to the Hierofalco/Peregrine clade (Figure 4) and cannot be regarded as a superspecies with *F. jugger* and *F. biarmicus* (del Hoyo *et al.* 1994). Distances between *F. mexicanus* and Hierofalcons range between 6 and 9% nucleotide substitutions, indicating that the New World *F. mexicanus* has diverged about 3 to 5 million years ago from an Old World ancestor (assuming a molecular clock calibration of 2% sequence divergence = 1 million years; Wilson *et al.* 1987; Tarr & Fleischer 1993).

F. peregrinus, of which more than 19 subspecies have been recognized (including the Barbary Falcon, *F. p. pelegrinoides*) (del Hoyo *et al.* 1994) does not show much haplotype variation (Figure 4, see Wink *et al.* 2000) and therefore either *F. peregrinus* represents a young taxon or a taxon with frequent gene flow between subspecies.

The Hierofalco group, which includes *F. rusticolus*, *F. cherrug*, *F. jugger* and *F. biarmicus*, appears as a closely related monophyletic species complex with recent speciation. According to our data, also the Black Falcon (*F. subniger*) from Australia appears to belong to this clade (Figure 4). The phylogenetic relationships in Hierofalcons have been studied in more in detail in Wink, Sauer-Gürth, Ellis & Kenward in this volume.

e) *Red-necked Falcon*

The Red-necked Falcon (*Falco chicquera*) occurs in two parapatric populations in Pakistan/India (*F.c. chicquera*) and in Africa south of the Sahara (*F. c. ruficollis* and *F.c. horsbrughi*) (del Hoyo *et al.* 1994). Differences in distribution and morphology are also reflected at the cytochrome b level, in that *F.c.chicquera* and *F.c. "horsbrughi"* (our samples derived from South Africa) differ by 1.8% nucleotide substitutions (equivalent to approximately 0.9 million years of divergence). It is unclear at the moment whether two

subspecies occur in Africa (the description of *F.c. horsbrughii* was based on a single bird and has been questioned). In any case, differences in morphology, distribution and cytochrome b suggest that both taxa have reached species level already and might be treated as distinct species, as *F. chicquera* and *F. ruficollis* or *F. horsbrughii*, respectively.

f) *Falco sparverius*

Falco sparverius of the New World does not cluster with kestrels as expected from behaviour and morphology, but either forms a clade together with *F. femoralis* or takes a basal position (Figure 4). Apparently, New World falcons must have diverged from the falcon tree a long time ago. The positions of the Australian Brown Falcon (*F. berigora*), the NewZealand Falcon (*F. novaeseelandia*) and the African Dickinson's Kestrel (*F. dickinsoni*) cannot be determined with certainty with the present cat b data set.

The caracaras and forest falcons of the New World cluster at the base of the Falconidae; their relationships have been discussed in Griffiths (1997).

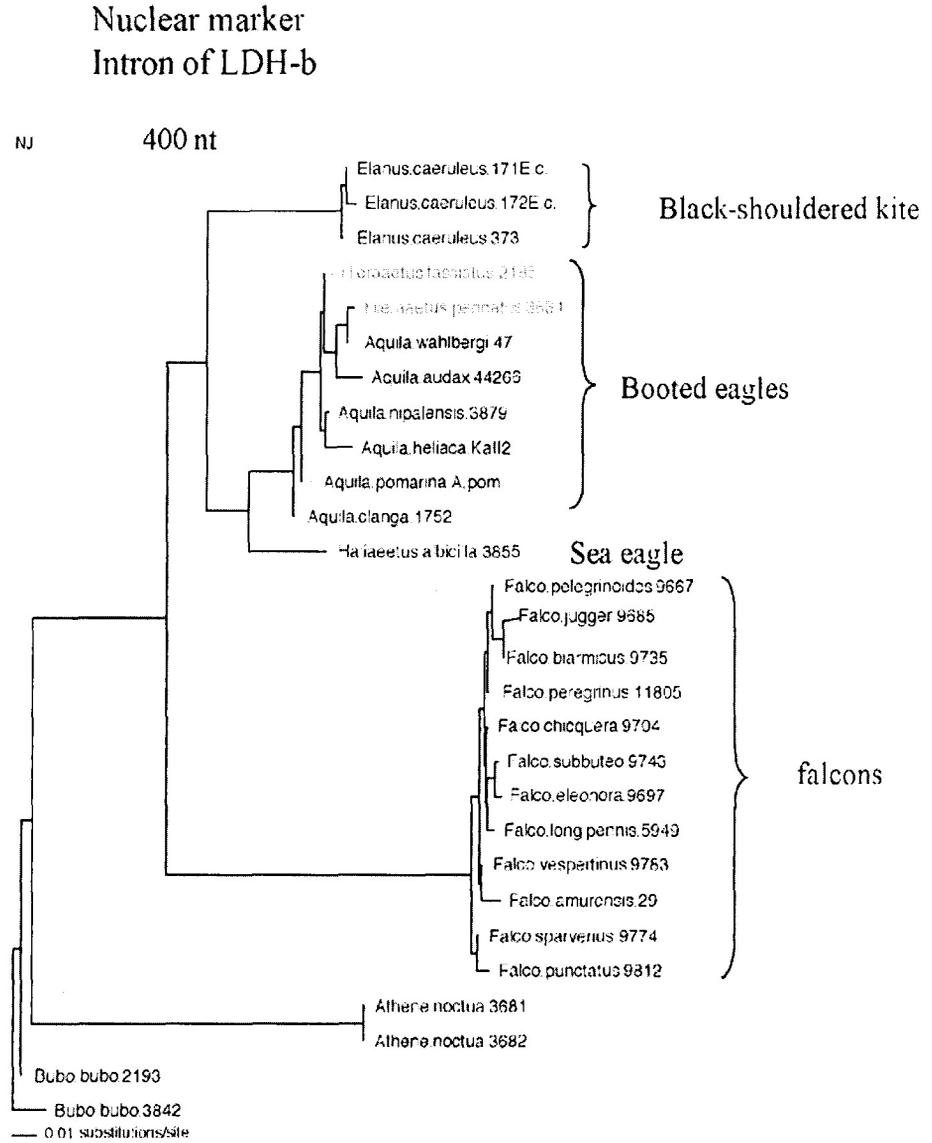
LDH Intron data

We have started to amplify and sequenced a nuclear marker gene. Since we had good results with LDHb intron DNA in our studies on owl phylogeny (Wink, Sauer-Gürth & Fuchs, in this volume) we have begun to use this marker for diurnal raptors. Figure 5 shows a preliminary tree, indicating that *Elanus* differs indeed from Accipitridae and clusters at its base.

CONCLUSIONS

Phylogenies which were reconstructed with the present data set support many relationships which had already been elaborated using detailed anatomical, morphological and behavioural data (summarized in del Hoyo *et al.* 1994). This finding shows that nucleotide sequences of even a single mitochondrial gene can reconstruct phylogenetic relationships within a family with a high degree of reliability. Relationships between families are more difficult to resolve since the cytochrome b gene is at its limits under these conditions (Meyer 1994). In many instances the DNA helps to decide longstanding systematic questions, as to which genus a species might belong or if a given subspecies differing in distribution, size and morphology can be regarded as a distinct species. The latter distinction is not only important for systematics but also for nature conservation. The Spanish Imperial Eagle, for example, is a good species (Seibold *et al.* 1996) and as its numbers are down to 150 pairs, it is one of the rarest birds of prey, whereas the Eastern Imperial Eagle still holds approximately 2000 pairs (del Hoyo *et al.* 1994). This means that conservation of *A. adalberti* gains highest priorities as a distinct

Figure 5. A molecular phylogeny of raptors inferred from nucleotide sequences of the nuclear LDHb intron. Reconstruction with NJ.



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