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Advances in the molecular systematics of African raptors

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ABSTRACT

The mitochondrial cytochrome b gene was amplified and sequenced from approximately 35% of all species and 50% of genera of diurnal raptors. Nucleotide sequence data were used to reconstruct their molecular phylogeny using the Maximum parsimony, Neighbour Joining, and Maximum Likelihood methods. A number of new phylogenetic relationships were discovered in African members of the Falconidae, Pandionidae and Accipitridae. In a number of instances, DNA supports the view that certain subspecies, which differ by morphology, size and distribution, can be regarded as distinct species: such pairs are: *Falco chicquera* and *F. (c.) horsbrughi*, *F. columbarius* and *F. (c.) aesalon*, *Circus maillardi* and *Circus (m.) macrosceles*, *Circus cyaneus* and *Circus (c.) hudsonius*, *Milvus migrans* and *Milvus (m.) parasitus*, *Aquila (Hieraaetus) fasciatus* and *A. (f.) spilogaster*, *Pandion haliaetus* and *P. (h.) ridgwayi*. The genera *Aquila* and *Hieraeetus* are also found to be paraphyletic and therefore *Hieraeetus* should be merged in *Aquila*.

INTRODUCTION

Diurnal raptors have been grouped into five families, Accipitridae, Pandionidae, Sagittariidae, Falconidae and Catharthidae, and are placed in a common order Falconiformes (del Hoyo *et al.* 1994) or the infraorders Falconides and Ciconiides (Cathartidae), 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 (Wink 1995; Wink *et al.* 1998), indicating that the order Falconiformes or infraorder Falconides are apparently artificial units which combine birds that share a common life style, especially in behaviour and ecology. Because convergent traits are abundant in raptors, molecular data, such as DNA sequences of marker genes, which provide many characters for comparison that are less biased by parallel evolution than morphological, ecological or behavioural traits (Avise 1994; Mindell 1997), offer an opportunity to elucidate evolutionary relationships.

Nucleotide sequences of the mitochondrial cytochrome b gene have already been employed to study the systematics and evolution of diurnal raptors (Avise *et al.* 1994; Griffiths 1997; Mindell 1997; and from our laboratory: Seibold *et al.* 1993, 1996; Helbig *et al.*, 1994; Wink 1995, 1998; Wink & Seibold 1996; Wink *et al.*, 1996, 1998; Seibold & Helbig 1995, 1996). 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, cover approximately 50% of the genera and 35% of

the species and provide a first idea of their evolutionary past. Because these conclusions rely on incomplete data sets (and may thus suffer from 'long branch attractions' and insufficient resolution) much more work is needed before we shall be able to understand the evolution of diurnal raptors with more precision.

In our continuing effort to elucidate the molecular systematics of raptors we have sequenced the cytochrome b gene of a number of African raptors for the first time. In this communication, emphasis was laid on phylogenetic relationships among African taxa that constitute a substantial subset on all raptors.

MATERIAL AND METHODS

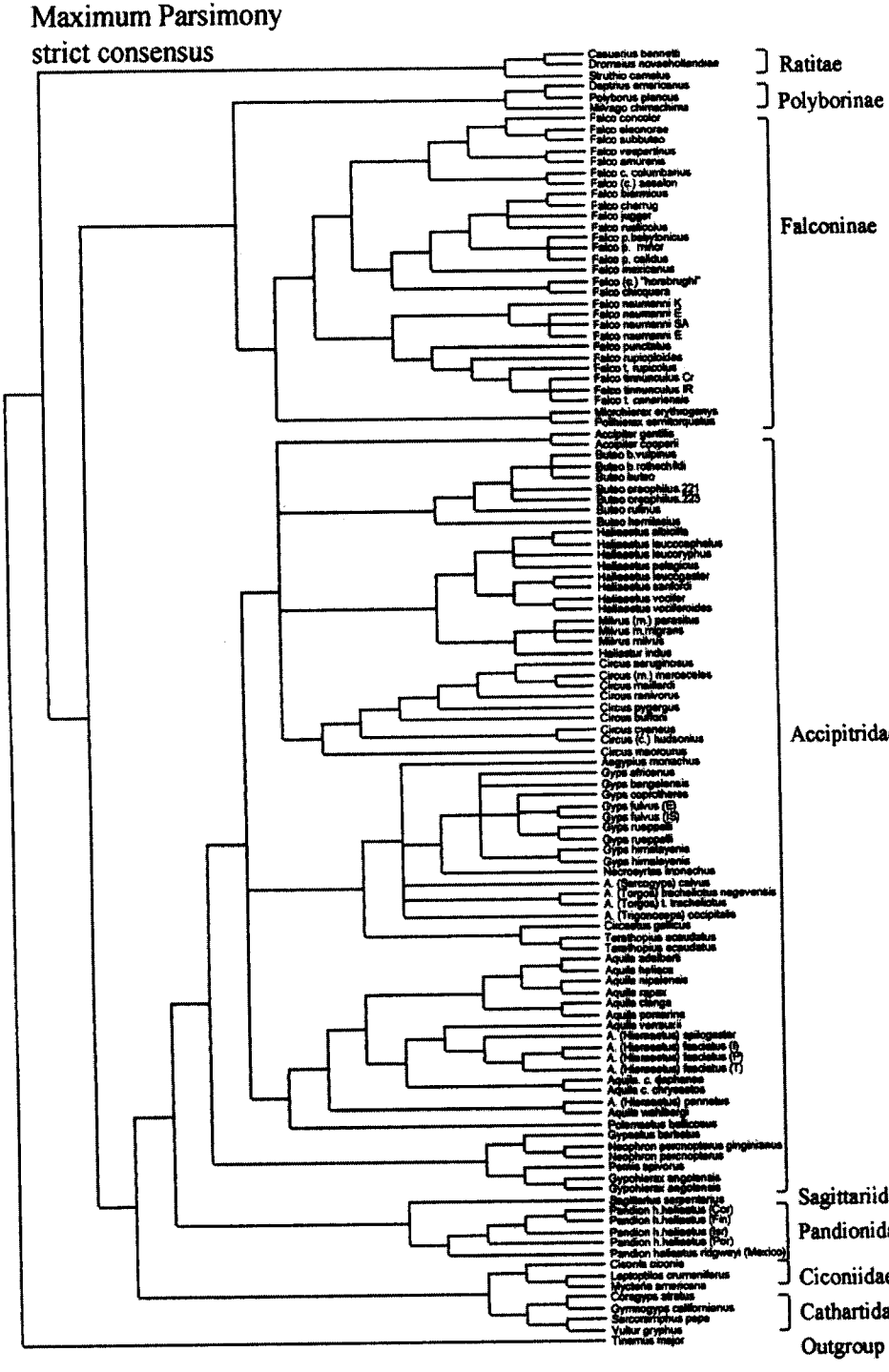
Origin of DNA, PCR and DNA-Sequencing

Blood and tissues were stored either in an EDTA buffer or in ethanol (Wink 1998) and stored at –20°C until processing. DNA was extracted using the proteinase K protocol. The mitochondrial cytochrome b gene was amplified by PCR using primers (Table 1). PCR products of at least two or more specimens were sequenced directly using the dideoxy chain termination method with the Sequenase PCR Product Sequencing Kit (Amersham Life Science, US70170) and [α-^{35}S] labelled dATP or using the cycle sequencing Kit (Amersham Life Science, RPN 2438/RPN 2538) in combination with CY5 labelled primers (Table 1). For cycle sequencing a two stage programme containing an initial denaturing step at 94°C for 4 min and 25 cycles at 60°C (40 sec), and 94° (30 sec) was used. Radioactive fragments were separated on a PAGE gel apparatus (Stratagene, Base Ace Sequencer) while CY5 labelled fragments were analysed on an automated Sequencer (Pharmacia, ALF-Express). Sequences of >1000 nt were read from autoradiograms or obtained directly from ALF-Express and aligned. Deletions, insertions or inversions were not encountered in cytochrome b. Part of the sequences used were based on earlier studies from our laboratory (Seibold 1994) or were taken from the literature [e.g., sequences of some Cathartidae from Avise *et al.*, (1994); of Polyborinae from Griffiths (1997)]. Usually two to over 10 sequences per species are available in our laboratory so that sequences used for this analysis can be regarded as representative for each species.

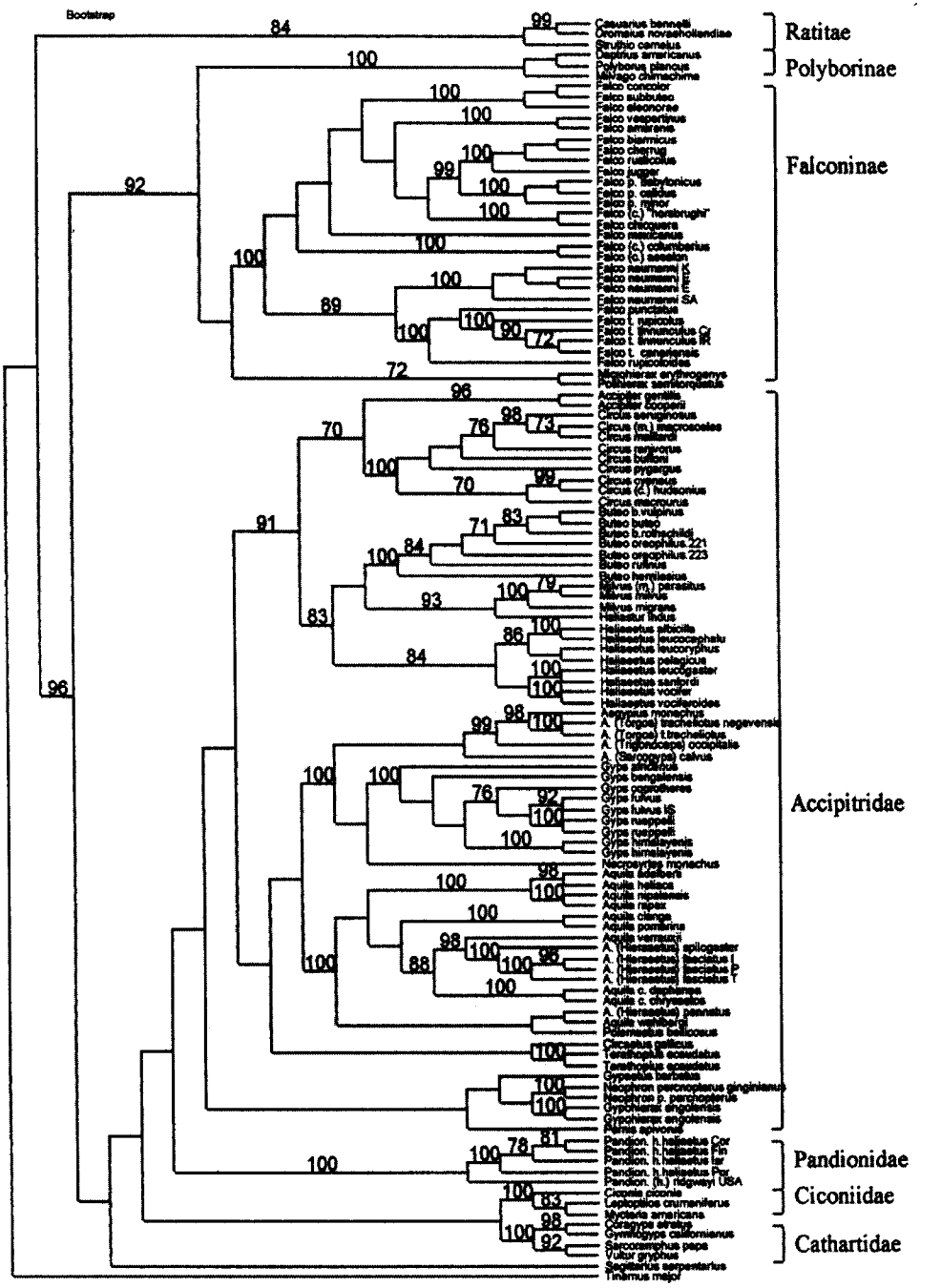
Table 1. Oligonucleotide primers used for PCR and sequencing. (x = CY5 fluorescent label. Sequence in 5'- 3' orientation)

PCR	
MT-A1	caacatctcagcatgatgaaacttcg
MT-A2	gccccatccaacatctcagcatgatgaaacttcg
MT-A3	ctcccagccccatccaacatctcagcatgatgaaacttcg
L14857	gggtctttcgccctatcaat
MT-F1	agggtggagtcttcagtttttggtttacaagaccaatg
MT-F2	ctaagaagggtggagtcttcagtttttggtttacaagaccaatg
SMT-B	tcaaatgatatttgtcctc
Cycle sequencing	
MT-C2-CY	xgaggacaaatatcattctgagg
MT-U2-CY	xggggtgaagttttctgggtc
MT-C4-CY	xagtgttgggttgtctactga
MT-U1-CY	xtccmggctcaaacaacccctagg
MT-CCY	xtaccatgaggmcaaacatc
SMTACY	xcaacatctcagcatgatgaaacttcg
MT-LeCY	xtcaaacccgaatgatayttcctatt
MT-V-CY	xtggagggcraaraatcgggt
SMT-FCY	xgtggagtcttcagtttttggtttacaagac
SMT-BCY	xtcaaatgatatttgtcctc

Figure 1. Molecular phylogeny of raptors.



Neighbour Joining



Strict consensus tree reconstructed using MP; 377 characters are constant, 267 variable characters are parsimony-uniformative and 616 parsimony-informative; tree lengths: 4691 steps; CI= 0.276; RI=0.683, HI= 0.724. Neighbour Joining bootstrap cladogram (1000 replications; p distance as distance algorithm)

Phylogenetic and statistical analysis

Phylogenetic relationships were analysed by different methods. The programme package MEGA (Kumar *et al.* 1993) and PAUP* were used to estimate genetic distances and to reconstruct trees with the Neighbour Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood methods (Swofford 1993). In MP, heuristic searches using the default settings were conducted without character weighting.

RESULTS AND DISCUSSION

For the present analysis, a data set was selected in which most species are represented by a single sequence. In a few instances, two or more sequences have been included from birds of different geographic origin in order to document phylogeographic variation (e.g., for *Hieraeetus fasciatus*, *Pandion haliaetus* or *Falco tinnunculus*).

Phylogenetic trees were reconstructed using the character state method MP (Fig. 1a) (represented as a strict consensus of 230 equally parsimonious trees) and the distance method NJ (Fig. 1b) (shown as a bootstrap cladogram; numbers are bootstrap frequencies (in %) at the respective furcation). ML analyses for the complete data set were not possible because of limited computation power. ML analyses which produce most reliable trees (Nei 1996), were constructed for partial phylogenies of smaller subsets. As can be seen from Figures 1a and 1b, the general topology of both MP and NJ trees is almost identical; differences can be seen in the placement of the genera *Sagittarius*, and *Circaetus/Terathopius*.

We have chosen *Tinamus* as a distant outgroup and the Ratitae 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.

Phylogenetic relationships between and within raptor families

The Falconidae, Cathartidae and probably the Accipitridae form monophyletic assemblages (Fig. 1), i.e. each of these families was derived from a common ancestor. The monotypic genera *Sagittarius* and *Pandion*, which are the sole representatives of their families, cluster at the base of the furcations which lead to the Accipitridae. 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 only small support for these furcations. 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 with a world-wide distribution. Four subspecies have been recognized, of which two are included in the present analysis. Within *P. h. haliaetus* populations (from Israel, Corsica, Portugal, and Finland) little phylogeographic differentiation can be seen, but a substantial difference (4% nucleotide divergence) can be detected between *P. h. haliaetus* and *P. h. ridgwayi* of the New World. Because similar distances have been found between distinct species and because of differences in distribution and morphology, it would be plausible to treat the New World Ospreys as a 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 a clear sister so far; storks never appear as a direct sister group to the *Sagittarius*, as suggested by Sibley and Ahlquist (1990).

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 (Figs. 1 & 2). 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.

Within the genus *Falco*, the following monophyletic clades can be distinguished: a) kestrels, b) merlins, c) hobbies (*Hypotriorchis*), d) desert falcons *Hierofalco* (Saker *F. cherrug*, Gyr *F. rusticolus*, Laggar *F. jugger*, Lanner *F. biarmicus*), and e) peregrines *F. peregrinus*.

Kestrels

Kestrels diverge at the base of the monophyletic *Falco* clade (Figs. 1 & 2). The Eurasian Kestrel (*F. tinnunculus*) has been subdivided into several subspecies, and some island forms have already been considered as distinct species, such as *F. newtoni*, *F. punctatus*, and *F. araea* (Sibley & Monroe 1990; del Hoyo *et al.* 1994). Our data set contains the subspecies *F. t. rupicolus* from South Africa, *F. t. canariensis* from Madeira, *F.t. tinnunculus* from Europe and *F. punctatus* from Mauritius. 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 *F. rupicoloides* from South Africa as a sister group. The Lesser Kestrel *F. naumanni*, which forms a sister group to the Eurasian Kestrel (Figs. 1 & 2), breeds in the Mediterranean and in parts of Eastern Europe and Asia. Cytochrome b data suggest that haplotypes of the populations of Spain and Kazakhstan differ and that sequence data can be used to determine the origin of wintering birds in Africa: A Lesser kestrel studied in South Africa came from the western Mediterranean (Figs. 1a & 2).

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).

Hobbies and red-footed falcons

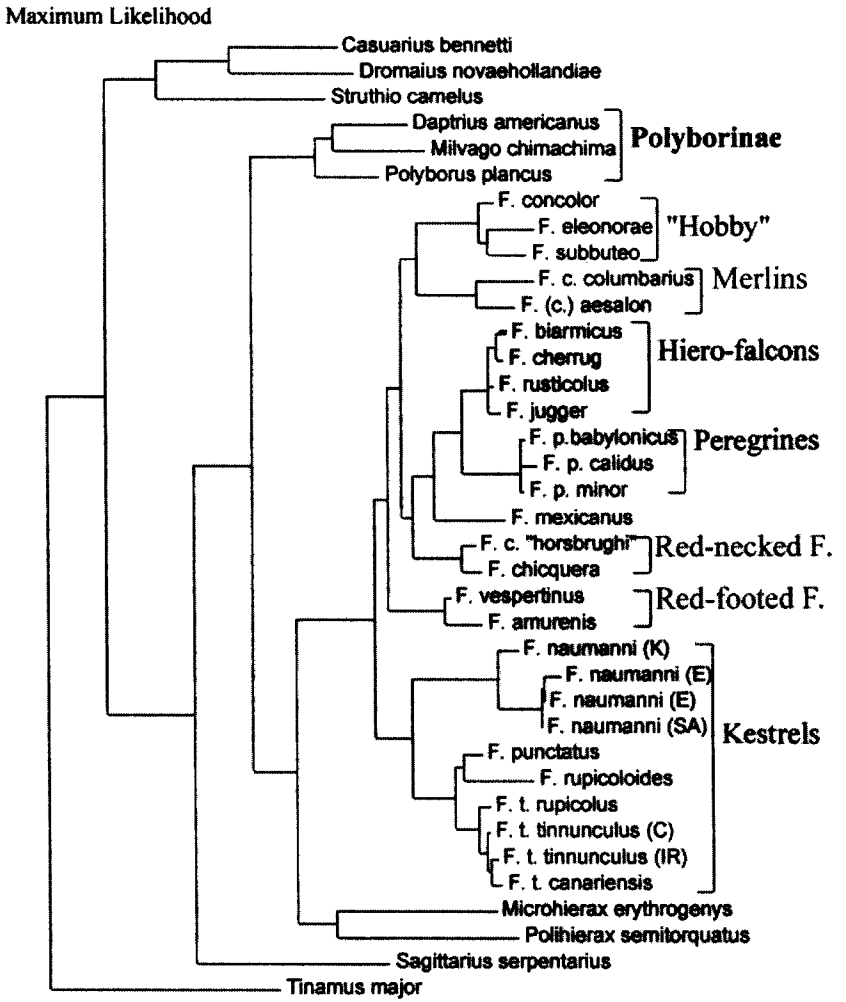
Sooty *F. concolor* and Eleonora's *F. eleanorae* Falcons and Hobby *F. subbuteo* share many similaries in ecology and behaviour (food of birds and insects; breeding distribution in the Mediterranean and Europe but wintering quarters in Africa), which is reflected at the cytochrome b level (Seibold *et al.* 1993). These species form an unambiguous monophyletic clade (Figs. 1 & 2). In several reconstructions (Figs. 1a & 2) Red-footed Falcon *F. vespertinus* and its sibling species, the Amur Falcon *F. amurensis*, cluster as a sister group. Both species share many similarities with the Hobby group, including a mixed diet, Eurasian breeding and South African wintering quarters. Plumage patterns in this group show a light and/or a dark type, suggesting that the ancestor of this assemblage could have been a hybrid between a dark and a light falcon species.

Desert hierofalcons and peregrines

The Prairie Falcon *F. mexicanus*, which has been viewed as a member of the *Hierofalco* complex, always clusters at the base of the Hierofalco-Peregrine clade (Figs. 1 & 2) and cannot be regarded as a superspecies with *F. jugger* and *F. biarmicus* (del Hoyo *et al.* 1994). Distances between *F. mexicanus* and *Hierofalco* 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 (Figs. 1 & 2; see also Wink *et al.* this volume). Therefore, either *F. peregrinus* represents a young taxon or a taxon with frequent gene flow between subspecies.

Figure 2. Molecular phylogeny of the Falconidae reconstructed with the Maximum Likelihood method.



Branch lengths are proportional to evolutionary distances.

The *Hierofalco* desert falcon group, which includes *F. rusticolus*, *F. cherrug*, *F. jugger*, and *F. biarmicus*, appears as a closely related monophyletic complex of species with recent speciation. In previous studies (Seibold *et al.* 1993; Helbig *et al.* 1994; Wink & Seibold 1996; Wink *et al.* 1998) we had reported that the *Hierofalco* group formed a monophyletic complex which clustered at the base of the falcon tree. Meanwhile we discovered that a paralogous cytochrome b gene exists in this group and that our earlier data were based on the nuclear copy of the cytochrome b gene. The present position of the *Hierofalco* group, as a sister group to the Peregrine, appears more likely (Figs. 1 & 2).

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. horsbrughi* 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. horsbrughi*, respectively.

ACCIPITRIDAE: *Haliaeetus*, *Milvus* and *Buteo*

Buzzards apparently form a monophyletic group with kites and sea-eagles (Fig. 1b). 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. However, *H. vociferoides* is clearly a sibling species of *H. vocifer* (Fig. 3). 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 (Figs. 1 & 3).

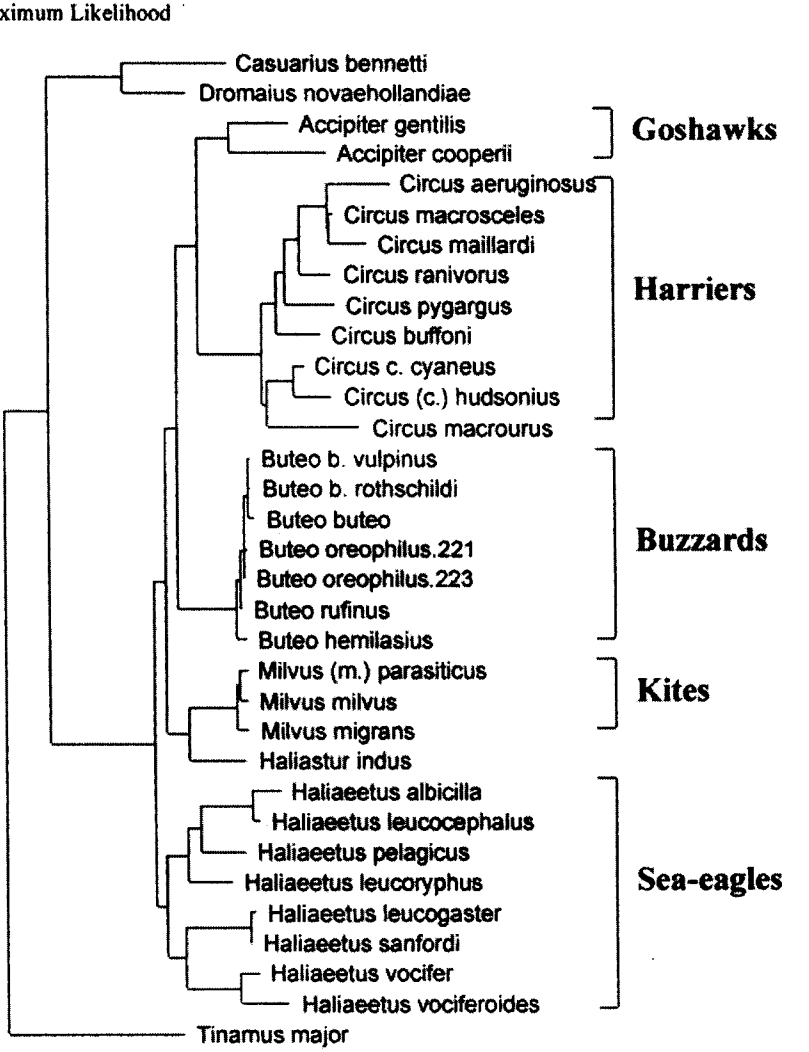
Kites of the genus *Milvus* are a closely related monophyletic group. The African Black Kite *M. m. parasitus* differs from the European and Australasian subspecies *M. m. migrans* by showing a yellow instead of a black beak. This difference is also reflected at the cytochrome b level (distances account for 1.6%). Because of geographic, morphological and genetic differences it would be plausible to treat African kites south of the Sahara as a distinct species (e.g., as *M. parasitus*, as suggested by some field guides). The Brahminy Kite *Haliastur indus*, which had been placed in the genus *Milvus*, is indeed the nearest relative of *Milvus* (distance 7%), so whether the genus *Haliastur* should be maintained is debatable (del Hoyo *et al.* 1994).

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 represent either a young group of raptors or that hybridisation between all of them has occured frequently. In other groups of raptors, such small differences were found in haplotypes of single species originating from different localities (see *Pandion*, *Hieraetus fasciatus*, *Falco peregrinus*, *Falco tinnunculus*). *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 is necessary to review the systematics of this genus which was always difficult because of strong plumage polymorphisms in the group.

Circus and *Accipiter*

Goshawks *Accipiter* usually cluster as a sister group to the monophyletic genus *Circus* (Figs. 1 & 3). Within species of the Marsh-harrier group, *C. aeruginosus* of Eurasia, *C. maillardi* of the Malagasy region, and *C. ranivorus* from South Africa, form a monophyletic group 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 (V. Bretagnolle, J.-C. Thibault pers. comm.). The Hen Harrier *C. cyaneus* and Pallid Harrier *C. macrourus* share close ancestry and both taxa share many similarities in plumage patterns. *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). *C. pygargus* and *C. buffoni* are not in the *cyaneus* clade, as could have been expected on account of different plumage patterns.

Figure 3. Molecular phylogeny of the buzzards, sea-eagles, kites and harriers, reconstructed with the Maximum Likelihood method.



Branch lengths are proportional to evolutionary distances.

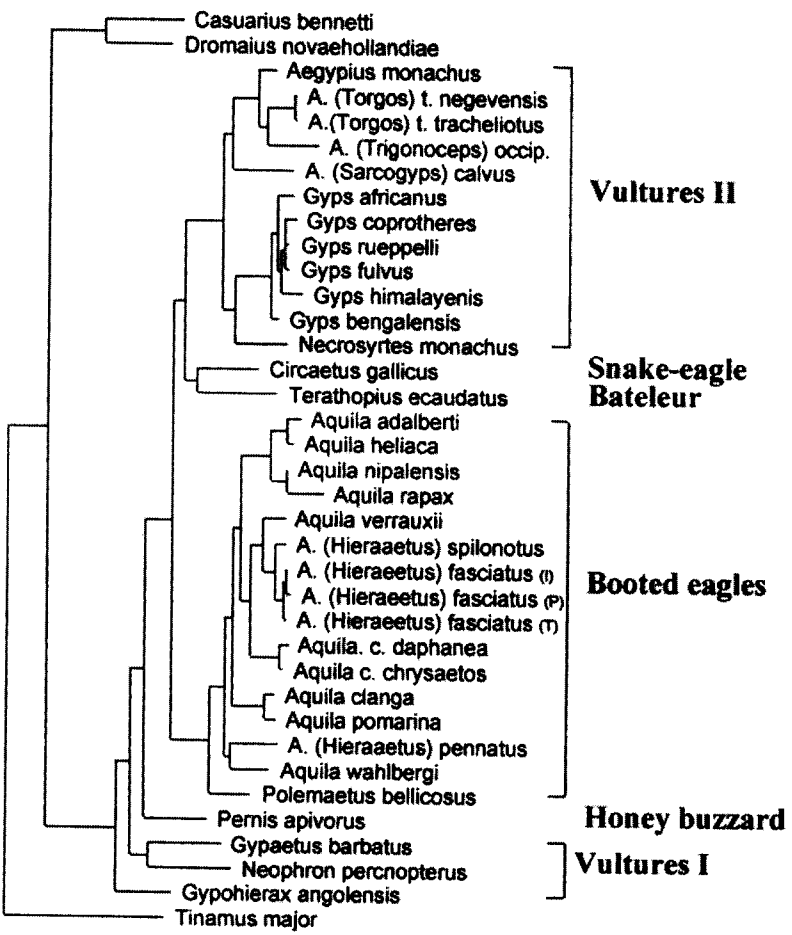
Old World Vultures

Within the group of Old world vultures two main evolutionary lineages are evident (Wink 1995; Wink & Seibold 1996; Wink *et al.* 1998; Seibold & Helbig 1995). One assemblage includes the Bearded Vulture *Gypaetus barbatus* and the Egyptian Vulture *Neophron percnopterus*. This clade shares many biological characters and is always positioned at the base of the Accipitridae cluster, indicating an evolutionarily old lineage of vultures. The Palm-nut Vulture *Gypohierax angolensis* of Africa had not yet been included in the molecular studies, 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 & 4). The Honey Buzzard *Pernis apivorus* never clusters with buzzards but always at the base of the Accipitridae tree, close to or in the *Gypaetus/Neophron* clade.

A second lineage includes the genera *Necrosyrtes* and *Gyps* which form a monophyletic clade, with *Aegypius*, *Torgos*, *Trigonoceps* and *Sarcogyps* as a sister group (Figs. 1 & 4). The latter group is comprised of monotypic genera which, because it constitutes a monophyletic clade of species which share many morphological and behavioural characters, could be placed in a single genus. The name *Aegypius* has already been proposed for this assemblage (Mundy *et al.* 1992; del Hoyo *et al.* 1994).

Gyps rueppellii and *G. himalayensis* were not included in previous molecular analyses, but form a closely related monophyletic group with *G. coprotheres* and *G. fulvus* (Figs. 1 & 4). Its members have been considered as a superspecies (Sibley & Monroe 1990; del Hoyo *et al.* 1994). Distances in this group are small, as observed in the *Buteo* complex, suggesting that they either represent young species or a species complex which shows some past and present hybridisation. For *G. africanus* and *G. bengalensis*, which had been placed in the genus *Pseudogyps* (having 12 and not 14 rectrices), genetic data imply a close relatedness to *Gyps* and their consideration as part of a common genus *Gyps* (as proposed by Sibley & Monroe 1990; del Hoyo *et al.* 1994)

Figure 4. Molecular phylogeny of the vultures and booted eagles reconstructed with the Maximum Likelihood method.



Branch lengths are proportional to evolutionary distances.

The example of Old 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.

Booted eagles and allies

Members of the genera *Aquila*, *Hieraaetus*, and *Polemaetus* share common ancestry (Figs. 1 & 4). This clade is apparently paraphyletic, indicating that the allocation of taxa to the genera *Aquila* and *Hieraaetus* does not reflect a phylogenetic sorting. Because *Hieraaetus* has been classified as a member of the genus *Aquila* before, the molecular data would support a merger of *Hieraaetus* into *Aquila*.

H. pennatus appears as a sibling species to *A. wahlbergi* (Figs. 1 & 4) and a relationship between both taxa has already been discussed, since the dark morph of *H. pennatus* is quite similar to *A. wahlbergi* (del Hoyo *et al.* 1994). *H. pennatus* breeds in Europe and Asia, but winters in Africa south of the Sahara (and in India) in the breeding grounds of *A. wahlbergi*. It could be argued that *A. wahlbergi* evolved from a dark morph of an ancestral *H. pennatus* that became resident in its winter quarters in Africa.

H. fasciatus (breeding in the Mediterranean and Asia) and *H. spilogaster* (breeding south of the Sahara) form sibling species (divergence 1.7%, Figs. 1 & 4). Morphological, geographical and genetical distances support the view to treat both taxa as distinct species (Sibley & Monroe 1990; del Hoyo *et al.* 1994). Within *H. fasciatus*, a low degree of haplotype differentiation can be seen (Cardia *et al.* this volume).

The relationships between *A. clanga*, *A. pomarina*, *A. rapax*, and *A. nipalensis*, and the recognition of *A. adalberti* as a distinct species from *A. heliaca* 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) (Figs. 1 & 4).

The Short-Toed Snake-eagle *Circaetus gallicus* and the Bateleur *Terathopius ecaudatus* form a monophyletic clade in all reconstructions (Figs. 1 & 4); this clade is not obvious as far as plumage patterns are concerned, but food and feeding, and especially breeding, show some similarities in both 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.

CONCLUSIONS

Molecular systematics of birds is still in its infancy (Mindell 1997) but will eventually provide a framework for the interpretation of anatomical, phylogeographic, and behavioural characters. 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 fact shows that 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 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 becomes one of the rarest birds of prey, whereas the Eastern Imperial Eagle still comprises approximately 2000 pairs (del Hoyo *et al.* 1994). This means that conservation of *A. adalberti* gains highest priorities as a distinct species.

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