Naturwissenschaften 80, 87–90 (1993)

Molecular Systematics of Falcons (Family Falconidae)

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The family Falconidae includes small to medium-sized diurnal birds of prey. According to morphological and biological criteria, the existing 60 species are divided into four subfamilies, 1. Polyborinae, 2. Herpetotherinae, 3. Micrasturinae, and 4. Falconinae. The latter group includes the genus *Falco* with 37 species [1, 2]. The members of the subfamily Falconinae are morphologically very homogeneous [2]. Their phylogenetic relationships are so far unresolved, because plumage and morphological characters are obvious adaptations to the way of life of a diurnal raptor and thus open to convergent evolution. Using these characters alone, it is very difficult to reconstruct the phylogeny of this group of birds [2]. We here present the first results of a DNA sequence analysis in an attempt to reconstruct a partial phylogeny of the genus *Falco*.

Recently, new methods, such as the polymerase chain reaction (PCR), have become available which allow the amplification and sequencing of marker

genes from total DNA of any organism [3]. Sequence variation of such genes can be used to evaluate relationships between species, genera, and higher taxa [4]. The ultimate goal is to reconstruct phylogenetic trees and to decipher the underlying evolutionary history or biogeographic migration patterns. In birds and other vertebrates the mitochondrial cytochrome b gene has become a useful and widely used marker gene, which displays enough sequence variation to assess phylogenetic relationships [4, 5].

We have determined and compared partial sequences of the cytochrome b gene (300 base pairs corresponding to positions 14846–15145 of the human mtDNA sequence; cf. [13]) to analyze the relationships among 17 taxa of the Falconidae. These include 16 members of the subfamily Falconinae (mostly Palearctic species) and the crested caracara (*Polyborus plancus*, subfamily Polyborinae). Small quantities of blood (100–200 µl) were collected and stored in an EDTA-NaF buffer [6]. Total DNA was isolated after digestion of protein with proteinase K. The cytochrome b gene was amplified by PCR using primers modified from [5]. PCR products were purified by agarose gel electrophoresis and reamplified asymmetrically. A portion of 300 nucleotides was sequenced directly employing T7 DNA polymerase and the chain termination method [3].

Sequences (1–4 per taxon) were aligned and analyzed with the program package PAUP 3.0 [7] using the maximum-parsimony method. Intraspecific variation was negligible with a maximum of two to three nucleotide differences, but usually none. The sequence of *Gallus domesticus* [8] was used as an out-group to root the partial phylogenetic tree of Falconidae. Figure 1 A illustrates the results of a heuristic analysis in form of a phylogram. Branch lengths are proportional to genetic distances between taxa. For comparison, Fig. 1 B gives the results of a bootstrap analysis (1000 replications) which provides probability values for each furcation (illustration as a cladogram).

The results (Fig. 1, Table 1) show that the genus *Falco* is monophyletic and that *Polyborus plancus* of the subfamily Polyborinae is paraphyletic. When sequence data from members of

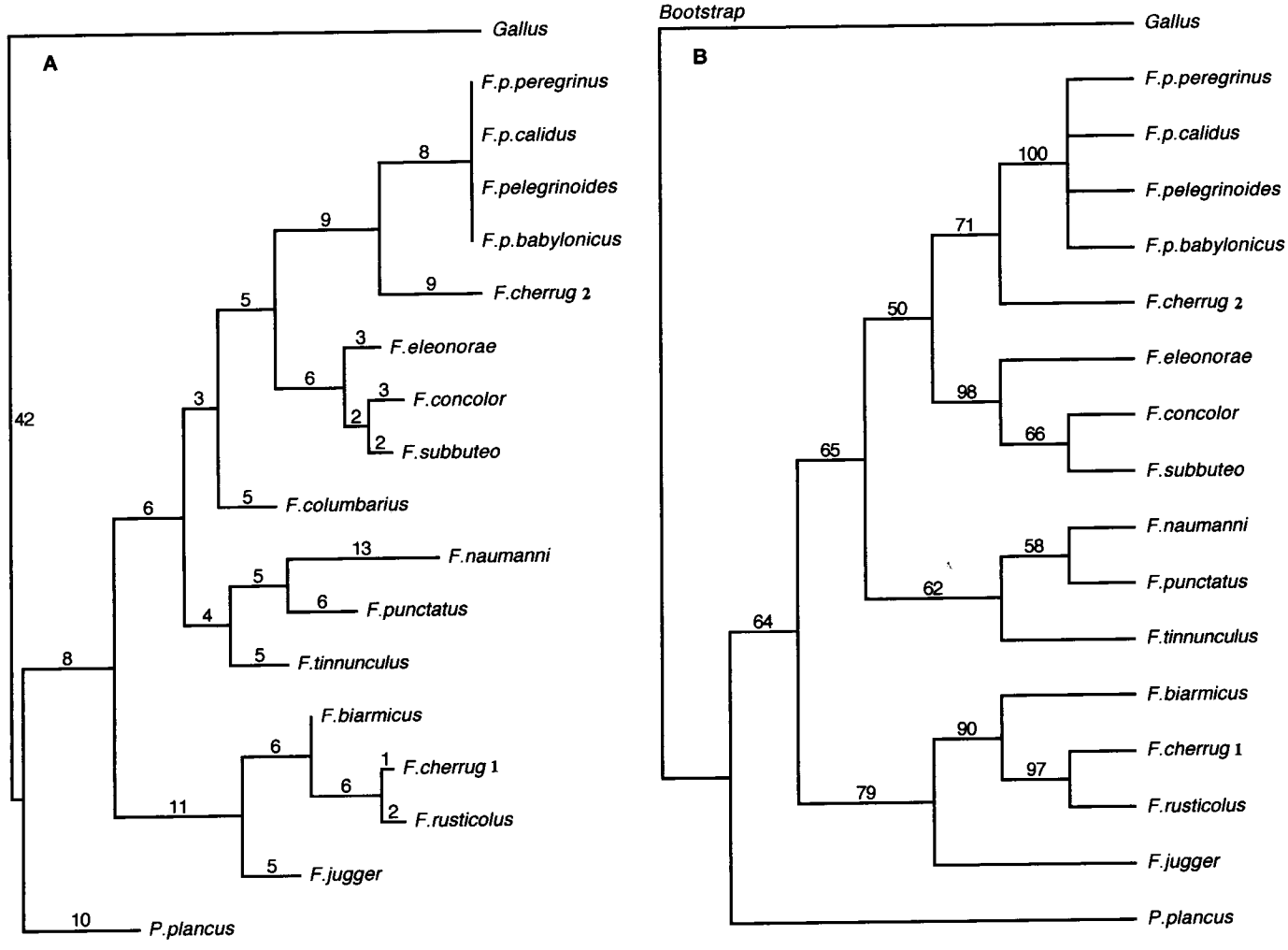

Fig. 1. Phylogenetic tree of the Falconidae (with *Gallus* as out-group) based on a maximum parsimony analysis [7] of nucleotide sequences (300 bp) of the cytochrome b gene. The following options were employed: heuristic search; sequence addition: closest, followed by a rearrangement of trees via "branch-swapping" [7]. A) Illustration as a phylogram. Numbers refer to nucleotide substitutions between nodes or nodes and terminal taxa. B) Results of a bootstrap analysis (1000 replications). Values are confidence estimates (in %) for each furcation

Table 1. Pairwise distances, i.e., nucleotide substitutions of cytochrome b gene between taxa. Below diagonal: absolute distances (number of substitutions); above diagonal: mean distances (adjusted for missing data) (in %)

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.	*F. p. peregrinus*	–	0.0	0.0	0.0	12.5	6.4	5.7	13.8	16.2	8.8	8.7	8.3	12.3	9.3	9.8	8.4	14.2
2.	*F. p. calidus*	0	–	0.0	0.0	12.5	6.4	5.7	13.8	16.2	8.8	8.7	8.3	12.3	9.3	9.8	8.4	14.2
3.	*F. pelegrinoides*	0	0	–	0.0	12.5	6.4	5.7	13.8	16.2	8.8	8.7	8.4	12.3	9.3	9.8	8.4	13.9
4.	*F. p. babylonicus*	0	0	0	–	12.5	6.4	5.7	13.8	16.2	8.8	8.7	8.4	12.3	9.3	9.8	8.4	14.2
5.	*F. biarmicus*	37	37	37	37	–	1.4	8.1	2.1	2.7	9.1	9.1	9.1	11.3	8.8	10.1	7.4	8.7
6.	*F. jugger*	19	19	19	19	4	–	3.4	3.8	6.2	7.1	6.4	6.4	8.5	6.4	7.8	3.7	8.0
7.	*F. cherrug* 2	17	17	17	17	24	10	–	10.0	12.0	9.1	9.0	9.3	11.3	7.7	9.5	7.1	11.8
8.	*F. cherrug* 1	40	40	40	40	6	11	29	–	1.0	11.0	10.3	10.7	12.4	10.0	11.7	8.6	10.8
9.	*F. rusticolus*	47	47	47	47	8	18	35	3	–	13.1	11.7	12.7	13.4	11.3	12.7	10.3	12.5
10.	*F. eleonorae*	26	26	26	26	29	21	27	32	38	–	2.7	2.4	10.6	7.8	7.8	6.4	12.5
11.	*F. concolor*	26	26	26	26	27	19	27	30	34	8	–	1.7	10.6	6.7	7.1	5.7	12.8
12.	*F. subbuteo*	25	25	25	25	27	19	28	31	37	7	5	–	10.9	7.7	8.1	6.8	13.2
13.	*F. naumanni*	36	36	36	36	33	25	33	36	39	31	31	32	–	7.2	6.5	7.8	12.5
14.	*F. tinnunculus*	28	28	28	28	26	19	23	29	33	23	20	23	21	–	4.7	5.7	10.8
15.	*F. punctatus*	29	29	28	29	30	23	28	34	37	23	21	24	19	14	–	6.4	11.8
16.	*F. columbarius*	25	25	25	25	22	11	21	25	30	19	17	20	23	17	19	–	9.0
17.	*P. plancus*	41	41	40	41	25	23	34	31	36	36	37	38	36	31	34	26	–

the Accipitridae are taken into account, Falconinae and Polyborinae obviously share a common ancestor and cluster outside the other Falconiformes [11].

The various taxa of the peregrine complex fall into a single clade. The underlying cytochrome b sequences are almost identical. The peregrines, *F. p. peregrinus* and *F. p. calidus*, have been classified as subspecies [2], a view which is supported by our sequence data. However, Barbary and Shaheen falcons, *F. pelegrinoides pelegrinoides* and *F. p. babylonicus*, are usually classified as a species separate from the peregrines [1, 2]. Our sequence data show that *F. peregrinus* and *F. pelegrinoides* have diverged very little, certainly less than other closely related species (Fig. 1, Table 1), and may thus be regarded as subspecies.

The subgenus *Hypotriorchis*, which includes Eleonora's falcon (*F. eleonorae*), sooty falcon (*F. concolor*), and Eurasian hobby (*F. subbuteo*) [2], is recognized as an independent clade and is paraphyletic to the peregrine complex (Fig. 1). These three species share a number of ecological and morphological features [1, 2], which may indeed reflect common descent: They are highly migratory, breeding in Europe, the Mediterranean region and the Middle East and wintering in E- and S-Africa or Madagascar; they feed on small birds and flying insects. Compared to other falcons, all are fairly late summer breeders with Eleonora's and sooty falcons feeding their young on Palearctic passage migrants.

The typical kestrels, which are traditionally regarded as a distinct subgenus *Tinnunculus*, are also recognized as an independent clade by our sequence analysis. Contrary to earlier hypotheses [2], they are not the phylogenetically oldest group within the genus *Falco*. Interestingly, the Mauritius kestrel (*F. punctatus*) seems to be more closely related to the lesser kestrel (*F. naumanni*), a Palearctic long-distance migrant, than to *F. tinnunculus*, which has resident populations in Africa much closer to Mauritius. The position of the merlin (*F. columbarius*) is still uncertain and we cannot say at present to which other species it is most closely related.

According to our parsimony analysis, the subgenus *Hierofalco* (gyrfalcon and related species [2, 9]) is the sister group of all other falcons studied. The inclusion of the lanner (*F. biarmicus*), lagger (*F. jugger*), and gyrfalcon (*F. rusticolus*) is in agreement with morphological, behavioral, and physiological data [1, 2]. However, results regarding the saker falcon (*F. cherrug*), usually grouped in the subgenus *Hierofalco*, are surprising. We found two distinct mitochondrial haplotypes: Type I (Fig. 1: *F. cherrug* 1) (six birds were analyzed) is closely similar to the gyrfalcon and supports this taxonomic view. Type II (Fig. 1: *F. cherrug* 2) (four birds) differs by 12% (Table 1) of the nucleotide positions from type I and clearly clusters with the peregrine complex. Birds of each genotype originated from SE Europe, Kasachstan, and Pakistan, so no geographic structuring of genotypes was apparent. According to plumage and morphology, they were all typical saker falcons. We assume that this discrepancy was caused by past hybridization [10] between male sakers and female peregrines and subsequent backcrosses with sakers. Interspecific hybrids are known to be fertile among several falcon species [2]. Since mitochondrial DNA is inherited clonally, the hybrids may have carried the peregrine mitochondrial genome into the saker population, where it persists today. The saker cytochrome b sequence of the peregrine type now differs substantially from that of peregrine (5.7%), indicating that the hybridization event took place in the relatively distant past.

Although the DNA phylogeny of falcons presented here comprises less than half of the living species, it allowed a more detailed resolution of relationships than a previous protein electrophoretic study [12] and is in good agreement with traditional morphological and biological data. The case of two highly divergent genotypes in the saker, however, illustrates how hybridization may lead to discrepancies between gene genealogy and species phylogeny. Ultimately, it is desirable to

base phylogenetic trees on both mitochondrial and nuclear gene sequences together with phenotypic characters to fully understand the evolutionary history of a taxon.

We thank M. Heidenreich, A. Schreiber, H. W. Prehn, M. Pomarol, H. Brüning, C. Fentzloff, D. Minnemann, P. Gaucher, D. Schmidl, C. Jones, W. Scharlau, and W. Bednarek for contributing blood or DNA samples for this study. Financial support by the Deutsche Forschungsgemeinschaft and the Landesgraduierten-Förderung Baden-Württemberg (to I.S.) is gratefully acknowledged.

Received October 26, 1992

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