Genetic vs. morphological differentiation of Old World buzzards (genus *Buteo*, Accipitridae)

Luise Kruckenhauser, Elisabeth Haring, Wilhelm Pinsker, Martin J. Riesing, Hans Winkler, Michael Wink & Anita Gamauf

Accepted: 20 May 2003

Kruckenhauser, L., Haring, E., Pinsker, W., Riesing, M. J., Winkler, H., Wink, M. & Gamauf, A. (2004). Genetic vs. morphological differentiation of Old World buzzards (genus *Buteo*, Accipitridae). — *Zoologica Scripta*, *33*, 197–211.

Here, a comprehensive phylogenetic investigation of Old World buzzards of the *buteo–vulpinus* complex and related taxa using morphological and genetic markers is presented. The morphometric analysis proved useful to discriminate taxa. Nevertheless, phylogenetic relationships cannot be resolved with these characters. Sequence comparisons between the control region and the pseudo-control region revealed that the latter is the most variable section of the mitochondrial genome. Consequently it was used as a marker sequence. In the genetic analysis, almost no sequence variability was found among taxa comprising the *buteo–vulpinus* complex as well as *Buteo rufinus* and *Buteo oreophilus*, suggesting gene flow and/or incomplete lineage sorting. Thus, rapid morphological differentiation in adaptation to different environments was not accompanied by genetic differentiation of the mitochondrial genomes of these taxa. In contrast, the East Palearctic taxa are well differentiated genetically. The 'superspecies' concept and taxonomic consequences of our results are discussed.

Luise Kruckenhauser, Elisabeth Haring, Martin J. Riesing & Anita Gamauf, Museum of Natural History Vienna, 1014 Vienna, Austria. E-mail: Luise.Kruckenhauser@univie.ac.at
Wilhelm Pinsker, Institute of Medical Biology, University of Vienna, 1090 Vienna, Austria
Hans Winkler, Konrad Lorenz-Institute for Comparative Ethology (KLIVV), Austrian Academy of Sciences (OEAW), Vienna, Austria
Michael Wink, Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Germany

Introduction

The 28 species of the genus *Buteo* (del Hoyo *et al*. 1994; Ferguson-Lees & Christie 2001) include 10 Old World species. Among those, the *buteo–vulpinus* complex, an assemblage of several subspecies of *Buteo buteo* (among them *B. b. buteo* and *B. b. vulpinus*), spans a huge distribution area including the West Palearctic as well as parts of northern, eastern and southern Africa. Some members of this complex occur on Atlantic and Mediterranean islands and have been classified as separate subspecies (Swann 1926; Vaurie 1961; Bannerman & Bannerman 1963–68; Brown & Amadon 1968). Even for the local population in Spain, subspecies status has been proposed (Vaurie 1961; Glutz von Blotzheim *et al*. 1971). However, this taxon (*B. b.* '*hispaniae*') is no longer used in the more recent literature (del Hoyo *et al*. 1994; Ferguson-Lees & Christie 2001). Furthermore, the relationships between *B. buteo* and the two closely related species *B. rufinus* and *B. oreophilus* have been a matter of debate (Meinertzhagen 1954; Rudebeck 1957; Siegfried & Frost 1973; James & Wattel 1983). In spite of the fact that considerable variation within taxa is observed with respect to plumage colour and pattern as well as body size, the classification of taxa is mainly based on these highly variable characters. Therefore, the still unsettled taxonomy of the Old World taxa of the genus *Buteo*, especially of the *buteo–vulpinus* complex, is in need of an extended taxonomic revision.

The utility of molecular vs. morphological approaches and the limitations of morphological investigations for resolving phylogenetic branching patterns have been discussed repeatedly (Felsenstein 1985, 1993; Patterson 1993). Nevertheless, the general systematic framework of most groups of organisms is based on morphological traits. Considering the high degree of variability observed within the *buteo–vulpinus* complex, the question arises whether the traits used for the taxonomic classification are suitable to differentiate the described taxa unambiguously. So far, several morphological studies have been performed (e.g. Vaurie 1961; James 1984, 1986, 1988; Eck 1991; Londai 1995; Clark & Davies 2000). The partly discordant classifications of species or subspecies reflect different views of the respective authors, i.e. whether they

belong to either the taxonomic 'splitters' or 'lumpers'. Nevertheless, an exhaustive morphological investigation using multivariate analyses that include all described taxa has not yet been accomplished.

Genetic analyses carried out so far on the taxa of the *buteo–vulpinus* complex were based on two different sections of the mitochondrial genome: (1) the *cytochrome b* (*cyt b*) gene (Clouet & Wink 2000; Wink *et al*. 2000), and (2) a non-coding mitochondrial sequence, the so-called pseudo-control region (ΨCR; Haring *et al*. 1999), which may have originated by duplication of the genuine mitochondrial control region (CR) and neighbouring sequences in the ancestor of the falconiform lineage (Mindell *et al*. 1998). Both DNA analyses suggested that the group of subspecies comprising the *buteo–vulpinus* complex cannot be clearly differentiated at the genetic level, and that the species status of *B. rufinus* and *B. oreophilus* seems at least debatable. Although in the *cyt b*-based tree of Clouet & Wink (2000) *B. rufinus*, *B. b. bannermani*, and *B. b.* '*socotrae*' share some derived substitutions, this marker is apparently not variable enough to resolve phylogenetic relationships among such closely related taxa. In the previous study by Haring *et al*. (1999), the more variable ΨCR was used, but only a limited number of specimens was analysed. Furthermore, the sampling area covered only a part of the entire geographical range of *B. buteo* and not all island forms were included. Although the ΨCR appeared more appropriate than the protein-coding *cyt b* gene, the possibility remained that with the aid of another non-coding mitochondrial marker sequence the differentiation of these closely related taxa could be achieved. With the sequencing of the complete mitochondrial genome of *B. buteo* (Haring *et al*. 2001) the genuine CR (generally referred to as the most variable part of the mitochondrial genome) was identified and thus became available as an additional marker for phylogenetic analyses at low taxonomic levels.

The present study comprised morphological (morphometric and plumage characters) as well as genetic investigations. A subset of the specimens studied genetically was also included in the morphological analysis. The morphological analysis of 92 morphological characters was carried out on 177 study skins from 19 taxa: 17 valid, one non-valid (*B. b.* '*hispaniae*'), as well as one not yet formally described taxon from the island of Socotra (*B. b.* '*socotrae*'). Altogether these taxa belong to five described species. In the genetic analysis, 81 individuals were analysed covering the whole distribution range of *B. buteo* (including all subspecies except *B. b. oshirioi*) and, in addition, the related species *B. rufinus*, *B. oreophilus*, *B. hemilasius*, and *B. brachypterus*. The other more distantly related Old World species have been analysed in the molecular phylogeny of the whole genus (Riesing *et al*. 2003). Both non-coding sections of the mitochondrial genome, the ΨCR and the CR, were employed as phylogenetic marker sequences.

The aims of the investigation were: (1) to determine whether all the described taxa can be unequivocally distinguished by their morphological characters; (2) to determine which of the two sequences (ΨCR/CR) is more variable and thus better for differentiating closely related taxa; (3) to characterize the Palearctic taxa of the genus *Buteo* genetically; and (4) to determine whether the morphological data are concordant with the genetic data.

Materials and methods

Taxon sampling

Morphometric measurements were taken from 177 study skins (see Appendix) representing five species (19 taxa) housed in five museum collections: Naturhistorisches Museum Wien (NMW, Austria), Zoologisches Forschungsinstitut und Museum Alexander Koenig (ZFMK, Bonn, Germany), Naturalis (RMNH, Leiden, the Netherlands), Zoological Museum, University of Amsterdam (ZMA, the Netherlands), and the Natural History Museum–Tring (NHM/BMNH, UK). For most taxa, between nine and 16 specimens of adult birds were analysed. However, from some rare taxa (*B. b. bannermani*, 'Socotra' buzzard, *B. b. toyoshimai*) the number was smaller (between two and four). Although there is no pronounced sexual dimorphism in buzzards, we tried to achieve an equal sex ratio within taxa (0.44–0.54). Specimens from different localities covering the whole distribution area of each taxon were analysed. Because some taxa are long-distance migrants, as defined by Berthold (2000) (*B. b. vulpinus*, *B. b. japonicus*, *B. hemilasius*) and some others are migrating at least to some extent (*B. b. buteo*, *B. r. rufinus*, *B. r. cirtensis*, *B. b. menetriesi*, *B. b. refectus*), our aim was to include only birds from their breeding grounds. Consequently, with some exceptions, the birds analysed morphologically were collected during their breeding season. Moreover, we used study skins that represented, as much as possible, the range of plumage patterns, to take into account the variability within populations. With regard to taxonomic nomenclature we followed primarily del Hoyo *et al*. (1994), and, for some of the European taxa, Glutz von Blotzheim *et al*. (1971). The 'Socotra' buzzard, an endemic form of Socotra Island (east of Somalia), was designated as *B.* (*b.*) *socotrae* by Clouet & Wink (2000). Although this taxon has not yet been formally described we will use the taxon name, *B. b.* '*socotrae*'. Specimens analysed genetically (geographical origins, abbreviations, source, and GenBank accession numbers) and those also included in the morphological analysis are listed in Table 1. In general, specimens analysed genetically were selected according to the same criteria as those of the morphological analysis.

Measurements

Sixty-four external morphological variables were measured as defined in Leisler & Winkler (1985, 1991) and Gamauf

Table 1 Specimens used for the genetic investigation. Designations of specimens correspond to the subspecies names.

Taxon and specimen	Geographical origin	Source	Accession no.
B. b. buteo			
b.but2*	Haringsee, Austria, 1997	H. Frey, Haringsee, Austria	AF202188
			AY299608
b.but5*	Haringsee, Austria, 1997	H. Frey, Haringsee, Austria	AF202189
			AY299609
b.but11*†	Göteborg, Sweden, 1904	NMW 54.050	AF202190
b.but12*†	Lauternbach/Rhön, Germany, 1975	NMW 73.801	AF202191
b.but14	Sicily, Italy, 1999	M. Riesing, Vienna, Austria	AY299604
b.but15	Sicily, Italy, 1999	M. Riesing, Vienna, Austria	AY299603
b.but29	London, UK, 2000	F. Steinheimer, Tring, UK	AY299569
b.but30	Wales, UK, 1972	F. Steinheimer, Tring, UK	AY299570
b.but39	Veenhuizermaden, the Netherlands, 2000	R. G. Bijlsma, Wapse, the Netherlands	AY299572
b.but40	Leemdijk, the Netherlands, 2000	R. G. Bijlsma, Wapse, the Netherlands	AY299573
b.but41	Sweden, 1877	RMNH 9.701 (181)	AY299574
B. b. 'hispaniae'			
b.his1	Alicante, Spain, 1999	J. A. Martinez, G. Lopez, Alicante, Spain	AY299565
b.his2	Alicante, Spain, 1999	J. A. Martinez, G. Lopez, Alicante, Spain	AY299566
b.his3	Alicante, Spain, 1999	J. A. Martinez, G. Lopez, Alicante, Spain	AY299567
b.his4	Alicante, Spain, 1999	J. A. Martinez, G. Lopez, Alicante, Spain	AY299568
b.his5	Madrid, Spain, 2000	R. Schirrmann, Madrid, Spain	AY299571
B. b. harterti			
b.har1	Madeira, Portugal, 1998	P. Sziemer, Vienna, Austria	AY299575
b.har2	Madeira, Portugal, 1998	P. Sziemer, Vienna, Austria	AY299576
b.har3*	Madeira, Portugal, 1998	P. Sziemer, Vienna, Austria	AF202194
			AY299611
b.har4†	Madeira, Portugal, 1913	ZFMK 7.632	AY299577
b.har5†	Madeira, Portugal, 2000	E. Vitek, K. Schrotta-Wiltschke, Vienna, Austria	AY299578
B. b. insularum			
b.ins1*†	Gran Canaria, Spain, 1909	NMW 30.482	AF202193
			AY299612
b.ins2†	Tenerife, Canary Islands, Spain, 1913	NMW 57.091	AY299579
b.ins3†	Canary Islands, 1996	M. Wink 2281, Heidelberg, Germany	AY299580
b.ins4	Canary Islands, 1996	M. Wink 2282, Heidelberg, Germany	AY299581
b.ins5	Canary Islands, 1996	M. Wink 2284, Heidelberg, Germany	AY299582
B. b. arrigonii			
b.arr1†	Sardinia, Italy, 1906	ZFMK 7.636	AY213049
			AY299610
b.arr2†	Sardinia, Italy, 1906	ZFMK 7.635	AY299563
b.arr3†	Sardinia, Italy, 2000	R. Bragioni, Roma, Italy	AY299564
B. b. rothschildi			
b.rot3†	Furnas, Azores, Portugal, 1907	ZFMK 523/159	AY299588
b.rot4†	Furnas, Azores, Portugal, 1907	ZFMK 572/158	AY299589
b.rot5†	Furnas, Azores, Portugal, 1907	ZFMK 571	AY299590
B. b. menetriesi			
b.men2*†	Tiflis, Georgia, 1902	NMW 50.038	AF202199
b.men3*†	Caucasus, Armenia, 1905	NMW 30.571	AY299613
			AF202200
b.men6	Eilat, Israel, 2000	R. Yosef, Eilat, Israel	AY299583
B. b. vulpinus			
b.vul1*	Eilat, Israel, 1998	R. Yosef, Eilat, Israel	AF202197
b.vul8*	Eilat, Israel, 1998	R. Rado, O. Hatzofe, Tel Aviv, Israel	AF202195
			AY299615
b.vul13*†	Orel, Russia, 1943	NMW 45.519	AF202196
b.vul14*†	Tomsk, Russia, 1901	NMW 57.040	AF202198
b.vul16	Tallinn, Estonia, 1990	J. Schergalin, Tallinn, Estonia	AY299584
b.vul17	Tallinn, Estonia, 1999	J. Schergalin, Tallinn, Estonia	AY299585

Table 1 *Continued.*

Taxon and specimen	Geographical origin	Source	Accession no.
B. b. 'socotrae'			
b.soc1†	Socotra, Yemen, 1934	BMNH 1934.8.12.3	AY213048
			AY299614
b.soc2†	Socotra, Yemen, 1999	R. Porter, Cambridge, UK	AY299601
B. b. bannermani			
b.ban2	Boa Vista, Cape Verde Islands, 2000	S. Hille, Vienna, Austria	AY299586
b.ban3	Sao Nicolas, Cape Verde Islands	AMNH 534.530	AY299605
b.ban5	Cape Verde Islands	M. Wink 5695, Heidelberg, Germany	AY299587
B. r. rufinus			
r.ruf1*	Hortobagy, Hungary, 1998	M. Dudas, Hortobagy, Hungary	AF202212
r.ruf3*	Israel, 1998	R. Rado, O. Hatzofe, Tel Aviv, Israel	AF202205
			AY299617
r.ruf9*†	Koj-Graig, Russia, 1900	NMW 15.543	AF202213
r.ruf11†	Narijn, Turkestan, 1910	G. Aubrecht, S. Weigl, Linz, Austria	AY299596
B. r. cirtensis			
r.cir1†	Algier, Algeria, 1839	NMW 57.033	AY299606
r.cir2†	Ouargla, Tunisia, 1893	ZFMK 638	AY299597
r.cir3†	Idelis, Algeria, 1914	ZFMK 641	AY299598
B. o. oreophilus			
o.ore1	Wlake Tanjanjika, Rep. Congo, 1910	NMW 3.3787	AY299616
o.ore3	Kinangop, Kenya, 1940	C. Gichuki, Nairobi, Kenya	AY299591
o.ore5	Mt. Elgon, Kenya, 1969	C. Gichuki, Nairobi, Kenya	AY213050
o.ore6	Mt. Kenya, Kenya, 1942	C. Gichuki, Nairobi, Kenya	AY299592
B. o. trizonatus			
o.tri1	Natal, South Africa, 1999	A. Jardine, Hout Bay, South Africa	AY299593
o.tri5*†	Pretoria District, South Africa	TM 45.636	AF202210
o.tri6*†	Knysna, South Africa, 1909	TM 6.280	AF202211
o.tri9†	Transvaal, South Africa, 1904	BMNH 1956.6.N.20.2197	AY299594
B. b. japonicus			
b.jap1*†	Japan, 1886	NMW 57.087	AF202201
b.jap2*†	Mt. Fuji, Japan, 1903	NMW 57.089	AY299602
b.jap4*	Kuangtun, China, 1915	ZMB 312.524	AF202204
b.jap5*	Kiantschun, China	ZMB B398N01	AF202206
b.jap6	Honshu, Japan, 1996	S. Weigl, Linz, Austria	AY213047
b.jap10	Kulusutay, Russia, 1999	S. Weigl, Linz, Austria	AY299599
b.jap12	Shanghai, China, 2001	E. D. H. Yasamaki, Tokyo, Japan	AY213066
B. b. toyoshimai			
b.toy1†	Bonin Island, Japan, 1889	BMNH 1955.N.20.2151	AY213063
b.toy3†	Bonin Island, Japan, 1889	BMNH 97.10.30.215	AY213064
B. b. refectus			
b.ref1	North India	A. T. S. Baker, London, UK	AY213059
b.ref2	North India	A. T. S. Baker, London, UK	AY213060
b.ref5†	Punjab, Dharmsala, India, 1921	BMNH 1949.Whi.1.472	AY213062
b.ref6	North Kunsu, Lau-hu-Kou, China, 1927	ZMB 35.364	AY213046
B. hemilasius			
hem1*†	Central Asia	NMW 10.785	AF202217
hem2*†	Altai, Russia, 1887	NMW 56.684	AF202218
hem8	Mongolia	M. Wink 3285, Heidelberg, Germany	AY299600
hem11	Altai, Russia, 2000	S. Ernst, Klingenthal, Germany	AY213067
B. brachypterus			
bra3	Madagascar, 1928	ZFMK 42.278	AY213072
bra4†	Tsiandro, Madagascar	M.-C. Dubois, Marseilles, France	AY299595
bra5†	Beroroka, Madagascar	M.-C. Dubois, Marseilles, France	AY213073
B. auguralis			
aug1	Vom, Nigeria, 1979	NMW 76.227	AY299607
			AY299618

*Individuals already analysed by Haring *et al.* (1999). †Individuals analysed morphologically. NMW, Naturhistorisches Museum Wien, Vienna, Austria; RMNH, Naturalis, Leiden, the Netherlands; ZFMK, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany; BMNH, the Natural History Museum, Tring, UK; AMNH, American Museum of Natural History, New York, USA; TM, Transvaal Museum, Pretoria, South Africa; ZMB, Zoologisches Museum Berlin, Germany.

et al. (1998). Measurements were made by only one person to avoid observer bias. The final data set for the discriminant analysis contained 44 variables. Apart from body length, these characters were assigned to one of three anatomical groups: (1) 28 characters were from the flight apparatus. The most important were wing length, Kipp's distance, length of each primary and selected secondary feathers, depth of the notches, tail length and its graduation. The correlation within this data set ranged from 0.0028 to 0.6885; (2) 11 variables were from the hind limb, namely tarsus length, length of the four toes and claws as well as claw diameters of digits I and IV. The correlation within this data set ranged from 0.0271 to 0.5289; (3) four bill dimensions were measured, including length (with and without cere), breadth, and height. The correlation within this data set ranged from 0.0835 to 0.5242. For the calculations, the measurements were not corrected for size, as this is an essential taxonomical criterion (Mayr 1963; Helbig 2000). Forty-eight characters of plumage pattern and colour nuances from the head, ventral side, and tail were recorded. On the body, 14 pattern types from the head and ventral side were distinguished, eight for the background colour, eight for the pattern itself, and three for the intensity of pattern colour. On the tail, seven pattern types and eight types of background colour were distinguished. No sex-related differences in plumage pattern or colour nuances were found. For the interrelationship between plumage variability and distribution range, the sizes of the geographical ranges were estimated from distribution maps (e.g. Ferguson-Lees & Christie 2001).

Morphometric analysis

We applied linear discriminant analysis to compute canonical axes and variates. UPGMA was based on a Euclidean distance matrix derived from the raw measurements and Wagner trees were calculated from plumage characters (program MIX; Felsenstein 1993). A matrix of taxonomic distances was derived from character means and used to construct a UPGMA phenogram to summarize overall external morphometric similarities. In addition, we subjected the correlation matrix of character means of all taxa to a discriminant analysis to identify the major axes of morphometric variation. *Buteo brachypterus* was used as an outgroup, because mitochondrial DNA analysis has shown this species to be basal to the other Old World buzzards (Riesing *et al*. 2003). Additionally, in comparison with the other taxa, *B. brachypterus* shows plumage peculiarities (in the ventral side and tail) and little intraspecific variation.

DNA extraction

DNA was extracted from study skins in a 10% Chelex solution (Biorad) containing proteinase K (0.5 mg/mL) for 3 h at 56 °C. After incubation the solution was heated for 5 min at 95 °C and centrifuged for 1 min. For purification and to remove short fragments of degraded DNA, the supernatant was purified using the QIA Quick PCR Purification Kit (Qiagen) with a final volume of 30–50 μL of elution buffer. DNA solutions were stored in aliquots to avoid frequent thawing. Control 'extractions' of pure extraction buffer were prepared.

Polymerase chain reaction (PCR), cloning and sequencing

PCR was carried out in a volume of 25 μL containing 0.5 units of DNA polymerase (Finnzymes OY), 0.5 μM of each primer and 0.2 mM of each dNTP. The solutions were heated to 95 °C (2 min) and put through 35 reaction cycles: 95 °C (10 s), annealing temperature (10 s), 72 °C (30 s), followed by a final extension at 72 °C (5 min). Optimal amounts of template DNA were determined empirically (2–10 μL of the DNA solution). If necessary, reamplifications were performed with 1–2 μL of the template. The following primers were used for amplification of sections of the CR or ΨCR region, respectively: CR: CR5+ (5′-CCCCCCCTTCCCCCCC-3′), CR7– (5′-GACCGACTAAGAGATAACCTA-3′); ΨCR: tGLUfwd (5′-CTCTCCAAAACCTACGACCTG-3′), YCR2–rev (5′-GGTTACATGGTTTGGTAGGGG-3′). Two negative controls were performed to detect contamination: control 'extractions' instead of the template and distilled water instead of the template. The PCR products were extracted from agarose gels using the Qiaquick Gel Extraction Kit (Qiagen) and cloned (TOPO TA Cloning Kit, Invitrogen). The sequencing of both strands was performed by MWG-Biotech (Ebersberg). GenBank accession numbers of sequences are listed in Table 1.

Intersimple sequence repeat (ISSR) profiles

ISSR–PCR is a method to detect interspecific variation, to analyse the genetic structure of populations (Damodar *et al*. 1999; Ge & Sun 1999), to generate species-specific genomic fingerprints (Gupta *et al*. 1994; Zietkiewicz *et al*. 1994), and to reveal hybridization (Wink *et al*. 2000). ISSR employs a single PCR primer, which is complementary to microsatellites, to amplify sections between adjacent microsatellite loci (one of which has to be inverted). ISSR fingerprints are variable mainly between species due to major genomic changes occurring over longer divergence times. Single PCR primers $(GACA)_4$ or $(CA)_{10}$ (10–20 nmol) were employed for ISSR–PCR. For technical details see Wink *et al*. (2000).

Sequence analysis

Alignments of DNA sequences were produced with the program CLUSTAL X (Thompson *et al*. 1997). Maximum parsimony dendrograms were calculated with the software package PAUP* (test version 4b6–10; Swofford 2002) with heuristic search using the tree bisection reconnection (TBR) algorithm and a random taxon addition sequence.

Gaps were treated as a fifth character; all characters were weighted equally. The minimum spanning network depicting the relationships among haplotypes was constructed manually.

Results

Morphometrics

First, the morphological differences among the 19 taxa were quantified. Scientific descriptions of the *Buteo* taxa are mostly based on plumage colour and pattern, and on some morphometric characters taken from a single or a few individuals. Nevertheless, these characters can be extremely variable, especially within the genus *Buteo*. In the present study, a discriminant analysis was performed using 44 morphometric characters (Fig. 1) to quantify the degree of differentiation among taxa. The differences among the groups in these characters were significant. The first component provided the best overall discrimination between groups (Wilk's lambda: 3.1×10^{-7}; $F_{18,157} = 3.670$, $P < 0.001$). The first component described 50.6% of the variation and was defined by characters related to size. The first canonical axis was negatively correlated mainly with body length, flight characters (wing length and breadth, Kipp's distance, depth of the notches, tail length), tarsus length, and feeding apparatus (bill breadth). The second component described 12.1% of the variation and correlated negatively with the killing apparatus (length of toes and claws). The third component described 8.4% of the variation and was defined by the depth of the notches of the outer primaries. Because the three components were approximately orthogonal, the measures of the axes were largely independent.

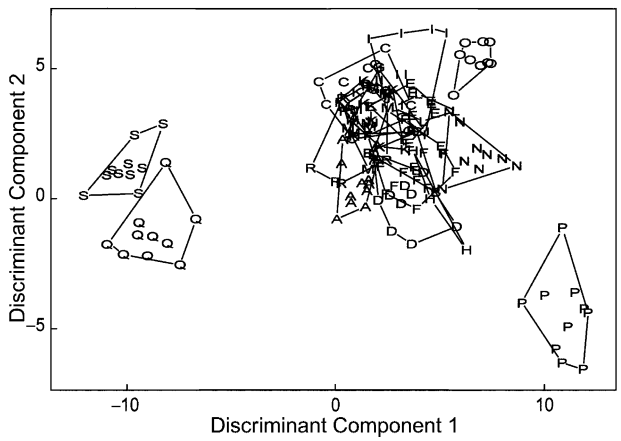

Fig. 1 Morphological separation of 19 Old World *Buteo* taxa according to discriminant component analysis. (A) *Buteo b. buteo*, (B) *B. b.* '*hispaniae*', (C) *B. b. harterti*, (D) *B. b. insularum*, (E) *B. b. arrigonii*, (F) *B. b. rothschildi*, (G) *B. b. bannermani*, (H) *B. b.* '*socotrae*', (I) *B. b. vulpinus*, (J) *B. b. menetriesi*, (K) *B. b. japonicus*, (L) *B. b. toyoshimai*, (M) *B. b. refectus*, (N) *B. o. oreophilus*, (O) *B. o. trizonatus*, (P) *B. brachypterus*, (Q) *B. r. rufinus*, (R) *B. r. cirtensis*, (S) *B. hemilasius*.

By plotting the data in a morphospace, the 19 different taxa of *Buteo* can be divided into three groups differentiated mainly by size-related characters (Fig. 1): (1) the two largest species *B. hemilasius* (weight up to 2000 g) and *B. r. rufinus* (weight up to 1500 g), which also are well separated from each other, (2) a main group containing 16 taxa, and (3) *B. brachypterus*, the smallest species with a weight up to 600 g. Generally, body size was positively correlated with the size of the distribution range ($r_{19} = 0.4140$, $P < 0.05$).

To resolve the main group further we performed a separate discriminant analysis including the taxa of group 2 only (Fig. 2A). Within the morphospace defined by the first two canonical axes there was still some overlap among these 16 taxa. Nevertheless, the differences proved significant (Wilk's lambda: 1.7×10^{-6}; $F_{15,130} = 3.054$, $P < 0.001$). The first component described 19.5% of the variation and was based on size-related characters. Besides body length, characters of the hind limb (tarsus length, length of hind and middle toe) and flight-dependent characters (wing length, length of outermost primaries and wing breadth, Kipp's distance) were negatively correlated along the first axis. The second component described 17.4% of the variation. Body length, wing parameters (length of innermost primaries, depth of the notches), and tail length correlated negatively with the second axis. The third component described 13.8% of the variation. This axis was positively correlated with some features of the hind limb (length of the innermost and outermost claws).

In spite of the generally high degree of morphological overlap in *Buteo*, it is remarkable that taxa from geographically adjacent breeding areas do not overlap morphologically, as shown in Fig. 2(B) (e.g. *B. b. buteo–B. b. vulpinus*, *B. b. buteo–B. b.* '*hispaniae*', *B. b. vulpinus–B. b. japonicus*). In contrast, the morphospaces of *B. b. japonicus* and *B. b. refectus*, which have disjunct distribution ranges on the Asian mainland, overlap extensively (Fig. 2C). The same is true for the subspecies found on small islands (e.g. *B. b. rothschildi–B. b. bannermani* and *B. b. insularum–B. b. harterti*) as shown in Fig. 2(D). Within the species *B. rufinus* the nominate subspecies *B. r. rufinus* belongs to group 1 in Fig. 1, but *B. r. cirtensis*, which is smaller and more *buteo*-like, is found in the very centre of the main cluster (group 2) (Figs 1, 2C). Only the widely distributed *B. r. rufinus* (southeast Europe to Central Asia) is locally sympatric to other taxa (*B. b. buteo*, *B. b. vulpinus*, *B. b. menetriesi*, *B. hemilasius*), whereas the North African subspecies *B. r. cirtensis* is geographically isolated.

The overall morphometric similarities within the Old World genus *Buteo* are reflected in a UPGMA phenogram (Fig. 3). Three main branches become apparent. The first clade comprises the four *B. buteo* subspecies from central and southwestern Europe, including the Canary Islands and Madeira, as well as *B. hemilasius* (Central Asia) and both

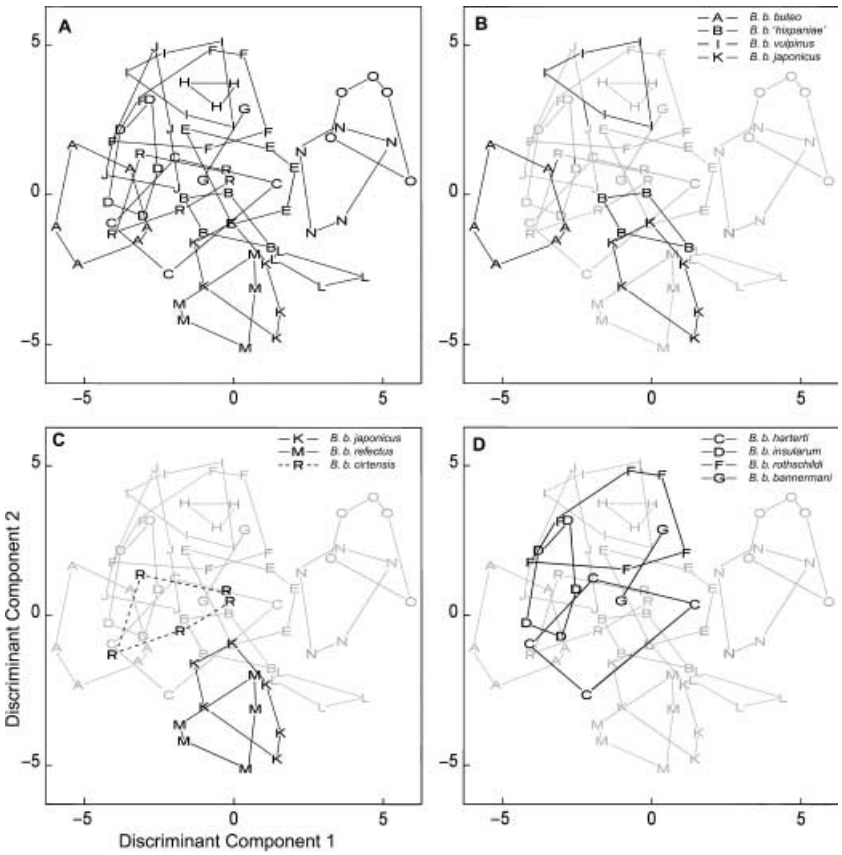

Fig. 2 A–D. Morphological separation of the main cluster containing 16 *Buteo* taxa using discriminant component analysis (abbreviations as in Fig. 1).

subspecies of *B. rufinus* (southeastern Europe to Central Asia). They represent inhabitants of mixed (*B. buteo* ssp.) and dry open habitats (*B. r. cirtensis*, *B. r. rufinus*, *B. hemilasius*). The second clade consists of the basal taxa *B. b. arrigonii* (Mediterranean) and *B. o. oreophilus* (East Africa), the East Asian *japonicus* group, and a cluster with *B. o. trizonatus* (southern Africa), *B. b. vulpinus* and *B. b. menetriesi* (eastern Europe). These taxa are well adapted to forested habitats. The third clade joins *B. brachypterus* (Madagascar), which splits off at the base, with the triad *B. b. bannermani* (Cape Verde Islands), *B. b. rothschildi* (Azores) and *B. b.* '*socotrae*' (Socotra). Whereas the triad comprises species from dry islands, *B. brachypterus* prefers tropical forests.

In addition, the interrelationships between migratory behaviour and morphology can be demonstrated. For this purpose the individuals were grouped in migratory (*B. b. vulpinus*, *B. b. japonicus*, *B. b. refectus*, *B. r. cirtensis*, *B. hemilasius*), non-migratory (*B. b. buteo*, *B. b. hispaniae*, *B. b. arrigonii*, *B. b. harterti*, *B. b. insularum*, *B. b. rothschildi*, *B. b. bannermani*, *B. b.* '*socotrae*', *B. b. menetriesi*, *B. o. oreophilus*, *B. o. trizonatus*), and individuals of unknown migratory behaviour (individuals of *B. b. buteo*, *B. r. rufinus* and *B. r. cirtensis* depending on the locality). In some taxa (e.g. *B. b. buteo*) the migratory behaviour varies from sedentary to long-distance migrants depending on the geographical area (Glutz von Blotzheim *et al.* 1971; Alerstam 1990). Therefore, we classified the individuals according to their geographical origin rather than their taxonomic assignment. The discriminant analysis divided these three groups very well. Slight overlapping (16%) occurred only between migratory and sedentary individuals (data not shown). The differences between the characters were significant (Wilk's lambda: 0.00001; $F_{2,173} = 5.394$, $P < 0.001$). The first component, comprising 72.5% of the variation, was characterized mainly by body and wing size (long and broad wings), number and depth of the notches, Kipp's distance, and tail length. Among the characters of the hind limb, only tarsus length and length of the hind toe proved relevant. The second component described 27.5% of the overall variation. It correlated negatively mainly with the depth of the notches, especially of the outermost primaries.

Plumage

The analysis of the highly variable colour and plumage pattern resulted in a different tree topology (data not shown). The basal node defined by the outgroup forms a polytomy in which most of the ingroup taxa branch off as single lineages.

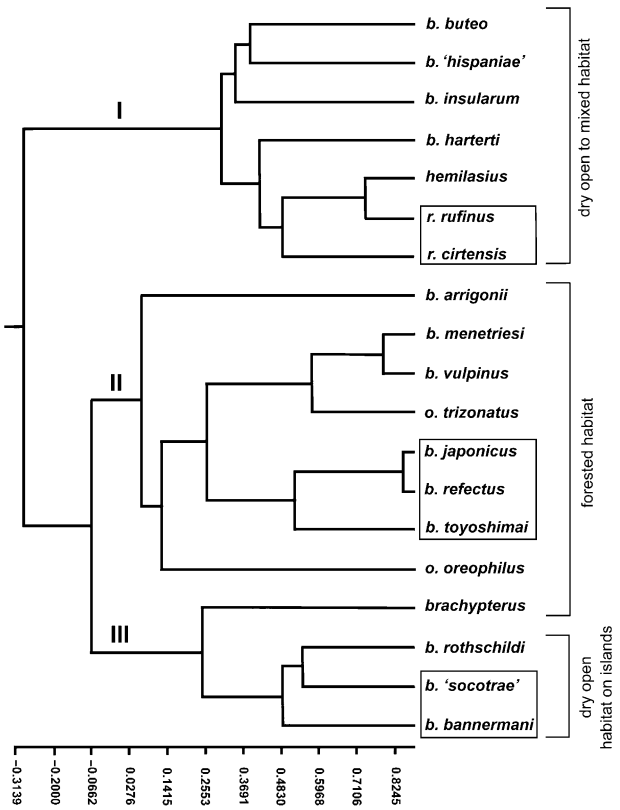

Fig. 3 UPGMA phenogram based on a correlation coefficient of all morphometrical variables of 19 Old World *Buteo* taxa. Remarkable is the low differentiation in deeper branches. Apart from *brachypterus*, the three groups (I, II, III) cluster according to three main types of habitat. Taxa also clustering in the analysis of plumage characters are framed.

Only three clusters become apparent: the two *B. rufinus* subspecies, the *japonicus* group (*B. b. japonicus*, *B. b. refectus*, *B. b. toyoshimai*), and *B. b. bannermani*/*B. b.* '*socotrae*'. The essential information of this tree is illustrated in Fig. 3 (framed taxa). Thus, colour and plumage pattern provide only limited information about phylogenetic relationships. Interestingly, the plumage variability seemed to be correlated with the size of the distribution area. Plumage variation is comparatively high in taxa with wide ranging distribution, e.g. *B. b. buteo*, *B. b. vulpinus*, *B. hemilasius*, but significantly lower in taxa with restricted distribution (oceanic and continental island habitats), e.g. *B. b. rothschildi* (see also James 1984), *B. b.* '*socotrae*' (see also Martins & Porter 1996), *B. b. menetriesi* and *B. o. trizonatus*, a phenomenon that could be explained by genetic drift.

Genetic analysis

In order to determine whether the genuine CR is more variable than the ΨCR, we sequenced a 389 bp section of the CR (5′-part) from a set of 11 individuals representing 10 taxa (b.ins1, b.but2, b.arr1, r.ruf3, b.but5, b.har3, b.men3, b.soc1, o.ore1, b.vul8, aug). From the ΨCR, which consists of a non-repetitive section followed by a cluster of 48 bp tandem repeats, a 279 bp section of the non-repetitive part (nrΨCR) was analysed from the same set of individuals. Although the ΨCR fragment is 110 bp shorter than the CR, it has twice as many variable sites (22 vs. 11). The distances ranged from 0.000 to 0.023 within the CR and from 0.000 to 0.059 within the ΨCR. Consequently, only the ΨCR was used as a marker sequence in the further analysis.

Using the primer pair tGLUfwd/YCR2-rev we amplified a 373 bp fragment (designated as ΨCR-L) from 53 different individuals. The amplified section comprised almost the entire nrΨCR spanning from the tRNA-Glu to 22 bp upstream of the repeat cluster. The alignment had a total length of 334 positions of which 260 sites were invariant and 36 were parsimony informative. The distances ranged from 0.000 to 0.049 (specimens of *B. brachypterus* not included). On the basis of this alignment we performed a maximum parsimony analysis using *B. brachypterus* as an outgroup. From the four most parsimonious trees a strict consensus tree was calculated. The main clusters are schematically presented in Fig. 4. The most basal lineage is composed of two identical *B. b. japonicus* sequences (b.jap10, b.jap12). A cluster consisting of two *B. hemilasius* and three *B. b. refectus* sequences splits off next, followed by another clade of two individuals of *B. b. japonicus* (b.jap6, b.jap2). Most sequences belong to the terminal main cluster which forms a trichotomy of (1) a large clade of 34 specimens representing 10 taxa designated as '*buteo* cluster' (among them 16 identical sequences), (2) a clade comprising

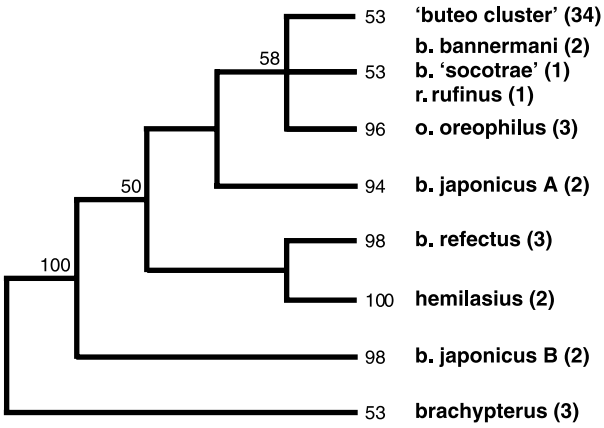

Fig. 4 Schematic presentation of the main clusters of a strict consensus tree of the ΨCR-L fragment (four equally most parsimonious trees). Bootstrap values (>50%, 1000 replicates) supporting the main clusters are given at the terminal branches; other bootstrap values are shown at the nodes. The number of specimens comprising the main clusters is in parentheses. Specimens representing the two *b. japonicus* lineages: (A) b.jap2 and b.jap6, (B) b.jap10 and b.jap12.

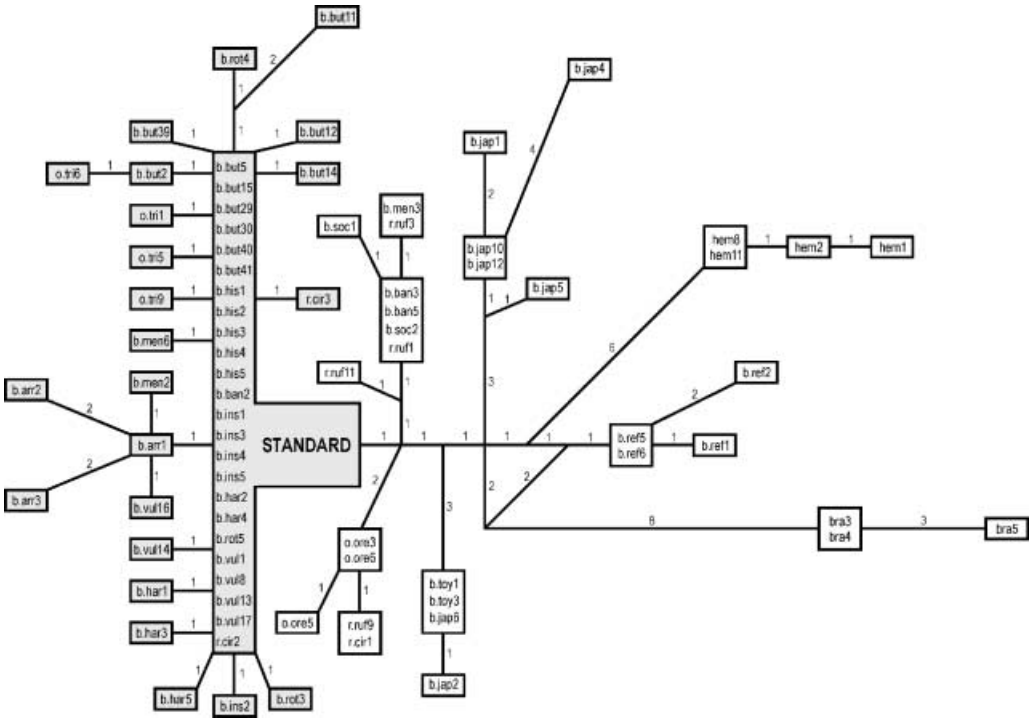

Fig. 5 Minimum spanning network of haplotypes (80 ΨCR-S sequences). The numbers at the branches denote mutational steps (substitutions or indels). Sample designation corresponds to Table 1. Haplotypes belonging to the '*buteo* cluster' are shaded grey.

B. b. bannermani, *B. b.* '*socotrae*' and *B. r. rufinus*, and (3) a clade containing three *B. o. oreophilus* sequences. Nevertheless, due to the small number of synapomorphic substitutions the clades of the West Palearctic buzzards have only low bootstrap support.

To increase the number of specimens we also included sequences determined in a previous study (Haring *et al*. 1999), which were obtained using a different primer pair. This fragment has a length of 379 bp and spans from ND6 to 116 bp upstream of the repeat cluster. The overlapping region (here designated as ΨCR-S) between the fragment analysed by Haring *et al*. (1999) and the ΨCR-L fragment measures 279 bp. The alignment of 80 ΨCR-S sequences has a length of 241 bp, 173 sites are constant and 33 are parsimony informative. The analysis of ΨCR-S yielded 16 most parsimonious trees. The topology of a strict consensus tree calculated from these 16 trees (not shown) differs from the ΨCR-L tree with respect to the basal lineages: the relationships among the four clades (two *japonicus*, *refectus*, *hemilasius*) as well as their relationships to the terminal main cluster are not resolved.

A minimum spanning network on the basis of the ΨCR-S alignment is shown in Fig. 5. From the 80 individuals, 24 had identical sequences which we designated as the 'standard haplotype'. There were 14 individuals with only one autapomorphic site and seven individuals with two or three substitutions with respect to the 'standard haplotype'. Altogether these 47 individuals represent the '*buteo* cluster' depicted in Fig. 4. This group contains all described subspecies of *B. buteo*, as well as individuals of the subspecies *B. r. cirtensis* and *B. o. trizonatus*. Two further clusters become apparent, representing the *B. b. bannermani*/*B. b.* '*socotrae*'/*B. r. rufinus* cluster and the *B. o. oreophilus* cluster of the dendrogram (Fig. 4). Members of these clusters are separated from the 'standard haplotype' by up to four substitutions. Clearly distinct from the 'standard haplotype' are five clusters represented by individuals of *B. b. japonicus*, *B. b. toyoshimai*, *B. b. refectus*, *B. hemilasius*, and the outgroup species *B. brachypterus*. The relationships among four of these clusters are not clearly resolved. The branch containing the Japanese *B. b. japonicus* and *B. b. toyoshimai* appears most closely related to the three West Palearctic clusters.

ISSR analysis of 22 individuals representing the taxa *B. b. buteo*, *B. b. vulpinus*, *B. rufinus*, *B. b. rothschildi*, *B. b.* '*socotrae*', *B. o. trizonatus*, and *B. hemilasius* was performed employing the primers $(GACA)_4$, and $(CA)_{10}$. Although up to 16 polymorphic bands were detected, no clear differentiation among the taxa was found (data not shown). For example, *B. o. trizonatus* clusters with *B. b. buteo* and *B. r. rufinus* with primer $(GACA)_4$, whereas in the $(CA)_{10}$ data set it is found basal

to the other taxa investigated. Together with two of the *B. b. buteo* individuals, *B. b.* '*socotrae*' clusters with *B. rufinus.* However, the other individuals of *B. b. buteo* are found on different branches.

Discussion

Morphological discrimination of taxa

In the morphometric analysis, four of the five currently recognized species (*B. brachypterus*, *B. hemilasius*, *B. oreophilus*, *B. buteo*) are well separated morphologically (Figs 1, 2). An exception is *B. rufinus*, of which only one subspecies (*B. r. rufinus*) is clearly distinct, whereas *B. r. cirtensis* falls into the morphospace of *B. buteo*. The two subspecies of *B. rufinus* differ considerably in size, which is the main factor in the first component of the discriminant analysis. While *B. r. cirtensis* resembles *B. buteo* in size, *B. r. rufinus* is almost twice as heavy. In the UPGMA dendrogram the two subspecies of *B. rufinus* appear much closer. Among the subspecies, at least the taxa with overlapping or adjacent breeding areas are differentiated. Moreover, the three East Palearctic subspecies *B. b. japonicus*, *B. b. refectus* and *B. b. toyoshimai* are slightly separated from the main cluster (Fig. 2).

Plumage characters are also useful to discriminate taxa, e.g. the two *B. rufinus* subspecies or the members of the *B. b. japonicus* group. Nevertheless, as is the case with the morphometric data, phylogenetic relationships among species are not resolved. Because most of the morphological differences concern adaptive characters they reflect mainly environmental conditions (see below).

Genetic characterization

In the genetic analysis, only the East Palearctic taxa (*B. b. japonicus*, *B. b. refectus*, *B. b. toyoshimai*, *B. hemilasius*) are well differentiated, whereas the taxa comprising the *buteo–vulpinus* complex (together with *B. rufinus* and *B. oreophilus*) cannot be discriminated unequivocally, even with the most variable mitochondrial marker sequence (ΨCR). This result is in accordance with the lack of differentiation in the ISSR profiles as well as the extremely low distances found among *cyt b* sequences (Wink & Sauer-Gürth 2000) ranging from 0.1 to 0.9% in species of the *buteo–vulpinus* complex. In comparison, *cyt b* distances between the undisputed species *B. buteo* and *B. hemilasius* range from 1.0 to 1.6% and between the New World species *B. jamaicensis* and *B. galapagoensis* from 3.8 to 4.4%.

The pattern observed in the minimum spanning network implies that the extant West Palearctic buzzards evolved within a comparatively short time frame. It can be assumed that the range of the ancestral buzzard was repeatedly reduced during the ice ages. Gene pools that had become differentiated in periods of interglacial territorial expansions might have been brought together and merged in the relict area. This hypothesis would explain the coexistence of differentiated haplotypes in the extant taxa, e.g. in *B. b. menetriesi* (b.men3, b.men6) and the observed incongruities between taxonomic grouping and haplotype distribution would thus be due to incomplete lineage sorting. However, the occurrence of the 'standard haplotype' and its direct derivatives in representatives of three species (e.g. *B. b. vulpinus*, *B. o. trizonatus*, *B. r. cirtensis*) suggests a severe bottleneck during the ice ages that reduced the mitochondrial variation considerably. The following rapid radiation and morphological differentiation were not accompanied by genetic differentiation of the mitochondrial genomes. Historic bottlenecks have been proposed by Schreiber *et al.* (2001) to explain low allozyme heterozygosity despite hyperpolymorphic plumage morphs in *B. b. buteo*. According to these results, the entire group of taxa comprising the *buteo–vulpinus–rufinus–oreophilus* complex (Fig. 5) can be considered as a bulk of potentially reticulating lineages where the cladistic approach does not provide an unequivocal solution (Helbig 2000). With respect to *B. rufinus*, which is morphologically clearly distinct and locally sympatric (*B. r. rufinus*) with *B. b. buteo*, two scenarios appear possible: (1) rapid morphological differentiation after the proposed bottleneck, or (2) acquisition of the '*buteo*' mitochondrial genome in the glacial relict area. The latter hypothesis could be tested through analysis of a nuclear marker sequence. ISSR analyses did not reveal a clear differentiation of these nuclear markers between *B. buteo* and *B. rufinus* (data not shown). Recent hybridization between *B. b. buteo* × *B. r. rufinus* (Dudas & Janos-Toth 1999) and *B. r. rufinus* × *B. hemilasius* (Pfänder & Schmigalew 2001) has been observed. Although nothing is known about the fertility of the hybrid offspring, such findings indicate that the gene pools might not be isolated completely. Moreover, field observations of hybrids between *B. buteo* × *Milvus migrans* (Corso & Gildi 1998) suggest that prezygotic barriers are not yet firmly established, even between accipitrid genera.

Habitat-dependent adaptations

Despite the lack of clear-cut genetic differentiation among the West Palearctic taxa, considerable morphological differences have been found which may be the result of adaptations to distinct environmental conditions imposed by habitat and food resources (Leisler 1980; Niemi 1985; Wiens 1989), e.g. tarsus length correlates well with body size, which is habitat dependent, whereas toes and claws (the real killing tools) are influenced by the size and type of the main prey (Voous 1969) and by hunting behaviour (Leisler *et al.* 1989; Carrascal *et al.* 1994; Gamauf *et al.* 1998). Additional morphological adaptations can be ascribed to migratory behaviour such as wing size and shape, namely a high aspect ratio and long pointed wings (Rayner 1988; Kerlinger 1989; Winkler & Leisler 1992; Pennycuick *et al.* 1994; Norberg 1995). Migratory

behaviour is an important strategy for birds to cope with changing environments and can evolve within relatively short time spans in some species, as has been shown for the blackcap *Sylvia atricapilla* (Berthold *et al*. 1992). Other examples of relatively rapid ecological adaptation (albeit not of migratory behaviour) are Darwin's finches (genus *Geospiza*) on the Galapagos Islands (Greenwood 1992), the Saker falcon (*Falco cherrug*; Pererva & Grazhdankin 1994) and the red-backed shrike *Lanius collurio* (Jacober & Stauber 2000).

The main types of habitat utilized by the West Palearctic taxa of the genus *Buteo* can be approximately categorized into either dry and open or forested (see also Fig. 3). Representatives of species that utilize dry open habitats are *B. rufinus* (ssp. *rufinus* and *cirtensis*), and the smaller island forms *B. b. rothschildi*, *B. b.* '*socotrae*', and *B. b. bannermani*. The big mainland birds have long and broad, deeply slotted wings and a long tail promoting their manoeuvrability in open habitats. They rely on soaring or perch on exposed structures from which they launch their attacks. Adaptations to small prey (Dement'ev *et al*. 1966; Flint *et al*. 1984) are weak feet and claws and a bill with the widest gape among the taxa compared enabling them to swallow their prey in one piece, whereas most buzzards usually pluck it before eating. The morphological similarity (Figs 1, 3) between *B. rufinus* and the other large species, *B. hemilasius*, has been considered by Hartert (1914) and, more recently, by del Hoyo *et al*. (1994) to be an indication of their close relationship. This view was not confirmed by our data and, therefore, the similarity may be the result of convergent evolution. On the other hand, the small island taxa, which occur in dry open habitats as well, differ considerably from the continental forms. Characters shared by this group are short and narrow wings to prevent drift to the open sea in windy areas and strong claws to catch relatively large, agile (mostly reptile) prey. A similar phenomenon is observed in another small island raptor, the kestrel (*Falco tinnunculus*), on the Cape Verde Islands, where trophic factors are largely responsible for the morphological differences among the island populations (Hille & Winkler 2000).

Raptor species of the forest interior are characterized by a small body size and a short wingspan (Gamauf *et al*. 1998). An example is *B. brachypterus*, which, as an inhabitant of tropical rain forests, is the smallest Old World *Buteo* species with short and rounded wings as a general adaptation to dense vegetation (Morris & Hawkins 1998). The two subspecies of *B. oreophilus* are also characterized by short, rounded, broad and slightly notched wings and a short tail (Kemp & Kemp 1998). The differences in body shape (*B. o. oreophilus* is more stocky), which are reflected in Figs 1 and 2, may be the consequence of different prey types and hunting behaviour (Clark & Davies 2000). The genetic data place the four individuals of *B. o. trizonatus* within the *B. buteo* cluster of the network (Fig. 5), whereas the three individuals of *B. o. oreophilus* are found on a separate branch that also contains two other taxa.

Taxonomic consequences

What are the implications of these results for the systematics and taxonomy of the genus *Buteo*? The species status of *B. hemilasius* and *B. brachypterus* remains undisputed at both the morphological and the genetic level. The proposed association between *B. brachypterus* and *B. oreophilus* (James 1986; del Hoyo *et al*. 1994) was not supported by our data.

Morphologically, *B. b. refectus* is not clearly separated from *B. b. japonicus*, whereas its mitochondrial haplotypes form a distinct cluster. In the network (Fig. 5), *B. b. refectus* appears even closer to *B. hemilasius*. Noteworthy in this context is the high proportion of melanistic specimens in museum collections of both species (Pfänder & Schmigalew 2001). *Buteo b. japonicus* and *B. b. toyoshimai* are differentiated morphologically by size and shape. The haplotypes of *B. b. japonicus* fall into two distinct lineages, which do not even cluster in the tree and the network. One also contains the haplotype of *B. b. toyoshimai*; the other also includes specimens from the mainland. With respect to the two haplotype clades of *B. b. japonicus*, only two specimens (b.jap1 and b.jap2) have been analysed morphologically. No obvious differentiation could be detected, although a sample of two individuals is certainly not representative.

Based on the genetic and morphometric data, the following modification of the current taxonomy of these three East Palearctic taxa can be proposed. *Buteo b. refectus* should be raised to species status (*B. refectus*), as previously suggested by Voous & Bijleveld (1964) and James (1988). With respect to *B. b. japonicus* and *B. b. toyoshimai*, the degree of genetic and morphological differentiation is similar, for example, to that found between *B. hemilasius* and the taxa of the *japonicus* group. There are two possible solutions: (1) a separate species *B. japonicus* with the two subspecies *B. j. japonicus* and *B. j. toyoshimai*. In this case the haplotypes of *B. japonicus*, which belong to two distinct evolutionary lineages, would not represent a monophyletic group; (2) *B. japonicus* and *B. toyoshimai* as separate species with occasional interspecific gene flow leading to introgression of *B. toyoshimai* haplotypes into *B. japonicus* (Brazil 1991). Nevertheless, to propose species status for *B. b. toyoshimai* would require further extensive investigations. The same is true for the two lineages of *B. b. japonicus*. At the present state of knowledge we suggest the provisional solution of treating *B. japonicus* as a distinct species with the two subspecies *japonicus* and *toyoshimai*.

For the West Palearctic taxa, the genetic situation appears rather complex as there is no unequivocal genetic differentiation within the *buteo–vulpinus–rufinus–oreophilus* complex. Under the biological species concept (Mayr 1940, 1963), the West Palearctic taxa (sharing a common gene pool) should

Table 2 Proposed classification of the taxa of the superspecies *B.* [*buteo*].

Genus *Buteo*		
Subgenus *Buteo*		
Superspecies	*Buteo* [*buteo*]	
	Buteo [*buteo*] *buteo* (with ssp. *buteo, hispaniae, harterti, insularum, arrigonii, rothschildi, menetriesi, vulpinus, trizonatus, 'socotrae'*, bannermani**)	
	Buteo [*buteo*] *rufinus* (with ssp. *rufinus, cirtensis, 'socotrae'*, bannermani**)	
	Buteo [*buteo*] *oreophilus*	
Species	*Buteo japonicus* (with ssp. *japonicus, toyoshimai*)	
	Buteo refectus	
	Buteo hemilasius	

*See Discussion for details.

be lumped into one species with 14 subspecies (including, e.g. *B. rufinus* and the African *B. oreophilus*). On the other hand, applying the phylogenetic species concept (Cracraft 1983) and considering the morphological data, the maintenance of the species status of *B. rufinus* and *B. oreophilus* (ssp. *oreophilus*) seems justified. As a possible compromise between these conflicting approaches, we suggest treating *B. buteo* as a superspecies (Mayr 1940, 1963; Haffer 1997; Helbig 2000). Accordingly, this superspecies should comprise the three allospecies: *B.* [*buteo*] *buteo*, *B.* [*b.*] *rufinus*, and *B.* [*b.*] *oreophilus*, with some of these subdivided further into subspecies (see Table 2).

With respect to the subspecies '*socotrae*' and *bannermani*, the morphological similarities, as already described by Naurois (1987) and James (1986), are paralleled by genetic affinities (Fig. 5). Geographically, a relationship between these island forms and *B.* [*b.*] *rufinus* appears possible. Sequence data of the *cyt b* gene (Clouet & Wink 2000) support this relationship (distance between '*socotrae*' and *B. r. rufinus*: 0.17%; between '*socotrae*' and *B. b. buteo*: 0.4–0.9%). These authors, as well as Hazevoet (1995), even proposed to classify *bannermani* as a distinct species, and the 'Socotra' buzzard could either be included into *B. rufinus* or, due to its isolated and remote distribution, raised to species status (Martins & Porter 1996; Clouet & Wink 2000; Aspinall 2001). Nevertheless, faced with the absence of unequivocal genetic affiliations in the ΨCR data, we leave the position of both taxa open (Table 2).

Buteo r. cirtensis appears clearly differentiated from *B. r. rufinus* in the morphometric analysis (Fig. 1), although this separation is based only on size-related characters. In the network (Fig. 5) the haplotypes of *B. r. cirtensis* are randomly distributed within the West Palearctic cluster. Because there is no convincing argument to connect *B. r. cirtensis* to any other species, we decided to retain the current taxonomy. With regard to the South African subspecies *trizonatus*, preliminary results of ISSR analysis (data not shown) give contradicting results when different ISSR primers are employed, either placing *trizonatus* within the *buteo–vulpinus* complex or clearly separated. On the other hand, it could be hypothesized that it might be a non-migrating form derived from *B.* [*b.*] *b. vulpinus*, a long-distance migrant that winters in South Africa. Taking into consideration that all *trizonatus* specimens are found within the '*buteo* cluster' of the ΨCR tree (Fig. 5), we suggest classifying *trizonatus* as a subspecies of *B.* [*b.*] *buteo.*

In the morphometric analyses, clear-cut differences among described taxa of the genus *Buteo* were found in some cases where the genetic data failed to reveal separation of the gene pools (e.g. the superspecies *B.* [*buteo*]). In contrast, some phylogenetic relationships became apparent only from the molecular data but were blurred by convergent evolution at the phenotypic level (e.g. *B. hemilasius*/*B.* [*b.*] *rufinus*, *B. japonicus*/*B. refectus*). As these results clearly demonstrate, speciation as a dynamic process cannot always be adequately dealt with by classical taxonomic approaches. The 'superspecies' concept may be a practical way to overcome the problems imposed by incomplete separation of lineages and reticulated evolution in a recent radiation.

Acknowledgements

This study was supported by the Austrian Science Foundation (FWF, Wien, project P14069-BIO). We are very much indebted to many people and institutions enabling this study in different ways. The morphological investigations were only possible through the excellent co-operation with the curators of different museums. Therefore, we wish to express our thanks to E. Bauernfeind (Museum of Natural History, Vienna, Austria), R. van den Elzen (Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany), R. Dekker (Naturalis, the Netherlands), R. Prŷs-Jones, M. Adams, F. Steinheimer (Natural History Museum, Tring, UK), C. Roselaar, and T. Prins (Zoological Museum, University of Amsterdam, the Netherlands). We are very much obliged to all those who provided tissues or DNA samples for the genetic analysis listed in Table 1. For additional material we thank H.-M. Berg (Vienna, Austria), H. Frey (University of Veterinary Medicine, Vienna, Austria), N. Teufelbauer (Vienna, Austria), R. Lindner (Salzburg, Austria), M. McGrady (Krems, Austria), W. Dolak (Schrems, Austria), S. Ernst (Klingenthal, Germany), S. Frahnert (Zoologisches Museum

Berlin, Germany), R. G. Bijlsma (Wapse, the Netherlands), M. Janicka, A. Adamski (Wroclaw, Poland), E. Potapov (Oxford, UK), R. Kenward, S. Walls (Institute of Terrestrial Ecology, Wareham, UK), A.-T. Bashta (Lviv, Ukraine), K. Saito (Wildlife Preservation Bureau, Hokkaido, Hokuto Kushiro, Japan), G. Barrowclough, P. Sweet (American Museum of Natural History, New York, USA), M. T. Cassidy (Transvaal Museum, Pretoria, South Africa). We are grateful to M. McGrady and W. Mayer for critical and useful comments on the manuscript.

References

Alerstam, T. (1990). *Bird Migration*. Cambridge: Cambridge University Press.

Aspinall, S. (2001). The *Buteo* population of Socotra. *Falco*, *17*, 8–9.

Bannerman, D. A. & Bannerman, W. M. (1963–68). *Birds of the Atlantic Islands*, Vols 1–5. Edinburgh: Oliver & Boyd.

Berthold, P. (2000). *Vogelzug — Eine Aktuelle Gesamtübersicht*, 4th edn. Darmstadt: Wissenschaftliche Buchgesellschaft.

Berthold, P., Helbig, A. J., Mohr, G. & Querner, U. (1992). Rapid microevolution of migratory behaviour in a wild bird species. *Nature*, *360*, 668–670.

Brazil, M. A. (1991). *The Birds of Japan*. London: Helm Edt.

Brown, L. H. & Amadon, D. (1968). *Eagles, Hawks, and Falcons of the World*. London: Country Life Books.

Carrascal, L. M., Moreno, E. & Valido, A. (1994). Morphological evolution and changes in foraging behaviour and mainland populations of blue tit (*Parus caeruleus*) — a test of convergence and ecomorphological hypotheses. *Evolutionary Ecology*, *8*, 25–35.

Clark, W. S. & Davies, R. A. G. (2000). Taxonomical problems in African diurnal raptors. In R. D. Chancellor & B.-U. Meyburg (Eds) *Raptors at Risk* (pp. 121–133). Surrey: World Working Group on Birds of Prey and Owls/Hancock House.

Clouet, M. & Wink, M. (2000). The buzzards of Cape Verde *Buteo* (*buteo*) *bannermani* Socotra *Buteo* (*buteo*) spp.: first results of a genetic analysis based on nucleotide sequences of the *cytochrome b* gene. *Alauda*, *68*, 55–58.

Corso, A. & Gildi, R. (1998). Hybrids between black kite and common buzzard in Italy 1996. *Dutch Birding*, *20*, 226–233.

Cracraft, J. (1983). Species concepts and speciation analysis. In R. F. Johnston (Ed.) *Current Ornithology*, Vol. 1 (pp. 159–187). New York: Plenum Press.

Damodar Reddy, K., Nagaraju, J. & Abraham, E. G. (1999). Genetic characterisation of the silkworm *Bombyx mori* by simple sequence repeat (SSR)-anchored PCR. *Heredity*, *83*, 681–687.

Dement'ev, G. P., Gladkov, N. A., Ptushenko, E. S., Spangenberg, E. P. & Sudilovskaya, A. M. (1966). *Birds of the Soviet Union*, Vol. 1. Jerusalem: Israel Program for Scientific Translation.

Dudas, M.-T. & Janos-Toth, I. (1999). [Natural hybridisation in buzzards]. *Természet*, *6*, 8–10 (in Hungarian).

Eck, S. (1991). Die Zoogeographische Art der Adlerbussarde *Buteo* [*lagopus*] (Accipitridae). Mitteilungen aus dem Zoologischen Museum Berlin (Suppl. 67). *Annalen für Ornithologie*, *15*, 137–146.

Felsenstein, J. (1985). Phylogenies and the comparative method. *American Naturalist*, *125*, 1–15.

Felsenstein, J. (1993). PHYLIP — *Phylogeny Inference Package*, Version 3.5p. Seattle: University of Washington, Department of Genetics.

Ferguson-Lees, J. & Christie, D. A. (2001). *Raptors of the World*. London: Helm Edt.

Flint, V. E., Boehme, R. L., Kostin, Y. V. & Kuznetsov, A. A. (1984). *Birds of the USSR*. Princeton: Princeton University Press.

Gamauf, A., Preleuthner, M. & Winkler, H. (1998). Philippine birds of prey: interrelations among habitat, morphology and behavior. *Auk*, *115*, 713–726.

Ge, X. J. & Sun, M. (1999). Reproductive biology and genetic diversity of a cryptoviviparous mangrove *Aegiceras corniculatum* (Myrsinaceae) using allozyme and intersimple sequence repeat (ISSR) analysis. *Molecular Ecology*, *8*, 2061–2069.

Glutz von Blotzheim, U. N., Bauer, K. & Bezzel, E. (1971). *Handbuch der Vögel Mitteleuropas*, Vol. 4, Falconiformes. Frankfurt/Main: Akademische Verlagsgesellschaft.

Greenwood, J. J. D. (1992). Theory fits the bill in the Galapagos Islands. *Nature*, *362*, 699.

Gupta, M., Chyi, Y.-S., Romero-Severson, J. & Owen, J. L. (1994). Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. *Theoretical Applied Genetics*, *89*, 998–1006.

Haffer, J. (1997). Species concepts and species limits in ornithology. In J. del Hoyo, J. A. Elliott & J. Sargatal (Eds) *Handbook of the Birds of the World*, Vol. 2 (pp. 11–24). Barcelona: Lynx Ediciones.

Haring, E., Kruckenhauser, L., Gamauf, A., Riesing, M. J. & Pinsker, W. (2001). The complete sequence of the mitochondrial genome of *Buteo buteo* (Aves, Accipitridae) indicates an early split in the phylogeny of raptors. *Molecular Biology and Evolution*, *18*, 1892–1904.

Haring, E., Riesing, M. J., Pinsker, W. & Gamauf, A. (1999). Evolution of a pseudo-control region in the mitochondrial genome of Palearctic buzzards (genus *Buteo*). *Journal of Zoological Systematics and Evolutionary Research*, *37*, 185–194.

Hartert, E. (1914). *Die Vögel der Paläarktischen Fauna*. Berlin: Friedländer & Sohn.

Hazevoet, C. J. (1995). *The Birds of the Cape Verde Islands*. B.O.U. Checklist no. 13. Dorchester: H. Lings.

Helbig, A. J. (2000). Was ist eine Vogel-'Art'? — Ein Beitrag zur aktuellen Diskussion um Artkonzepte in der Ornithologie. *Limicola*, *14*, 57–79, 172–184, 220–247.

Hille, S. & Winkler, H. (2000). Ecomorphology of island populations of the kestrel *Falco tinnunculus* on Cape Verde. In R. D. Chancellor & B-U. Meyburg (Ed.) *Raptors at Risk* (pp. 729–736). Surrey: World Working Group on Birds of Prey and Owls/Hancock House.

del Hoyo, J., Elliott, J. A. & Sargatal, J. (1994). *Handbook of the Birds of the World*, Vol. 2. Barcelona: Lynx Edicions.

Jacober, H. & Stauber, W. (2000). Werden die Neuntöter (*Lanius collurio*) kleiner? *Journal für Ornithologie*, *141*, 408–417.

James, A. H. (1984). Geographic variation in the buzzard *Buteo buteo* (Linnaeus, 1758): mid-Atlantic and west Mediterranean islands (Aves: Accipitridae). *Beaufortia*, *34*, 101–116.

James, A. H. (1986). Review of taxonomic characters in African buzzards (genus *Buteo*). *Beaufortia*, *36*, 1–12.

James, A. H. (1988). Geographic variation in the buzzard *Buteo buteo* (L.): *japonicus*-group (Aves: Accipitridae). *Beaufortia*, *38*, 57–74.

James, A. H. & Wattel, J. (1983). The nomenclature of *Buteo oreophilus*. *Bulletin of the British Ornithologists' Club*, *103*, 128–133.

Kemp, A. & Kemp, M. (1998). *Birds of Prey of Africa and its Islands*. Cape Town: New Holland.

Kerlinger, P. (1989). *Flight Strategies of Migrating Hawks*. Chicago: University of Chicago Press.

Leisler, B. (1980). Morphological aspects of ecological speciation in bird genera. *Ecology of Birds*, *2*, 9–22.

Leisler, B., Ley, H.-W. & Winkler, H. (1989). Habitat, behaviour and morphology of *Acrocephalus* warblers: an integrated analysis. *Ornis Scandinavica*, *20*, 181–186.

Leisler, B. & Winkler, H. (1985). Ecomorphology. In R. F. Johnston (Ed.) *Current Ornithology*, Vol. 2 (pp. 155–186). New York: Plenum Press.

Leisler, B. & Winkler, H. (1991). Ergebnisse und Konzepte ökomorphologischer Untersuchungen an Vögeln. *Journal für Ornithologie*, *132*, 373–425.

Londai, T. (1995). Field observations on the Azores buzzard *Buteo buteo rothschildi*. *Bulletin of the British Ornithologists' Club*, *115*, 48–53.

Martins, R. P. & Porter, R. F. (1996). The *Buteo* population in Socotra. *Sandgrouse*, *17*, 134–137.

Mayr, E. (1940). Speciation phenomena in birds. *American Naturalist*, *74*, 249–278.

Mayr, E. (1963). *Animal Species and Evolution*. Cambridge, MA: Harvard University Press.

Meinertzhagen, R. (1954). *Birds of Arabia*. Edinburgh: Oliver and Boyd.

Mindell, D. P., Sorenson, M. D. & Dimcheff, D. E. (1998). Multiple independent origins of mitochondrial gene order in birds. *Proceedings of the National Academy of Sciences of the USA*, *95*, 10693–10697.

Morris, P. & Hawkins, F. (1998). *Birds of Madagascar*. Sussex: Pica Press.

de Naurois, R. (1987). Les oiseaux de l'archipel du Cap Verd: peuplements, adaptations, endémisme. *Bulletin de la Société Zoologique de France*, *112*, 307–326.

Niemi, G. J. (1985). Patterns of morphological evolution in bird genera of New World and Old World peatlands. *Ecology*, *66*, 1215–1228.

Norberg, U. M. (1995). How a long tail and changes in mass and wing shape affect the cost for flight in animals. *Functional Ecology*, *9*, 48–54.

Patterson, C. (1993). Congruence between molecular and morphological phylogenies. *Annual Review of Ecology and Systematics*, *24*, 153–188.

Pennycuick, C. J., Fuller, M. R., Oar, J. J. & Kirkpatrick, S. J. (1994). Falcon versus grouse: flight adaptation of a predator and its prey. *Journal of Avian Biology*, *25*, 39–49.

Pererva, V. I. & Grazhdankin, A. V. (1994). Possible effect of anthropogenic environmental changes on morphological variation of some European birds of prey. In R. D. Chancellor & B-U. Meyburg (Eds) *Raptor Conservation Today* (pp. 667–675). Sussex: World Working Group on Birds of Prey and Owls/Pica Press.

Pfänder, P. & Schmigalew, S. (2001). Umfangreiche Hybridisierung der Adler-*Buteo rufinus* Cretz. und Hochlandbussarde *Buteo hemilasius* Temm. et Schlegel. *Ornithologische Mitteilungen*, *53*, 344–349.

Rayner, J. M. V. (1988). Form and function in avian flight. In R. F. Johnston (Ed.) *Current Ornithology*, Vol. 5 (pp. 1–66). New York: Plenum Press.

Riesing, M. J., Kruckenhauser, L., Gamauf, A. & Haring, E. (2003). Molecular phylogeny of the genus *Buteo* based on mitochondrial marker sequences. *Molecular Phylogeny and Evolution*, *27*, 328–342.

Rudebeck, G. (1957). *Buteo buteo trizonatus*: a new buzzard from the South African Union. *South African Animal Life*, *4*, 415–437.

Schreiber, A., Stubbe, A. & Stubbe, M. (2001). Common buzzard (*Buteo buteo*): a raptor with hyperpolymorphic plumage morphs, but low allozyme, heterozygosity. *Journal für Ornithologie*, *142*, 34–48.

Siegfried, W. R. & Frost, P. G. H. (1973). Systematic notes on the small African Buteos. *Ardea*, *61*, 123–127.

Swann, H. K. (1926). *A Monograph of the Birds of Prey (Order Accipitres)*, Version 1. London: Wheldon & Wesley.

Swofford, D. L. (2002). *PAUP* — Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4.0b6–10. Sunderland, MA: Sinauer Associates.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The Clustal–windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, *25*, 4876–4882.

Vaurie, Ch. (1961). Systematic notes on Palearctic birds, 47. Accipitridae: the genus *Buteo*. *American Museum Novitates*, *2042*, 1–14.

Voous, K. H. (1969). Predation potential in birds of prey from Surinam. *Ardea*, *57*, 17–148.

Voous, K. H. & Bijleveld, M. F. I. J. (1964). A note on the Himalayan buzzards *Buteo buteo*. *Beaufortia*, *11*, 37–43.

Wiens, J. A. (1989). *The Ecology of Bird Communities*, Vol. 1. Cambridge: Cambridge University Press.

Wink, M., Guicking, D. & Fritz, U. (2000). Molecular evidence for hybrid origin of *Mauremys iversoni* Pritchard et McCord, 1991 and *Mauremys pritchardi* McCord, 1997 (Reptilia: Testudines: *Bataguridae*). *Zoologische Abhandlungen*, *Staatliches Museum für Tierkunde Dresden*, *51*, 41–49.

Wink, M. & Sauer-Gürth, H. (2000). Advances in the molecular systematics of African raptors. In R. D. Chancellor & B.-U. Meyburg (Eds) *Raptors at Risk* (pp. 135–147). Surrey: World Working Group on Birds of Prey and Owls/Hancock House.

Winkler, H. & Leisler, B. (1992). Morphological aspects of habitat selection in birds. In M. L. Cody (Ed.) *Habitat Selection in Birds* (pp. 415–434). Orlando: Academic Press.

Zietkiewicz, E., Rafalski, A. & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (ISSR)-anchored polymerase chain reaction amplification. *Genomics*, *20*, 176–183.

Appendix

Museum study skins used for the morphological investigation. Taxonomy according to Table 2. Detailed information can be obtained upon request.

Buteo b. buteo	RMNH 67.418, RMNH 97.184, RMNH 56.013, RMNH 56.068, RMNH 67.421, RMNH 67.424, RMNH 56.022, RMNH 67.419, NMW 57.050, NMW 69.340, NMW 87.585, NMW 73.801, NMW 87.577, NMW 89.366.
Buteo b. hispaniae	BMNH 1961.17.107, BMNH 75.11.22.73, BMNH 1934.1.1.1116, BMNH no data.
Buteo b. harterti	BMNH 1955.6.N-20.2213, BMNH 91.7.19.2, BMNH 1955.6.N-20.2218, BMNH 91.7.19.1, BMNH 1956.6.N-20.2214, BMNH 1904.7.22.2, BMNH 1944.12.1.315, BMNH 1904.4.22.4, BMNH 1904.7.22., BMNH 92.5.2.1, ZFMK 7632, ZFMK 7633.
Buteo b. insularum	BMNH 1965.M.1268, BMNH 1905.12.22.235, BMNH 1905.12.22.234, NMW 30.482, NMW 57.073, RMNH 1888, RMNH 487.363.3, ZMA 43.938, ZMA 43.939, ZMA 43.940.
Buteo b. arrigonii	BMNH 75.5.1.32, BMNH 1951.13.82, RMNH 56.081, RMNH 56.084, RMNH 56.085, RMNH 56.082, ZFMK 7635, ZFMK 7636, ZMA 43.941, ZMA 14.983.
Buteo b. rothschildi	BMNH 1904.12.31.286, BMNH 1905.1.-26.17, BMNH 1904.12.31.287, BMNH 1904.12.31.285, BMNH 1904.12.31.291, BMNH 1904.12.31.283, BMNH 1904.12.31.285, BMNH 1904.12.31.289, BMNH 1904.12.31.290, BMNH 1934.1.1.1118, BMNH 1904.12.31.292, BMNH 1904.12.31.284, RMNH 3762, ZFMK 493.35.45, ZFMK 485.353.34, ZFMK 485.352.34.
Buteo b. bannermani	BMNH 1919.8.15.148, BMNH 1911.12.23.436.
Buteo b. 'socotrae'	BMNH 99.8-11.10, BMNH 1934.8.12.2, BMNH 1934.8.12.3.
Buteo b. vulpinus	NMW 57.041, NMW 78.389, RMNH 3.762 (55), RMNH 3.762, RMNH 3.762, RMNH 3762 (39), ZFMK 41.157, ZMA 27.535, ZMA 43.936, ZMA 22.298.
Buteo b. menetriesi	BMNH 1905.6.28.90, BMNH 1965.M.1265, BMNH 86.3.25.97, NMW 30.571, NMW 57.037, NMW 57.038, ZFMK 599, ZFMK 7628, ZFMK 7639, ZFMK 7631.
Buteo b. trizonatus	BMNH 1965.M.1273, BMNH 47.7.6.54, BMNH 1905.12.29.113, BMNH 45.7.6.54, BMNH 1905.12.29.114, BMNH 1956.6.N-20.2175, BMNH 1905.12.29.110, BMNH 1905.12.29.115, BMNH 1956.6.N-20.2197.
Buteo j. japonicus	BMNH 1909.11.20.27, BMNH 1914.5.1.90, BMNH 1955.6.N-20.2143, BMNH 1902.8.5.372, BMNH 1955.6.N-20.22147, BMNH 1909.11.20.28, BMNH 97.10.30, BMNH 1966.6.N.20.2158?, NMW 57.089, NMW 57.088, ZFMK 7637, ZMA 34.702, ZMA 34.721.
Buteo j. toyoshimai	BMNH 97.10.30.20, BMNH 97.10.30.215, BMNH 1955.N.20.2151, BMNH 97.10.30.214.
Buteo refectus	BMNH 1908.11.10.11, BMNH 1937.1.17.86, BMNH 85.8.19.837, BMNH 1968.6.N-20.2149, BMNH 1949-Whi-1.465, BMNH 82.4.1.14, BMNH 85.8.19.835, BMNH 85.8.19.850, ZMA 2298, ZMA 2299.
Buteo oreophilus	BMNH 1947.7.100.4, BMNH 1906.12.23. 1916, BMNH 1934.11.21.1, BMNH 1900.12-23. 1914, BMNH 1965.M.1272, BMNH 1939.10.1.564, BMNH 1965-M-1274, BMNH 85.6.14.5, ZFMK 237.
Buteo r. rufinus	BMNH 1922.12.8.338, BMNH 85.8.19.727, BMNH 1922.12.8.335, BMNH 85.8.19.2096, NMW 22.791, NMW 31.929, ZMA 43.922, ZMA 43.923, ZMA 43.924, ZMA 43.926.
Buteo r. cirtensis	BMNH 1919.12.11.1, BMNH 1955.6.N.20.2183, BMNH 1965.M.1234, BMNH 1905.6.28.89, RMNH 14, RMNH 2, ZFMK 642, ZFMK 641, ZFMK 638, ZFMK 640.
Buteo hemilasius	BMNH 1914.5.1.94, BMNH 1912.9.23.17, BMNH 43.1.13.52, BMNH 1908.1.4.15, BMNH 1908.1.4.414, BMNH 1914.5.1.93, BMNH no data, NMW 10.785, NMW 56.684, ZFMK 7659.
Buteo brachypterus	BMNH 1931.8.18.240, BMNH 1931.8.18.229, BMNH 1956.6.N.20.2244, BMNH 1931.8.18.234, BMNH 1931.8.18.230, BMNH 1931.8.18.220, BMNH 1921.8.18.233, BMNH 1931.8.18.237, ZFMK 42.278, ZMA 14.689.

RMNH, Naturalis, Leiden, the Netherlands; NMW, Naturhistorisches Museum Wien, Vienna, Austria; BMNH, the Natural History Museum, Tring, UK; ZFMK, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany; ZMA, Zoological Museum, University of Amsterdam, the Netherlands.