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Molecular Phylogenetics and Evolution 27 (2003) 328–342

MOLECULAR PHYLOGENETICS AND EVOLUTION

www.elsevier.com/locate/ympev

Molecular phylogeny of the genus *Buteo* (Aves: Accipitridae) based on mitochondrial marker sequences

Martin J. Riesing, Luise Kruckenhauser, Anita Gamauf, and Elisabeth Haring*

Museum of Natural History Vienna, Burgring 7, A-1014 Vienna, Austria

Received 26 April 2002; revised 11 September 2002

Abstract

DNA sequences of the mitochondrial *nd6* gene and the non-repetitive part of the pseudo-control region (ΨCR) were isolated from 101 individuals to analyze the phylogenetic relationships among all buzzards of the genus *Buteo* and other buteonine genera. Comparisons of the two marker sequences indicate that the ΨCR evolved two times faster than the *nd6* gene. The ΨCR proved to be an efficient, neutral genetic marker sequence for phylogenetic analyses at the intrageneric level, especially suitable for analyses based on old tissues, where only short fragments can be obtained. The molecular data set implies a neotropical origin of the genus *Buteo*. Monophyly of the genus *Buteo* as currently defined is contradicted due to the positions of *Asturina nitida*, *Geranoaetus melanoleucus*, *Buteo magnirostris*, and *Buteo leucorrhous*. These findings suggest several taxonomic consequences. *A. nitida* and *G. melanoleucus* should be included into the genus *Buteo*. Moreover, *B. leucorrhous* should be transferred into the genus *Percnohierax* (which clusters with *Parabuteo*), and *B. magnirostris* into the genus *Rupornis*. According to this classification of the genus *Buteo*, the basal lineage of the genus is formed by a clade containing *Buteo polyosoma*, *Buteo poecilochrous*, and *Buteo melanoleucus*. The "woodland buteos" form a paraphyletic assemblage with *B. magnirostris* as a clearly separated lineage basal to the genus *Buteo*.

Keywords: *Buteo*; Phylogeny; mtDNA; Pseudo control region; *nd6*; Accipitridae; Buzzard

1. Introduction

Buzzards of the genus *Buteo* represent a large group of diurnal birds of prey of the family Accipitridae. *Buteo* species are almost globally distributed (Thiollay in del Hoyo et al., 1994; Ferguson-Lees and Christie, 2001) being absent only in the Australian region, and in Antarctica. The distribution range also includes several remote oceanic islands in the Atlantic and Pacific. According to the Handbook of Birds of the World (del Hoyo et al., 1994) 12 of the 28 species are polytypic with up to 14 subspecies (e.g., *Buteo jamaicensis*). *Buteo* species use various habitats ranging from well forested areas (from boreal to tropical regions) to open tundra and steppes.

Various classifications of the genus *Buteo* with different numbers of species (25–28) exist (Brown and Amadon, 1968; Clements, 2000; Thiollay in del Hoyo et al., 1994; Ferguson-Lees and Christie, 2001; Peterson, 2002; Stresemann and Amadon, 1979) and various subgenera have been proposed in the past, although Friedmann (1950) stated that the genus *Buteo* defies subdivision into subgenera. So far, classification is based on osteological and other morphological traits, but the phylogenetic information of those characters may be blurred by convergence. Up to now, no phylogenetic analysis has been presented to clarify intrageneric relationships of *Buteo*. Particularly obscure are the evolutionary affinities of the island taxa *Buteo galapagoensis*, *Buteo solitarius*, *Buteo polyosoma exsul*, and *Buteo ridgwayi* and a group of species designated as "American woodland buteos" by Johnson and Peeters (1963) (*Buteo lineatus*, *Buteo ridgwayi*, *Buteo platypterus*, *Buteo magnirostris*, and *Asturina nitida*). Phylogenetic relationships between *Buteo* and related genera are also not

* Corresponding author. Fax: +43-1-5235254.
E-mail address: elisabeth.haring@nhm-wien.ac.at (E. Haring).

1055-7903/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S1055-7903(02)00450-5

well understood. Uncertainties refer to the positions of *A. nitida* and *B. magnirostris*, which have been either included into *Buteo* or placed into separate genera (Brown and Amadon, 1968; Stresemann and Amadon, 1979). Another assemblage of genera are the sub-buteonine hawks (Amadon, 1982) which are mainly distributed in the Americas, especially in the Neotropics. Various hypotheses have been proposed about inter and intrageneric relationships of these genera: *Kaupifalco*, *Butastur*, *Busarellus*, *Buteogallus*, *Geranoaetus*, *Harpyhaliaetus*, *Leucopternis*, and *Parabuteo* (Amadon, 1982; Brown and Amadon, 1968; Holdaway, 1994; Johnsgard, 1990; Jollie, 1977).

In previous molecular studies on the phylogeny of other groups of birds of prey, taxa of the genus *Buteo* were included only sporadically. For example, in their investigation on the phylogeny of vultures, which was based on the *cytochrome b* (*cytb*) gene, Seibold and Helbig (1995) included several accipitrid genera. In their trees a basal sister group relationship of *Buteo* to the *Milvus-Haliaeetus* clade is evident, next to the separate sister group of *Accipiter* and *Circus*. Like Sibley and Ahlquist (1990), Seibold and Helbig (1995) associate *Parabuteo* and *Geranoaetus* with *Buteo*, although they did not include these genera in their tree. A sister group relationship between the *Buteos* and the *Milvus-Haliaeetus* clade has been also found by Mindell et al. (1997) in a study based on mitochondrial (mt) sequences (12S rRNA gene). In another analysis of *cytb* sequences including eight buteonine taxa Wink et al. (1998) obtained a tree topology with *P. unicinctus* placed basal to the *Buteo* radiation. Old World species of the genus *Buteo* were investigated by Haring et al. (1999), Clouet and Wink (2000), Wink and Sauer-Gürth (2000), and Kruckenhauser et al. (in prep.). The results indicated a recent radiation of the Palearctic taxa. Nevertheless, a DNA sequence analysis comprising all members of the genus *Buteo* has not been carried out so far. Elucidating the phylogeny of *Buteo* and related taxa should also help to provide the basis for a deeper understanding of the evolution of behavior and morphology within this large group of raptors.

The present study on the molecular phylogeny of the genus *Buteo* includes 101 individuals representing 61 taxa. Two molecular marker sequences of the mitochondrial (mt) genome were used: the nicotinamide adenine dinucleotide dehydrogenase subunit 6 gene (*nd6*), and the non-repetitive part of the pseudo-control region (ΨCR), a highly variable non-coding section of the mt genome (Haring et al., 1999). The following questions will be addressed: (1) Is the genus *Buteo*, as currently defined, monophyletic? Especially the phylogenetic relationships of the "American woodland buteos" should be clarified. (2) In which geographic region did the genus *Buteo* originate? (3) What are the relationships of some sub-buteonine taxa to the genus *Buteo*? (4) Is the ΨCR a suitable marker to resolve relationships among *Buteo* species? (5) What are the evolutionary relationships of several island taxa within *Buteo*?

2. Material and methods

2.1. Sampling

Sequences from 101 individuals (see Table 1) of the genera *Buteo*, *Asturina*, *Parabuteo*, *Geranoaetus*, *Buteogallus*, *Busarellus*, *Kaupifalco*, *Aquila*, and *Accipiter* were included in the study. Samples were taken from both museum collections and tissue collections as well as from feathers collected in the field. Whenever possible, at least two individuals per taxon were analyzed. Sequence alignments are available upon request.

2.2. DNA extraction, PCR amplification and sequencing

DNA was extracted from all samples by a proteinase K digestion in a 5% CHELEX 100 (Bio-Rad) solution at 56 °C for 4 h with agitation (Walsh et al., 1991). After incubation at 98 °C for 3 min the supernatant was purified with spin columns (Qiagen) as described in Haring et al. (1999). PCR was performed on a Master gradient thermocycler (Eppendorf) in 25 μl with 0.5 U Dynazyme DNA polymerase (Finnzyme, OY), 1 μM of each primer and 0.2 mM of each dNTP (Boehringer–Mannheim). Control reactions of both DNA extraction and PCR amplification were performed. The following primers were used: tPROfwd (ATCACCAACTCCCAAAG CTGG), tGLUrev (AAGTTTACAACGGCGATTT TTC), ND6int (CTAACCCGTCGCCTTATTATGG), ND6int1 (CAGTAACCACCCCAATCTTC), ND6int2 (GCAGGGATGGTTCTGGTGCT), tGLUfwd (CTC TCCAAAACCTACGACCTG; Haring et al., 1999), YCR1rev (GGAACTCCAGTGGTGTTTGG), YCR2-rev (GGTTACATGGTTTGGTAGGGG), YCRint2fwd (CTTACTTTCCAAACAACACCCAAT).

Using these primers in different combinations, four different fragments (Fig. 1) were amplified for the phylogenetic analysis (positions refer to the complete sequence of the mt genome of *B. buteo*, Haring et al., 2001; GenBank Accession No. AF380305; sample Bbutbut2 in this study): nd6 (pos. 16627–17145), PNEΨ (pos. 16635–17514), ΨCR1 (pos. 17186–17421), and nd6s1 (pos. 16817–17062). The actual size of the PNEΨ section used in the phylogenetic analysis was 38 bp shorter since some shorter fragments from previous studies (Haring et al., 1999) were included, which had been obtained with a different set of primers. An additional 182-bp fragment (not indicated in Fig. 1) of the ΨCR was amplified with the primer pair YCRint2fwd/YCR2rev from two older specimens from which no other fragments could be obtained (see Section 4). PCR fragments

Table 1
Sample list

Taxon	Code of sample	Tissue[a]	Country, locality, year	Source, voucher[b]	GenBank	Data set[c]
Accipiter nisus nisus	Anisnis3	mu	Austria, Vienna, 1999	C. Roland	AY216924	nd6
Aquila chrysaetos chrysaetos	Achrchr2	fe	Austria, Tyrol, Achensee, 1996	A. Mayrhofer	AY216922	nd6
	Achrchr3	fe	Austria, Salzburg, Oberpinzgau, 2000	A. Mayrhofer	AY216923	nd6
Asturina nitida costaricensis	Anitcos1	mu	Ecuador, Maniba/G, Rio Ayampe, 1991	ZMUC FS3	AY213033	PNEΨ, S
Asturina nitida nitida	Anitnit1	ba	Brazil, Rio Grande do Norte Paraguá, 1906	NMW 40143	AY213085	ΨCR
Asturina nitida plagiata	Anitpla1	ba	Costa Rica, Guanacoste, 1930	NMW 6847	AY213057	ΨCR
Busarellus nigricollis ssp.	Bnig3	DNA	–, 1996	M. Wink	AY216919	nd6
Busarellus nigricollis leucocephalus	Bnig5	mu	Paraguay, Rio Negro, 1999	KU 3140	AY216920	nd6
Buteo archeri	Barc1	ba	Eritrea, Habesch, 1909	RMNH 4758	AY216930	nd6s1
Buteo albicaudatus colonus	Balccol1	fe	Venezuela, Amazonas, La Esmeralda, 1996	A. Gamauf	AY213037	PNEΨ, S
	Balccol2	fe	Netherlands Antilles, Curaçao, 1996	A. Gamauf	AY213088	ΨCR
Buteo albicaudatus hypospodius	Balchyp2	fe	USA, southern Texas, 2001	TZ	AY213038	PNEΨ,
Buteo albigula	Balb1	ba	Argentinia, Chubut, El Bolson, 1984	NMW 84192	AY213025	PNEΨ, S
	Balb2	ba	Ecuador, Antisana, 1914	A.T. Ramirez	AY213081	ΨCR
Buteo albonotatus albonotatus	Baln1	ba	USA, Texas, 1901	BZ-Linz 744	AY213077	ΨCR
	Baln4	mu	Paraguay, Alto Py., Rio Negro, 1999	KU 3142	AY213029	PNEΨ, S
Buteo albonotatus abbreviatus	Balnabb1	ba	Surinam, Commewijne Maastroorn, 1965	RMNH 37977	AY213076	ΨCR
Buteo augur	Baug1	bl	Tanzania, 2000	H. Frey (UVMV)	AY213014	PNEΨ, S
	Baug4	DNA	–	M. Wink	AY213015	PNEΨ
Buteo auguralis	Bagl1	ba	Nigeria, Vom, 1979	NMW 76227	AY213016	PNEΨ, S
Buteo brachypterus	Bbra3	ba	Madagascar, Tsiandro, 1928	ZFMK 42278	AY213072	ΨCR
	Bbra5	ba	Madagascar, Beroroka, –	H.-C. Dubois	AY216916 AY213073	nd6, ΨCR
Buteo brachyurus brachyurus	Bbrubru1	ba	Brazil, Santa Catharina, 1911	NMW 40121	AY216928 AY213056	nd6, ΨCR
	Bbrubru2	ba	Brazil, Rio Grande do Sul, 1908	BZ-Linz, INR831	AY213055	ΨCR
Buteo buteo "socotrae"	Bbutsoc1	ba	Yemen, Socotra Is., Momi, 1934	BMNH 1934.8.12.2	AY213048	ΨCR
Buteo buteo arrigonii	Bbutarr1	ba	Italy, Sardinia, Ogliastra, 1906	ZFMK 7636	AY213049	ΨCR
Buteo buteo buteo	Bbutbut2	bl	Austria, Haringsee, 1997	H. Frey (UVMV)	AF380305	PNEΨ, S
Buteo buteo vulpinus	Bbutvul8	bl	Israel, Eilat, 1998	R. Rado, O. Hatzofe	AF202187	PNEΨ
	Bbutvul14	ba	Russia, Tomsk, 1901	NMW 57040	AF202198	ΨCR
Buteo galapagoensis	Bgal1	DNA	Ecuador, Galapagos Is., 1992	C. Farquhar	AY213079	ΨCR
	Bgal3	bl	Ecuador, Galapagos Is., 1992	C. Farquhar	AY213026	PNEΨ, S
	Bgal4	fe	Ecuador, Galapagos Is., 1995	M. Vosser	AY213078	ΨCR
Buteo hemilasius	Bhem8	DNA	Mongolia, 1997	M. Wink	AY213012	PNEΨ, S
	Bhem11	fe	Russia, Altai Mts., Tashanta, 2000	S. Ernst	AY213067	ΨCR

Table 1 (*continued*)

Taxon	Code of sample	Tissue[a]	Country, locality, year	Source, voucher[b]	GenBank	Data set[c]
	Bhem12	fe	Mongolia, S Ulaanbaatar, 2000	E. Potapov	AY216915	nd6
Buteo jamaicensis borealis	Bjambor6	mu	USA, New York, Rockland Co., 1996	AMNH PRS 1303	AY213020	PNEΨ
	Bjambor7	mu	USA, New Jersey, Somerset Co., 1996	AMNH PRS 1331	AY213019	PNEΨ
	Bjambor8	fe	Captive (USA), 2000	R. Schmid, R. Probst	AY213021	PNEΨ, S
	Bjambor9	DNA	Captive (Canada), –	M. Wink	AY213022	PNEΨ
	Bjambor10	DNA	Captive (Canada), –	M. Wink	AY213023	PNEΨ
Buteo jamaicensis calurus	Bjamcal3	fe	USA, California, Napa Country, 2001	S. Moore	AY213053	PNEΨ
Buteo jamaicensis costaricensis	Bjamcos1	ba	Guatemala, 1911	BZ-Linz INR 806	AY213054	ΨCR
Buteo jamaicensis harlani	Bjamhar3	ba	USA, N Dakota, 1890	BMNH 91.7.15.9	AY213052	ΨCR
Buteo jamaicensis jamaicensis	Bjamjam1	ba	Jamaica, Stony Hill, 1882	BMNH 1906.12.7.575	AY213051	ΨCR
Buteo japonicus japonicus	Bbutjap6	fe	Japan, Honshu, Nagano-ken, Omachi, 1996	BZ-Linz	AY213047	ΨCR
	Bbutjap10	fe	Russia, Ostoscho, S Kulusutay, 1999	S. Weigl	AY213011	PNEΨ, S
	Bbutjap11	fe	China, Shanghai, 2000	E. Yasamaki	AY213065	ΨCR
Buteo japonicus toyoshimai	Bbuttoy1	ba	Japan, Bonin Is., 1889	BMNH 1955.N.20.2151	AY213063	ΨCR
	Bbuttoy3	ba	Japan, Bonin Is., Chichishimna, 1889	BMNH 97.10.30.215	AY213064	ΨCR
Buteo lagopus lagopus	Blaglag1	ba	Austria, Lower Austria, Seyring, 1961	NMW 87604	AY213069	ΨCR
Buteo lagopus menzbieri	Blagpal1	ba	Russia, NE-Sibirien, Kolyma, 1905	NMW 8855	AF202216	ΨCR
Buteo lagopus sanctijohannis	Blagsan3	ba	N Greenland, Cape York, 1937	BMNH 1938.10.24.11	AY213071	PNEΨ
	Blagsan4	ba	Canada, NW Terr., Southampton Is., 1937	BMNH 1947.6.567	AY213070	ΨCR
Buteo lagopus kamtschatkensis	Blagkam2	fe	Russia, Kamchatka, 2001	E. Haring	AY213017	PNEΨ, S
Buteo leucorrhous	Bleu1	ba	Brazil, Sta. Catharina, Joinville, 1911	NMW 40186	AY213091	ΨCR
	Bleu7	mu	Ecuador, Cotopaxi, Cerro Parcato, 1991	ZMUC NK5	AY213040	PNEΨ, S
Buteo lineatus alleni	Blinall2	mu	USA, Florida, Martin Co., 1997	AMNH PRS 1591	AY213030	PNEΨ
Buteo lineatus elegans	Blinele1	mu	USA, California, Shasta Co., 1995	AMNH PRS 1224	AY213031	PNEΨ
	Blinele2	fe	USA, California, Novato Co., 2001	S. Moore	AY213083	ΨCR
Buteo lineatus lineatus	Blinlin5	mu	USA, Kansas, –	KU –	AY213082	PNEΨ
	Blinlin6	mu	USA, New Jersey, Morris Co., 1995	AMNH PRS 1065	AY213032	PNEΨ, S
Buteo magnirostris griseocauda	Bmaggri1	ba	Costa Rica, Catalina, Bebedero, 1930	NMW 6915	AY213089	ΨCR
Buteo magnirostris magniplumis	Bmagmap1	ba	Brazil, Sao Paulo, Pirassununga, 1940	ZFMK 56458	AY213058	ΨCR
	Bmagmap2	ba	Brazil, Sta. Catharina, Joinville, 1911	NMW 40183	AY213090	ΨCR
Buteo magnirostris saturatus	Bmagsat2	le	Paraguay, Depto. Alto Paraguay, 1998	KU 2972	AY213039	PNEΨ, S
Buteo oreophilus oreophilus	Boreore5	ba	Kenya, Mount Elgon, 1969	C. Gicucki	AY213050	ΨCR

Table 1 (*continued*)

Taxon	Code of sample	Tissue[a]	Country, locality, year	Source, voucher[b]	GenBank	Data set[c]
Buteo platypterus platypterus	Bplapla4	he	USA, Missouri, Howell Co., 1999	KU 2713	AY213084	ΨCR
	Bplapla5	le	USA, New Jersey, Morris Co., 1996	AMNH PRS 1293	AY213033	PNEΨ, S
Buteo platypterus insulicola	Bplains1	ba	Antigua, Mt. Joshua, 1937	J. Framington	AJ536267	ΨCR
Buteo polyosoma poecilochrous	Bpoe1	DNA	Ecuador, Monte Antisana, –	M. Wink	AY213036	PNEΨ, S
	Bpoe3	skin	Peru, Puno, 1964	C. Farquhar	AY213087	nd6, ΨCR
Buteo polyosoma exsul	Bpolexs3	skin	Chile, Mas Afuera Is., 1875	C. Farquhar	AY216926	nd6s1
Buteo polyosoma polyosoma	Bpolpol1	ba	Argentinia, Chubut, 1983	NMW 80329	AY213035	PNEΨ, S
	Bpolpol17	skin	Ecuador, Prov. Pichincha Chiriboga, 1990	C. Farquhar	AY213086	ΨCR
Buteo refectus	Bbutref3	ba	N India, –	A.T.S. Baker	AY216914	nd6
	Bbutref4	ba	India, Kashmir, Wardwan Valley, 1937	BMNH 1949.Whi.1.475	AY213061	PNEΨ
	Bbutref5	ba	India, Punjab, Dharmsala, 1921	BMNH 1949.Whi.1.472	AY213062	ΨCR
	Bbutref6	ba	China, N Kunsu, Lau-hu-Kou, 1927	ZMB 35364	AY213046	ΨCR
Buteo regalis	Breg1	ba	Mexico, 1902	BZ-Linz, INR830	AY213074	ΨCR
	Breg5	mu	USA, Minnesota, –	AMNH PRS 1529	AY213018	PNEΨ, S
Buteo ridgwayi	Brid3	ba	Domican Rep., St. Domingo, –	BMNH 956.6.N.20.2558	AY216927	nd6s1
Buteo rufinus rufinus	Brufruf1	fe	Hungary, Hortobagy, 1998	M. Dudas	AF202212	ΨCR
	Brufruf3	bl	Israel, Eilat, 1998	R. Rado, O. Hatzofe	AF202185	PNEΨ, S
Buteo rufofuscus	Brfcrfc3	DNA	South Africa, 1994	M. Wink	AY213068	PNEΨ
	Brfcrfc5	DNA	–	M. Wink	AY213013	PNEΨ, S
Buteo solitarius	Bsol5	ba	USA, Hawaii, Puniki Waimea, 1887	BMNH 1955.6.N.20.2245	AY216929	nd6s1
Buteo swainsoni	Bswa3	mu	USA, Kansas, Cheyenne Co., 1996	KU 1731	AY213080	PNEΨ
	Bswa4	mu	USA, New Mexico, Las Vegas, 1995	AMNH PRS 1520	AY213027	PNEΨ, S
	Bswa5	mu	USA, New Mexico, Eddy Co., 1996	AMNH PRS 1521	AY213028	PNEΨ
Buteo ventralis	Bven1	ba	Argentinia, Rio Negro, El Bolson, 1986	NMW 81840	AY213024	PNEΨ, S
	Bven2	ba	Argentinia, Rio Negro, El Bolson, 1985	NMW 81426	AY213075	ΨCR
Buteogallus aequinoctialis	Baeq1	ba	Brazil, Piauhy, 1903	NMW 40199	AY213092	ΨCR
Buteogallus meridionalis	Bmer1	ba	Ecuador, –	NMW 78353	AY213093	ΨCR
	Bmer4	he	Paraguay, Depto. Presidente Hayes, 1999	KU 3265	AY213044	PNEΨ, S
Buteogallus u. urubitinga	Buruuru1	he	Paraguay, Alto Py., Rio Negro, 1999	KU B 3141	AY213045	PNEΨ, S
Geranoaetus m. melanoleucus	Gmel1	bl	Ecuador, Quito, 1992	M. Wink	AY216918	nd6
Geranoaetus melanoleucus australis	Gmelaus2	fe	Argentinia, 1992	T.S. Gonzales	AY216917	nd6
Kaupifalco monogrammicus	Kmon1	DNA	–, (Africa), 1995	M. Wink	AY216921	nd6
Parabuteo unicinctus harrisi	Punihar3	fe	Captive (USA), 2000	F. Mohr	AY213041	PNEΨ
Parabuteo u. unicinctus	Puniuni4	DNA	–, 1990	M. Wink	AY213042	PNEΨ
	Puniuni6	DNA	Ecuador, –	M. Wink	AY213043	PNEΨ, S

Table 1 (*continued*)

Note. Samples are are listed in alphabetic order following the taxonomy of del Hoyo et al. (1994) as well as the A.O.U. (1998) Check-list of North American Birds (7th edition), with some adaptations according to Kruckenhauser et al. (in prep.). – = no data.

[a] Abbreviations of sample types used: bl, blood; fe, basal feather quill; ba, skin from foot pad; DNA, genomic DNA extracts provided by M. Wink, Univ. Heidelberg, D; mu, muscle; he, heart; le, liver. Locality and year of collection according to information from specimen's labels or tissue data bases.

[b] Names of collectors or vouchers from institutions (plus contact persons). *Abbreviations*: AMNH (American Museum of Natural History, NY, USA; G. Barrowclough, P. Sweet), BMNH (British Museum of Natural History/The Natural History Museum, The Bird Group, Tring, UK; R. Prŷs-Jones, M. Adams, F. Steinheimer), BZ-Linz (Biologiezentrum des OÖ Landesmuseums, Linz-Dornach, A; G. Aubrecht, S. Weigl), KU (University of Kansas, USA; A.T. Peterson), NMW (Naturhistorisches Museum, Wien, A; E. Bauernfeind, A. Gamauf), RMNH (Rijks Museum voor Natuurlike Historie/Naturalis, Leiden, NL; R. Dekker), TZ (The Texas Zoo, Houston, USA; C. Grutzmacher, D. Jahn), UVMV (Veterinärmedizinische Universität, Wien, A; H. Frey), ZFMK (Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn, D; R. van den Elzen), ZMB (Zoologisches Museum Berlin, D; S. Frahnert), ZMUC (Zoological Museum, University of Copenhagen, DK; J. Fjeldså).

[c] S (26 sequences used in the comparative molecular sequence analysis of the PNEΨ data set); for other abbreviations, see text.

were isolated from agarose gels with the Qiagen Gel extraction kit and sequenced directly. Sequencing (both strands) was performed on an ABI 3100 Prism Gene Analyzer (Applied Biosystems) at VBC-genomics, Vienna, using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit 2.0 according to producer's instructions (Applied Biosystems). All sequences are deposited in GenBank. Accession numbers are given in the sample list (Table 1).

2.3. Sequence alignment and phylogenetic analysis

Sequences were first aligned with CLUSTAL X (Thompson et al., 1997) and further adjusted manually using the BioEdit package version 5.0.9 (Hall, 1999). Tajima's relative rate test was performed with DnaSP vers. 3.53 (Rozas and Rozas, 1999). Distances were calculated with PAUP* (version 4b10, Swofford, 2002). If not otherwise stated, distances are uncorrected *p*-distances. We used *p*-distances for the calculation of NJ trees for the following reason: application of corrected distances, e.g., HKY (Hasegawa et al., 1985) for the ΨCR1, did not improve trees with respect to resolution, and even led sometimes to obviously wrong topologies. This may be explained by high distances of the outgroup taxa in comparison to the ingroup, indicating that for such basal relationships the ΨCR1 is too variable. The intrageneric relationships, which were of main interest, were resolved best applying *p*-distances for the calculation of NJ trees. Patristic distances (''tip-to-tip distances'') were calculated using the first MP tree of a heuristic search as topological source. These were compared with *p*-distances in a saturation plot as in Philippe et al. (1994). Neighbour joining (NJ, Saitou and Nei, 1987) and maximum parsimony (MP) trees were calculated with PAUP*. MP analyses were performed with heuristic search (with 1000 random addition replicates), accelerated character transformation (ACCTRAN), and tree bisection–reconnection (TBR) branch swapping algorithm. Gaps were treated as fifth character state in the ΨCR1 because sequence similarity was high enough to consider them as phylogenetically informative. Insertions >8 bp were treated as single mutations in the calculations of phylogenetic trees. All substitutions were weighted equally. Bootstrap analyses were performed with 1000 replicates (10 random addition replicates).

We also conducted heuristic searches using the maximum-likelihood (ML) criterion. The optimal evolution model for the ML searches was determined separately for two different data sets (coding nd6, non-coding ΨCR1) with Modeltest version 3.06 (Posada and Crandall, 1998). For the ΨCR1 data set the hierarchical likelihood ratio tests selected the HKY + Γ model with the following parameters: empirical base frequencies (A = 0.36, C = 0.33, G = 0.08, T = 0.23), transition/transversion ratio = 8.37 and gamma distribution shape

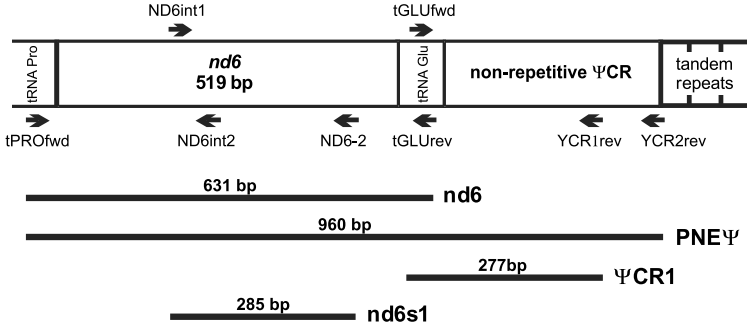

Fig. 1. Analyzed section of the mt genome and primer binding sites. Resulting PCR fragments and expected sizes (according to *B. buteo*) are given below the scheme. The fragment designation PNEΨ is derived from the abbreviations for tRNA-Pro (P), nd6 (N), tRNA-Glu (E), and ΨCR (Ψ).

parameter = 1.51. For the *nd6* data set the TrN + I + Γ model was selected with the following parameters: empirical base frequencies (A = 0.39, C = 0.37, G = 0.10, T = 0.14), proportion of invariable sites (I) = 0.56, rate matrix = (1.00 42.68 1.00 1.00 43.65), and gamma distribution shape parameter = 3.66. In general (except Fig. 3) we present NJ trees to make branch lengths evident which would not be apparent in a MP consensus tree. The differences in topology found in ML and MP trees are explained in the text.

3. Results

3.1. Data sets

An essential (~50%) part of the samples (taxa from which it was impossible to get fresh material) was derived from museum specimens. From these it was not feasible to amplify fragments larger than 350 bp. On the other hand, we used fresh samples to sequence larger fragments. This resulted in four different data sets (Fig. 1) to infer the phylogeny of the genus *Buteo* and related taxa at different taxonomic levels: (1) The complete *nd6* gene which was used to obtain information about the basal relationships of the genus *Buteo* and related buteonine taxa. This more conserved section could also be amplified from outgroup taxa for which the binding of the ΨCR primers failed. (2) A section designated PNEΨ containing the sequences *nd6–tRNA-Glu–*ΨCR, giving better resolution of the internal branches. It was employed to define relationships among major clades within *Buteo* as well as for comparing sequence variation in the different sections. (3) The ΨCR1 fragment containing the 3′-end of *tRNA-Glu* gene and the non-repetitive part of the ΨCR. This small but highly variable section was used for the extensive analysis including almost all extant *Buteo* species (except *Buteo archeri*, *B. solitarius*, and *B. ridgwayi*). (4) The nd6s1 fragment, a short section of the *nd6* gene, was amplified from old specimens of some endemic taxa from which neither the ΨCR1 nor the complete nd6 fragments could be obtained.

To avoid (co-)amplification of nuclear copies (numts) instead of the authentic mt sequences several measures were taken. Amplification of four different PCR fragments (Fig. 1) with different primers enabled evaluation of congruency between the resulting topologies and comparison of the different sections (nd6, tRNA-Glu, and ΨCR). With respect to coding regions the reading frames were intact in all *nd6* sequences (no matter which PCR fragment) with the highest variability found in the third positions. The *tRNA-Glu* section proved highly conserved. The tissues used in this study were mainly feathers or muscles, which, in contrast to blood samples, are less likely to give rise to numt amplification in birds (Bensasson et al., 2001; Sorenson and Quinn, 1998). Direct sequencing of PCR products was preferred to detect duplets in the pherograms, which are expected in the presence of co-amplified numts, contaminations or heteroplasmy. Finally, sequences derived from different individuals of the same taxon were always found in the same cluster (diagnostic haplotypes).

3.2. Sequence variation

To assess differences in sequence variability among the various sections (*nd6*, *tRNA-Glu*, and ΨCR) the PNEΨ fragment was used. Only one sequence per taxon was selected (26 of the 37 sequences indicated in Table 1). The alignment of these sequences reveals several larger insertions/deletions (indels): Puniuni6 contains a 12 bp insertion at position (pos.) 677 of the alignment, a 10 bp deletion at pos. 721, and a 7 bp deletion at pos. 777; Bplapla5 contains a 9 bp insertion at pos. 803; Blinlin6 contains two nested deletions of 5 and 6 bp at pos. 700 and 708, respectively. No sections had to be excluded because of ambiguous homology.

The observed patterns of sequence evolution in the three sections of PNEΨ are concordant with previous studies of mt DNA sequences. In the *nd6* fragment substitutions differ among codon positions: pS (segregating sites per site) is 0.187 in first, 0.100 in second, and 0.694 in third positions (average *p*-distance: first: 0.040; second: 0.020; and third: 0.169). Amino acid replacements are mainly conservative, i.e., within groups of amino acids with similar physicochemical properties (31 out of 40 changes). In *Busarellus* the start codon (ATG) of the *nd6* gene is followed by insertion of the triplet ATA (Met), which may serve as an alternative translation start.

As expected, among the three sections of the PNEΨ fragment the ΨCR shows the highest variability, whereas the short and highly conserved *tRNA-Glu* gene did not provide any useful phylogenetic information. Table 2 gives ranges of distances for the three sections. The *tRNA-Glu* gene has the slowest rate with nine variable sites (six of them parsimonious informative). Nucleotide diversity (π, the mean of pairwise uncorrected sequence

Table 2
Sequence comparisons of different sections of the PNEΨ fragment

	nd6	tRNA Glu	ΨCR
No. positions (alignment)	511	67	338
Range of *p*-distances (%)	0.58–13.50	0–7.46	1.66–31.34
Nucleotide diversity π	0.0762	0.0207	0.1475
Segregating sites per site pS	0.3268	0.1364	0.6423
R (overall mean)	17.33	0.70	6.45
GC content (%)	49.65	38.71	36.74

Note. Values were calculated from a set of 26 taxa. *R* (transition/transversion ratio).

differences per site) for the three sections of PNEΨ, transition (ts)/transversion (tv) ratio (R), and base composition are also summarized in Table 2. Pairwise p-distances among ΨCR sequences are on average 2.04 (± 0.55) times higher than those among *nd6* sequences. Saturation within coding and non-coding sections, respectively (*nd6*/ΨCR), was estimated by plotting patristic distances inferred from MP analysis versus p-distances according to Philippe et al. (1994) (not shown). Complete saturation is reached at ~12% (p-distance) in *nd6*, whereas in the ΨCR the curve is linear until ~18% and does not reach complete saturation.

3.3. Phylogenetic relationships

The *nd6* data set was used to define the basal relationships and to determine appropriate outgroup species for the analysis of the genus *Buteo*. The alignment of the 43 *nd6* sequences (representing 39 taxa) has a length of 522 nucleotide positions with 305 constant and 214 variable characters (166 parsimonious informative sites and 48 singletons). The NJ tree derived from this data set is shown in Fig. 2. *Accipiter nisus* has the highest distances to all other taxa (mean p-distance: 0.1638 ± 0.0065) and was consequently used as outgroup. The topology of the NJ tree proved congruent with two equally parsimonious MP trees (tree length TL = 571; not shown). The next shortest trees are only one or two substitutions longer (two trees of TL = 572, one tree TL = 573). Some short internal branches, which are only weakly supported in the NJ tree (e.g., node I), collapse in the MP bootstrap consensus tree. The ML tree derived from this data set has the same topology except that the *B. jamaicensis* clade and the *Buteo albigula*/*Buteo swainsoni* clade change positions.

The Old World species *Kaupifalco monogrammicus* and *Aquila chrysaetos* are placed basal to the *Buteoninae* ingroup, which mainly comprises New World taxa. A

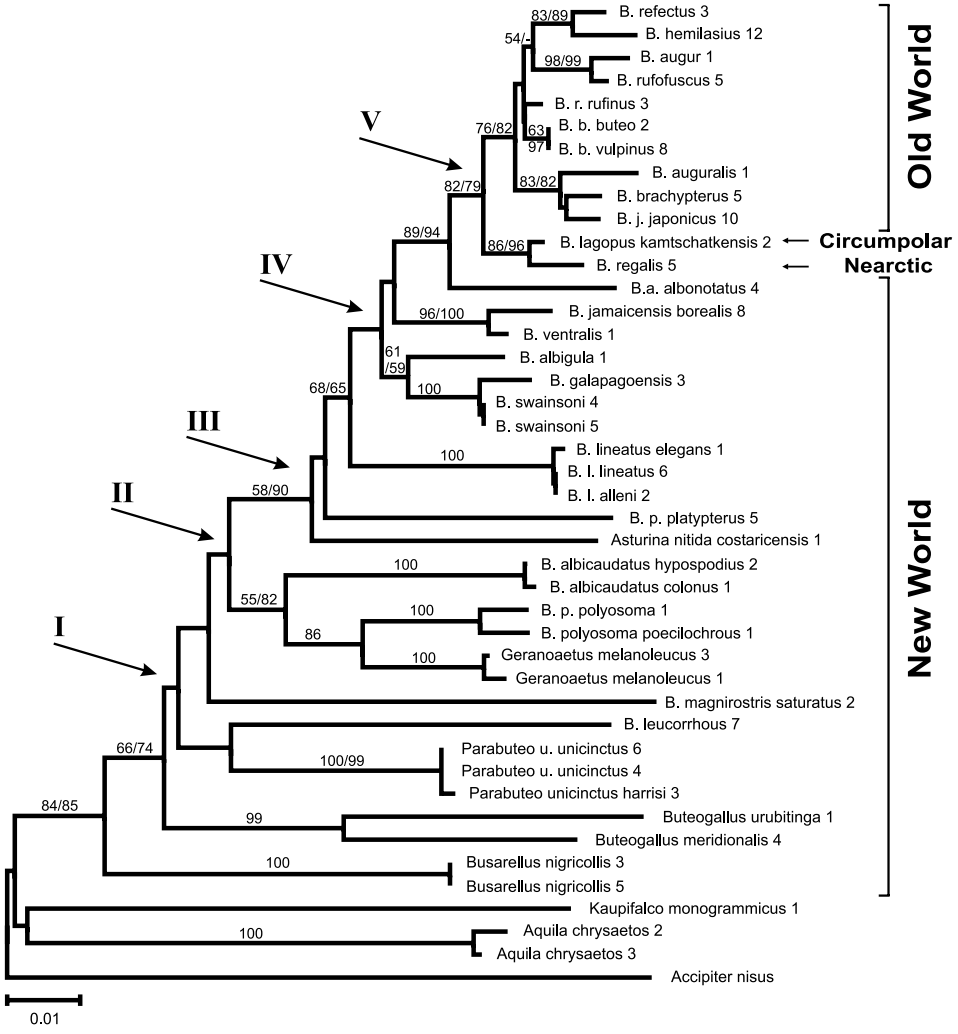

Fig. 2. Relationships among buteonine hawks. NJ tree (p-distances) of 43 taxa based on *nd6* (519 bp). Branch support (bootstrap values >50%, MP left, NJ right) is indicated above each branch. Single bootstrap values indicate that the same value was obtained with both algorithms. Arrows mark the five important steps in the evolution of the genus *Buteo* (I–V). Numbers are according to specimen numbers (Table 1).

closer relationship of *Kaupifalco* to the buteonine ingroup, as suggested by Amadon (1982), is not supported by our data. Within *Buteoninae* the neotropical *Busarellus nigricollis* represents the basal lineage followed by a clade formed by *Buteogallus urubitinga*/*Buteogallus meridionalis*. The next node (designated I) defines the clade containing all extant *Buteo* species. However, this clade also includes the genera *Parabuteo* (which clusters with *Buteo leucorrhous*), *Geranoaetus*, and *Asturina*. Thus, the genus *Buteo* appears paraphyletic in this tree. Node V defines a clade containing the Nearctic *Buteo regalis* and the circumpolar *Buteo lagopus* standing basal to the remaining Old World taxa. Thus, the buteos restricted to the Old World form a monophyletic group representing the most recent radiation within the genus. Since all basal buteonine taxa belong to the Neotropics, the *nd6* tree implies a Neotropic origin of the genus *Buteo*. For the detailed analysis of clade I (including nodes II–V) the PNEΨ and the ΨCR1 fragments were used and *Buteogallus* was chosen as outgroup.

The PNEΨ tree was calculated from the sequences of 37 specimens representing 32 taxa. The alignment has a length of 899 positions with 509 constant and 390 variable characters (297 parsimonious informative sites and 93 singletons). From *Geranoaetus* no ΨCR fragment (and thus no PNEΨ fragment) could be amplified, probably due to mutations at the binding site of the 3′ primer. Fig. 3 shows a strict consensus tree of 158 equally parsimonious trees. The topology of this cladogram is congruent with the NJ tree, but several nodes collapse in the MP consensus tree. Compared to the *nd6* tree the topology of the PNEΨ tree is similar, but higher bootstrap values are obtained for the nodes I–V (Fig. 3). As in the *nd6* tree (Fig. 2) the Old World taxa form a clade (within node V) that is clearly separated from the circumpolar and New World taxa. Node II has high bootstrap support and apparently delimitates the genus *Buteo*. Thus, on the basis of our sequence data, it seems appropriate to define the *Buteo albicaudatus*/*Buteo polyosoma* clade as

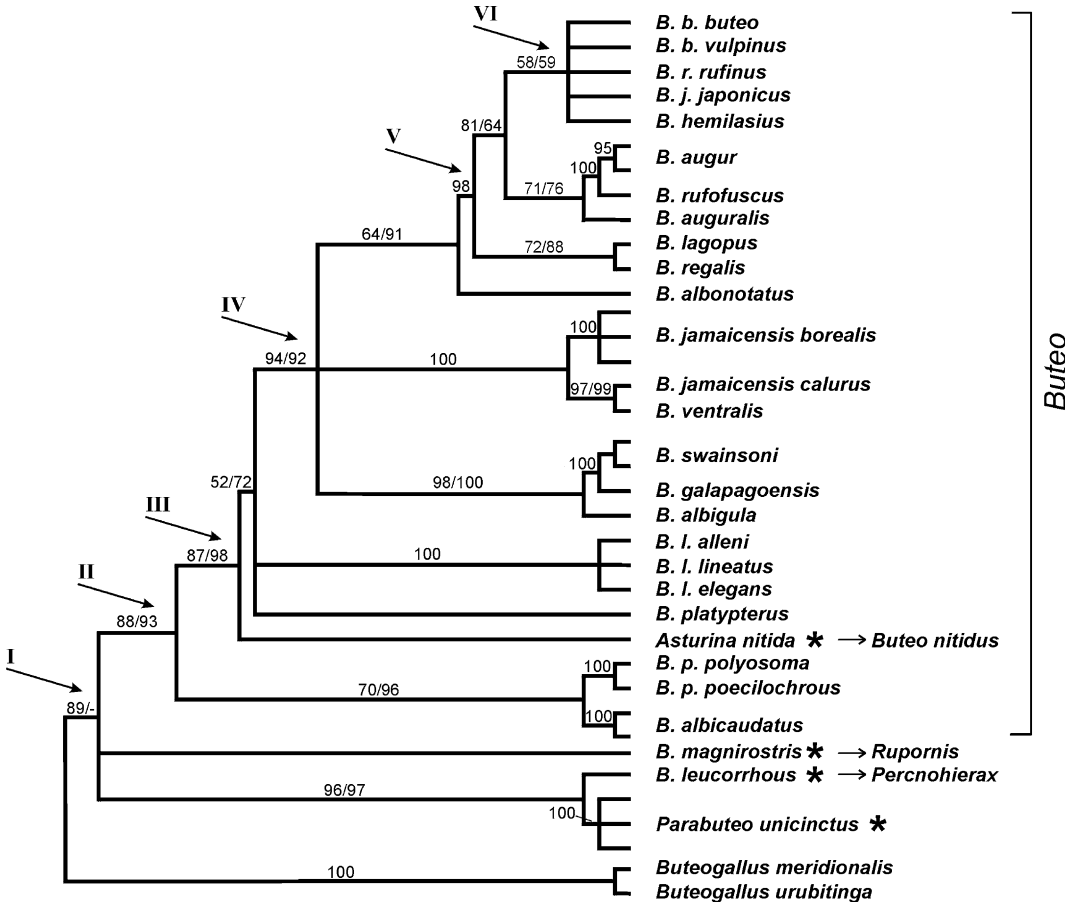

Fig. 3. Major lineages of the genus *Buteo*. Strict consensus tree based on the PNEΨ fragment of 158 shortest trees (844 steps) found in an equally weighted parsimony heuristic search analysis with 1000 random addition replicates. Clade support (bootstrap values >50%, MP left, NJ right) is indicated above each branch. Single bootstrap values indicate that the same value was obtained with both algorithms. Outgroup taxa: *Buteogallus urubitinga* and *B. meridionalis*. Arrows mark six (I–VI) important steps in the evolution of the genus *Buteo*. Taxa which implicate polyphyly of the genus *Buteo* are indicated with an asterisk. Proposed taxonomic changes are indicated (see Section 4).

the basal lineage of the genus *Buteo* and to exclude *B. magnirostris* and *B. leucorrhous* from the genus.

The relationships within clade VI of the *Buteo* lineage, which represents the youngest Old World radiation, cannot be resolved satisfactorily from the data set used in the PNEΨ tree. Thus we attempted an intrageneric analysis of *Buteo* from a much larger taxonomic sample based on 84 highly variable (compared to nd6) ΨCR1 sequences. Each of the 53 taxa included, was represented by at least two individuals (except some Old World taxa, e.g., *Buteo auguralis*). The alignment has a length of 253 positions, the fragment lengths of ΨCR1 varied between

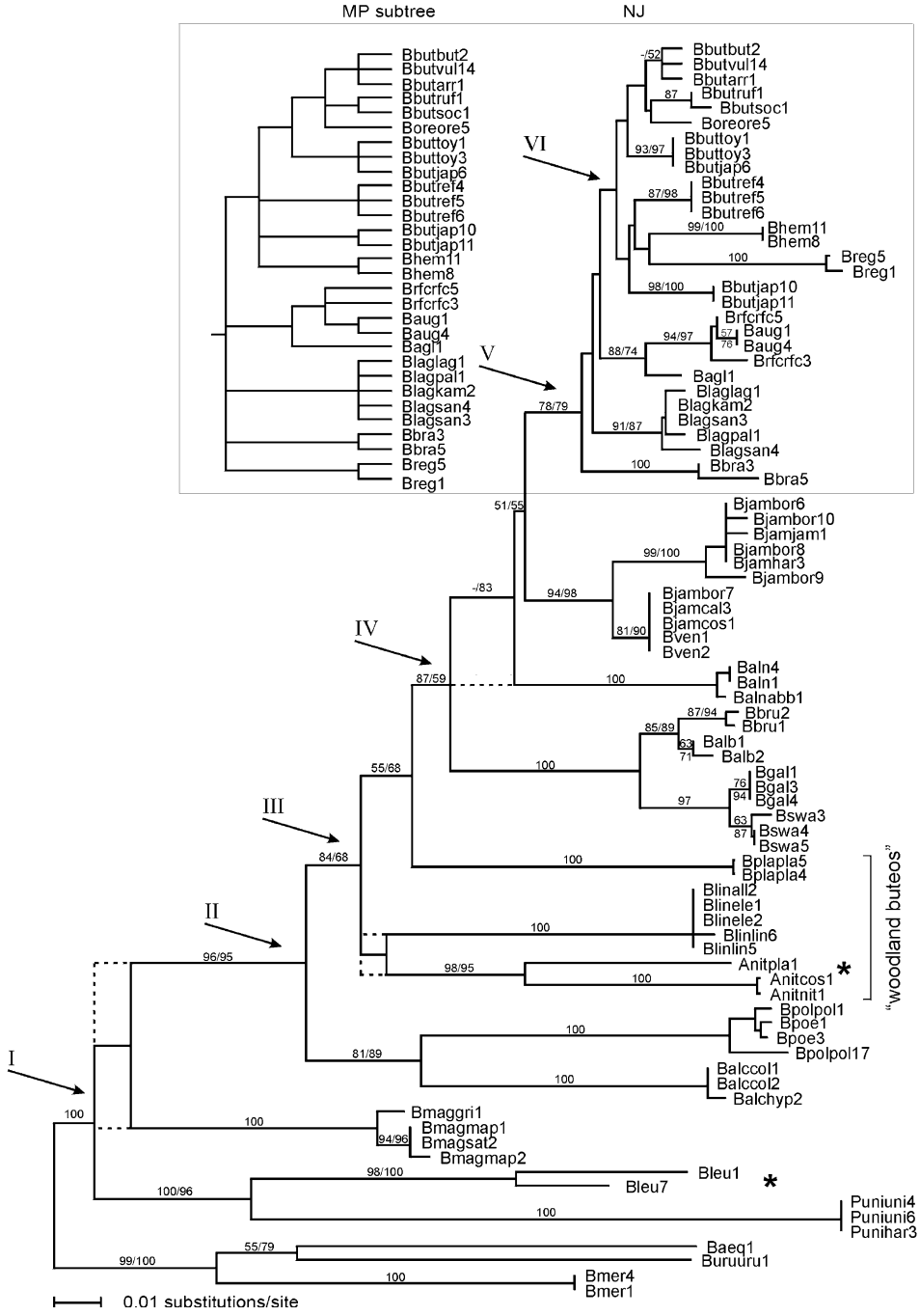

Fig. 4. Intrageneric relationships in the genus *Buteo*. NJ tree of 84 taxa based on 253 pos. of the ΨCR1 fragment. Clade V is also shown as a MP subtree of the strict consensus tree of 92 shortest trees (TL, tree length = 546; CI = 0.53; RI = 0.87; RC = 0.46) found in an unweighted parsimony analysis including gaps as fifth character state. Bootstrap values are given as defined in Fig. 2. Arrows mark the six major clades which are referred to in the text. Asterisks indicate taxa which are discussed in the text because of their high intraspecific diversity. Branches depicted as broken lines (e.g., Baln cluster) indicate nodes collapsing in the MP strict consensus tree.

229 and 247 bp due to indels of up to 14 bp. Intraspecific variability in the ΨCR1 is generally low within the species studied (mostly not exceeding 1%), even when geographically well separated subspecies are compared. A more detailed analysis of the Old World *Buteo* species will be presented by Kruckenhauser et al. (in prep.).

Higher intraspecific diversity (>1%) in the ΨCR1 was found within the New World species *B. leucorrhous*, *A. nitida*, and *B. jamaicensis*. In both the NJ and the MP trees the genus *Buteo* (node II, as defined in Fig. 3) as well as all sub-buteonine species are supported by high bootstrap values. In Fig. 4 differences between the NJ tree and a MP strict consensus tree (of 255 trees) are indicated as dotted lines in the major tree and shown in an additional MP subtree. Beside the three trichotomies at nodes I, III, and IV differences are also found in clade V concerning the position of *B. regalis*, which clusters with *Buteo hemilasius* in the NJ tree (although not supported in the bootstrap analysis). This grouping, which is due to two neighboring synapomorphic substitutions shared by these species, is neither found in the MP analysis (Fig. 4 subtree) nor in the ML tree where *B. regalis* stands at the base of the Palearctic clade (not shown). It is also not found in the *nd6* and PNEΨ trees (Figs. 2 and 3). Additional topological changes with respect to the other trees (nd6, PNEΨ) are (1) the position of *Buteo albonotatus*, (2) *B. lineatus* and *B. platypterus* which swap positions (clade III).

The branch leading to *B. magnirostris* is comparatively shorter in the ΨCR1 tree. A generally slower substitution rate within *B. magnirostris* is not likely, since in the nd6 tree the branch length of *B. magnirostris* does not appear shorter. Furthermore, Tajima's relative rate test (Tajima, 1989) conducted with various groups indicated no significant deviations in the substitution rate of *B. magnirostris*. Yet, the ΨCR1 section might be too short to evaluate relative rates (Bromham et al., 2000). Thus, the shorter branch in the ΨCR1 tree may rather reflect the uncertain position of this species within the tree (In the ML analysis *B. magnirostris* groups with the *B. leucorrhous*/*P. unicinctus* clade).

Phylogenetic relationships of four "island" taxa, are presented in Fig. 5 in a NJ tree based on the nd6s1 fragment. This tree indicates a close relationship of *B. p. exsul*, endemic to the Alejandro Selkirk Island of the Juan Fernández Archipelago off the Chilean coast, with *B. polyosoma* from the mainland. *B. ridgwayi*, native to Hispaniola, is found basal to the *B. lineatus* group (average $D = 3.46\%$). This is consistent with earlier presumptions (Johnson and Peeters, 1963). The Hawaiian *B. solitarius* is located within the clade containing *B. galapagoensis*, *B. swainsoni*, *Buteo brachyurus* and *B. albigula*, a result which is in accordance with Clarkson and Laniawe (2000). *B. archeri*, endemic to the coastal highlands of Somalia, appears not related to the *Buteo*

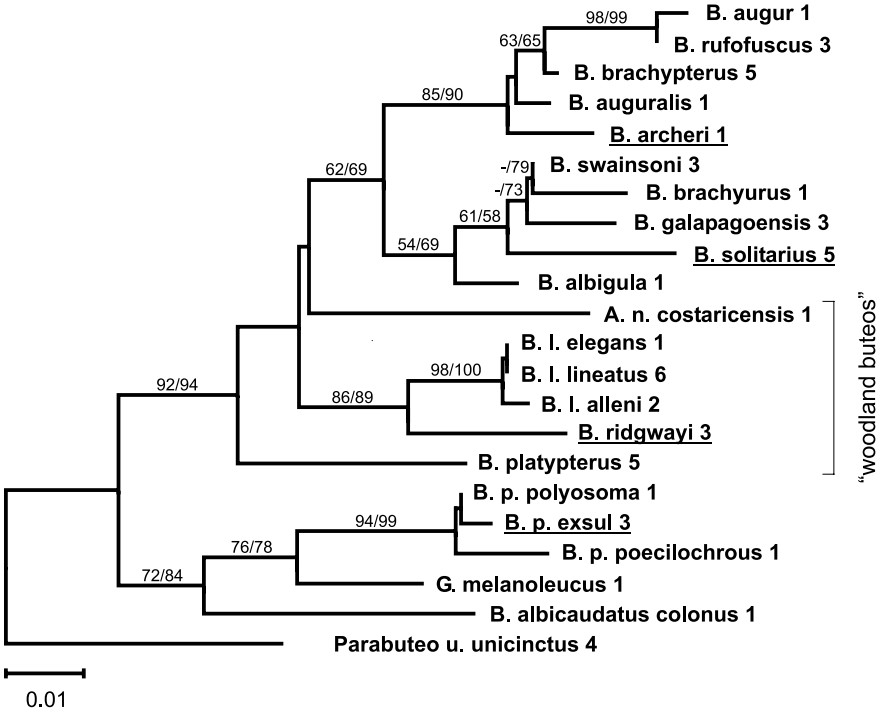

Fig. 5. Endemic *Buteo* taxa with restricted distribution ("island" taxa). NJ tree calculated from the nd6s1 fragment (246 bp) of 22 taxa. Bootstrap values are given as defined in Fig. 2. The four relevant endemic taxa are underlined. Numbers are according to specimen numbers (Table 1).

augur/*Buteo rufofuscus* clade, which would be expected from plumage characters. Instead, it splits off at the base of the African clade.

4. Discussion

4.1. Marker sequences

The non-repetitive section of the ΨCR is an efficient, neutral genetic marker sequence to infer intrageneric relationships within the genus *Buteo*. The main advantages in comparison to *nd6* are: (1) The higher substitution rate (2.04 ± 0.55 times higher) makes it suitable for analyses of old material, where only short fragments can be obtained. (2) Rate variation among sites is more uniformly distributed compared to *nd6* (Table 2). (3) Clades of species and species groups are supported by high bootstrap values. Thus the marker appears useful for diagnosis of unidentified samples. Nevertheless, with both marker sequences (either single or in combination) some nodes are not clearly resolved (in particular nodes III and IV). Furthermore, the high variability of the ΨCR might cause problems with primer binding in more diverged taxa (e.g., *Geranoaetus*).

4.2. Origin of the genus Buteo and taxonomic considerations

In our trees all basal lineages of the genus *Buteo* and related genera are restricted to the Neotropics. This finding suggests a South American origin of the genus *Buteo* which is in accordance with previous hypotheses (Amadon, 1982; Voous and de Vries, 1978).

The apparent paraphyly of the genus *Buteo* can be eliminated in two ways: (1) inclusion of *Parabuteo* into the genus *Buteo*, or (2) exclusion of *B. leucorrhous* and *B. magnirostris* together with inclusion of *A. nitida* (as *B. nitidus*) and *Geranoaetus melanoleucus* (as *Buteo melanoleucus*). Although the first possibility appears more parsimonious, the treatment of *Parabuteo* (Ridgway, 1899) as a separate genus is widely accepted (Amadon, 1982). Based on our molecular data we prefer the second solution, i.e., to define the genus *Buteo* as the taxa originating from node II. As a consequence of this definition of *Buteo* we suggest the following classification which we will use in the subsequent discussion (see also Fig. 3): *B. magnirostris* should be placed in the genus *Rupornis* Kaup, 1844, and *B. leucorrhous* into *Percnohierax* Ridgway, 1920, because these two genera have previously been established for these taxa. Their exclusion from the genus *Buteo* is also supported by their aberrant external morphology as well as plumage characters.

4.3. Sub-buteonine taxa

According to our definition of the genus *Buteo* the sub-buteonines include also the genera *Rupornis* and *Percnohierax*. In the nd6 tree the most basal sub-buteonine genus is *Busarellus*. It is followed by *Buteogallus* (Amadon, 1982), which represents a monophyletic group (Fig. 4). The distances among the three *Buteogallus* species investigated are quite high ranging from 16.5 to 17.8% (ΨCR1 fragment). This finding indicates that they represent old lineages. Evaluation of the phylogenetic relationships within *Buteogallus*, which has been split into several other genera (Brown and Amadon, 1968) requires a more comprehensive analysis comprising all *Buteogallus* species.

In the ΨCR1 data set three of the 12 subspecies of *Rupornis magnirostris* are included. *Rupornis m. griseocauda* (specimen Bmaggri1) from Costa Rica is separated (average $D = 1.4\%$) from the two South American subspecies *R. m. magniplumis* and *R. m. saturatus* (Bmagmap1, Bmagmap2, and Bmagsat2) which are almost identical. Between the two specimens of *Percnohierax leucorrhous* from two disjunct populations (Fjeldså and Krabbe, 1990) a higher sequence divergence (average $D = 5.5\%$) was detected. This divergence exceeds that found between *Buteo* species, e.g., *B. brachyurus* and *B. swainsoni*, suggesting that *P. leucorrhous* is not a monotypic species. Another individual of the western population (data not shown) differs from Bleu7 by only one transition in the 182 bp 5′-section of the ΨCR. Surely more information about distribution and breeding biology is needed to make a conclusive decision about *P. leucorrhous*. In our trees *P. leucorrhous* appears as the closest relative of *Parabuteo* with which it shares clear similarities in plumage characters (including the juvenile plumage). Interestingly, intraspecific variability of *Parabuteo unicinctus* is not detected in the five specimens of our study, although the samples investigated cover much of the species distribution in North and South America. This finding indicates a rapid and recent expansion from South America.

4.4. Relationships within the genus Buteo

The basal clade (Fig. 5) of extant *Buteo* species comprises the white-tailed and red-backed hawks *B. albicaudatus*, and *B. polyosoma* as well as *B. melanoleucus*. We detected an average distance of 1.28% between the subspecies *polyosoma* and *poecilochrous* in ΨCR1, whereas no genetic variability was found in an unpublished *cytb* data set of 606 bp (C. Farquhar, pers. comm.). These data suggest *B. p. poecilochrous* and *B. p. exsul* should not be treated as distinct species but considered as conspecific with *B. polyosoma*. This has already been suggested by Farquhar (1998) but is in contrast to del Hoyo et al. (1994) and Ferguson-Lees

and Christie (2001). Within *B. albicaudatus* variability in the ΨCR1 is very low. The two investigated subspecies *Buteo albicaudatus colonus* and *Buteo albicaudatus hypospodius* differ by one transition. The samples from Curaçao of the Netherlands Antilles (Balccol2) and from Venezuela (Balccol1) share the same haplotype supporting the hypothesis of a recent colonization of that island as suggested by Voous and de Vries (1978).

According to our data, the "woodland buteos" (Johnson and Peeters, 1963) do not represent a monophyletic group. While *B. nitidus* (specimens Anitnit1, Anitcos1, and Anitpla1), *B. platypterus*, *B. lineatus*, and *B. ridgwayi* form a paraphyletic assemblage, *B. magnirostris* appears as a clearly separated lineage basal to the genus *Buteo*. Substantial variability was detected within *B. nitidus*. The subspecies *B. n. plagiatus* is 9% apart from *B. n. nitidus* and *Buteo jamaicensis costaricensis*, respectively. Thus, the earlier proposed species status of *plagiatus* (Millsap, 1989) is supported by our data. Intraspecific diversity within *B. platypterus* and *B. lineatus* is low. From a single sample of *B. p. insulicola* (Bplains1), a subspecies endemic to Antigua, which differs considerably in size and plumage, a 182 bp 5′-section of the ΨCR was analyzed, which, interestingly, proved to be identical to that of the nominate form.

The clade comprising the two pairs of mainly Neotropic sister species, *B. brachyurus*/*B. albigula* and *B. swainsoni*/*B. galapagoensis*, also includes the Hawaiian hawk *B. solitarius* (Fig. 5). The only North American species within this cluster is *B. swainsoni*, which winters in South America. It can be assumed that the ancestor of this clade was a South American long distance migrant morphologically similar to *B. swainsoni* which could easily reach remote islands. An allospecific relationship of *B. galapagoensis* with *B. albicaudatus*, as suggested first by Voous (1968) and later by Voous and de Vries (1978), can be excluded. Taxonomic affinities of the morphologically distinct Zone-tailed Hawk *B. albonotatus* are uncertain (Palmer, 1988). Its position in our trees differs between data sets: either basal to *B. jamaicensis* (ΨCR1, Fig. 4) or basal to the species of node V (*nd6*, PNEΨ, Figs. 2 and 3), whereby the latter topology is better supported in the bootstrap analyses.

Within *B. jamaicensis* significant intraspecific diversity is detected, splitting the clade into an eastern and a western group (mean distances between groups: $3.19 \pm 0.21\%$ (ΨCR1); 1.16 % (*nd6*)). The western group includes *Buteo jamaicensis calurus*, *B. j. costaricensis*, and *Buteo ventralis*, while the nominate form, *jamaicensis* from terra typica (island of Jamaica), is part of the eastern group together with the subspecies *borealis* and *harlani*. Yet, the sample Bjamhar3 was not collected in the breeding area (Mindell, 1983), and thus the attribution to *Buteo jamaicensis harlani* remains dubious. A single specimen (Bjambor7) from New Jersey clusters with the western clade which is not concordant with the division of the *B. jamaicensis*. Nevertheless, this sample is derived from a captive bird of unknown origin. Missing diversity within the western clade indicates a severe recent bottle neck and subsequent rapid expansion across the western coasts of the Americas. A close relationship of *B. ventralis* to *B. jamaicensis* was assumed already by Clark (1986). Considering the lack of genetic differentiation, species status of *B. ventralis* is questionable. It could be hypothesized that the distribution range of *B. ventralis* represents the southern refuge of this western group during the last glacial. Higher diversity within the eastern clade could indicate different refugial areas, probably on Caribbean islands. To clarify the taxonomic status of the divergent populations, individuals from potential hybrid zones should be analyzed.

The remaining taxa represent a radiation which includes all Old World buteos and the Nearctic *B. regalis*. Within this group the following divergent lineages can be distinguished: (1) *B. regalis*, (2) *Buteo brachypterus*, the endemic species of Madagascar, (3) *B. lagopus*, (4) the *auguralis* cluster of the Afro-tropical species (*B. auguralis*, *B. augur*, *B. rufofuscus*, and *B. archeri*), and (5) a cluster comprising the Asian taxa (*B. hemilasius*, *Buteo refectus*, and *Buteo japonicus*) and the very recent radiation of the superspecies *B.* [*buteo*]. This clade (node VI in Figs. 3 and 4) comprises a plentitude of taxa which have been a matter of debate for decades. It is addressed in detail by Kruckenhauser et al. (in prep.). The basal lineage of the group defined by node V (either *B. regalis*, *B. brachypterus*, *B. lagopus* or the *auguralis* cluster) cannot be defined unambiguously which may also be ascribed to a rapid radiation of this group. A close relationship between *B. regalis* and *B. lagopus* was assumed from morphological similarities (Johnsgard, 1990) and was also found in our trees (Figs. 2 and 3). Subtle intraspecific genetic variation of the Nearctic subspecies of *B. lagopus* was detected which is consistent with differences in plumage found by Cade (1955), who assumed *B. l. sancti-johannis* to be the basal rough-legged hawk. The author suggested two refugia where the differentiation of *B. lagopus* subspecies may have occurred during the last glaciation: one in Beringia, which was connected with the large unglaciated region of Siberia, the other in the south-eastern parts of North America. These refuges were separated by thousands miles of ice. An analogous model can be put forward for the differentiation of the Old Word species. The common ancestor of the Palearctic taxa could have split off from the *B. regalis*/*B. lagopus* lineage as an isolated Beringian population in an earlier glacial period. Proceeding from this ancestral refuge the descendants of this population may have invaded the Old World in the course of several waves under the influence of cyclical climatic changes during the ice ages. This gave rise to the extant lineages: the two African branches

(*B. brachypterus* and the *auguralis* cluster), a cluster of Asian species (*B. japonicus*, *B. refectus*, and *B. hemilasius*), and their most recent offshoot, the superspecies *B.* [*buteo*].

Acknowledgments

This research was funded by the Austrian Science Foundation (FWF; Project No. P14069-BIO). We thank many people and institutions (refer to names given in Table 1) for providing samples. We are grateful to S. Mueller and S. Moesl (VBC-genomics) who introduced M.J.R. into state of the art of automated DNA sequencing. We are very much obliged to W. Pinsker and W. Mayer for their extensive support of the project, valuable discussions, and contributions to the manuscript. We thank B. Mayer (Emergentec) for providing computing facilities. We also thank C. Sturmbaver and C. Farquhar for helpful comments on the manuscript and two anonymous reviewers for valuable criticism and numerous detailed suggestions.

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