Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 37 (2005) 327–346
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogeny of eagles, Old World vultures, and other Accipitridae based on nuclear and mitochondrial DNA

Heather R.L. Lerner *, David P. Mindell

Department of Ecology and Evolutionary Biology, University of Michigan Museum of Zoology, 1109 Geddes Ave., Ann Arbor, MI 48109-1079, USA

Received 16 November 2004; revised 31 March 2005
Available online 31 May 2005

Abstract

We assessed phylogenetic relationships for birds of prey in the family Accipitridae using molecular sequence from two mitochondrial genes (1047 bases ND2 and 1041 bases cyt-*b*) and one nuclear intron (1074 bases β-fibrinogen intron 7). We sampled representatives of all 14 Accipitridae subfamilies, focusing on four subfamilies of eagles (booted eagles, sea eagles, harpy eagles, and snake eagles) and two subfamilies of Old World vultures (Gypaetinae and Aegypiinae) with nearly all known species represented. Multiple well-supported relationships among accipitrids identified with DNA differ from those traditionally recognized based on morphology or life history traits. Monophyly of sea eagles (Haliaeetinae) and booted eagles (Aquilinae) was supported; however, harpy eagles (Harpiinae), snake eagles (Circaetinae), and Old World vultures were found to be non-monophyletic. The Gymnogene (*Polyboroides typus*) and the Crane Hawk (*Geranospiza caerulescens*) were not found to be close relatives, presenting an example of convergent evolution for specialized limb morphology enabling predation on cavity nesting species. Investigation of named subspecies within *Hieraaetus fasciatus* and *H. morphnoides* revealed significant genetic differentiation or non-monophyly supporting recognition of *H. spilogaster* and *H. weiskei* as distinctive species.

Keywords: Accipitridae; Avian systematics; Phylogenetics; Molecular evolution; Cytochrome-*b*; ND2; β-Fibrinogen intron 7

1. Introduction

Accipitridae is a diverse avian family, comprising up to 14 subfamilies, 65 genera, and 231 species (see Table 1, Dickinson, 2003; Stresemann and Amadon, 1979). Of the Accipitridae species, some of the largest and most threatened by anthropogenic factors belong to four eagle subfamilies (Circaetinae, Haliaeetinae, Aquilinae, and Harpiinae) and two Old World vulture subfamilies (Gypaetinae and Aegypiinae). All Accipitridae species are protected under the Convention on International Trade in Endangered Species (CITES) and four eagles are listed as top priority species (CITES I, CITES-Secretariat, 2003). As ecologically sensitive predators, birds of prey are valuable indicators of habitat quality. The Accipitridae are found in a variety of habitats from primary rainforest to arctic tundra throughout the world. Some taxa are restricted in distribution such as the snake eagles (Circaetinae) which are found only in the Old World, while others, such as the sea eagles (Haliaeetinae), are global in distribution. Thorough phylogenetic analyses are needed to delineate the genetic and overall biological diversity of this family, and to inform conservation programs which aim to preserve genetic diversity of distinguishable taxonomic units.

Phylogeny for Accipitridae based on morphological traits has been difficult to resolve (e.g., Brown and Amadon, 1968; Jollie, 1976, 1977a,b). The few published molecular studies have been limited in sampling and have proposed some previously unrecognized relationships (see below). The goal of the present study is to identify phylogenetic relationships within and among the six subfamilies of eagles and Old World vultures in

* Corresponding author. Fax: +1 734 763 4080.
E-mail address: hlerner@umich.edu (H.R.L. Lerner).

1055-7903/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2005.04.010

Table 1
Accipitridae subfamilies

Subfamily	Common name	Genera	Brief description	Genera placed in the subfamily by this analysis
Elaninae[a]	Kites	*Elanus*, *Gampsonyx*, *Chelictinia*	Kites noted for having a bony shelf above the eye, *Elanus* is cosmopolitan, *Gampsonyx* is restricted to the New World and *Chelictinia* is found in Africa	*Elanus* (*Gampsonyx* and *Chelictinia* not sampled)
Perninae[a]	Kites	*Pernis Aviceda*, *Leptodon*, *Chondrohierax*, *Henicopernis*, *Elanoides*, *Machaerhamphus* (?)	Kites mainly found in the tropics and specializing on insects and bee or wasp larvae, all lack the bony eye shield found in the Elaninae	*Pernis*, *Leptodon*, *Chondrohierax*, *Elanoides*, *Hamirostra* and *Lophoictinia* (*Aviceda*, *Henicopernis* and *Machaerhamphus* not sampled)
Milvinae[a]	Milvine or Brahminy kites	*Milvus*, *Rostrhamus*, *Harpagus*, *Ictinia*, *Lophoictinia*, *Hamirostra*, *Haliastur*	Diverse kites found in the New and Old World, all species have fusion of joints of the second and third toes (Brown and Amadon, 1968)	*Milvus* and *Haliastur*
Aegypiinae[a]	Old World vultures	*Gyps*, *Pseudogyps*, *Necrosyrtes*, *Aegypius*, *Torgos*, *Trigonoceps*, *Sarcogyps*	Largest Old World vultures, scavengers, most with long necks and lightly feathered to bare heads	*Gyps*/*Pseudogyps*, *Necrosyrtes*, *Aegypius*, *Torgos*, *Trigonoceps* and *Sarcogyps*
Gypaetinae[b]	Old World vultures	*Neophron*, *Gypaetus*, *Gypohierax*	Generally smaller vultures found in the Old World with more restricted ranges, various specialized feeding behaviors, vocalizations, breeding displays, *Gypohierax* and *Neophron* similar to each other in plumage coloration and molt stages	*Neophron*, *Gypaetus*, *Eutriorchis* and *Gypohierax*
Circaetinae[a]	Snake eagles	*Circaetus*, *Terathopius*, *Dryotriorchis*, *Eutriorchis*, *Spilornis*	Old World species feeding mainly on snakes, other reptiles and small mammals, have a reticulate pattern of heavy scales on the tarsi and relatively short toes	*Circaetus*, *Terathopius*, *Dryotriorchis*, *Spilornis* and *Pithecophaga*
Polyboroidinae[c]	Harrier hawks	*Polyboroides*, *Geranospiza*	One New World and one Old World species, both exploit species found in tree cavities for prey, have short outer toe, increased mobility and length of the tarsus, relatively weak bill	*Polyboroides*
Aquilinae[b]	Booted eagles, hawk-eagles	*Aquila*, *Spizaetus*, *Hieraaetus*, *Stephanoaetus*, *Polemaetus*, *Ictinaetus* (considered a kite[d]), *Spizastur*, *Oroaetus*	Large eagles with feathered tarsi, globally distributed in diverse habitats taking a wide variety of prey, the hawk-eagles have crests	*Aquila*, *Spizaetus*, *Hieraaetus*, *Stephanoaetus*, *Polemaetus*, *Ictinaetus*, *Spizastur*, *Oroaetus*, and *Lophaetus*
Accipitrinae[b]	Sparrowhawks and (?) Chanting goshawks	*Accipiter*, *Urotriorchis*, *Megatriorchis*, *Erythrotriorchis*, *Melierax*, *Heterospizias* (?)	Small, fast fliers specializing on small birds as prey, long and slim tarsometatarsus and toes	*Accipiter* (*Urotriorchis*, *Megatriorchis*, *Erythrotriorchis* and *Heterospizias* not sampled)
Circinae[a,f]	Harriers	*Circus*	Broad and long-winged birds with facial feather disks, found mainly in open habitat such as fields or marshes, have specialized outer ears and related bone structures	*Circus*
Haliaeetinae[d]	Sea and Fish eagles	*Haliaeetus*, *Ichthyophaga*	Large eagles found in riverine and coastal habitat throughout the world, all have fused basal joint of middle toe	*Haliaeetus*, *Ichthyophaga*

Table 1 (*continued*)

Subfamily	Common name	Genera	Brief description	Genera placed in the subfamily by this analysis
Buteoninae[b]	Hawks, buzzards, (usually includes booted eagles, sea eagles and harpy eagles which we have separated out here)	*Buteo*, *Geranoaetus*, *Parabuteo*, *Kaupifalco*, *Buteogallus*, *Harpyhaliaetus*, *Busarellus*, *Heterospizias* (?), *Leucopternis*, *Butastur*	Predominately New World species of soaring hawks with long broad wings and relatively short tails and legs	*Buteo*, *Geranoaetus*, *Parabuteo*, *Buteogallus*, *Harpyhaliaetus*, *Leucopternis*, *Ictinia*, *Geranospiza* and *Rostrhamus*, (*Busarellus*, *Heterospizias*, *Kaupifalco* and *Butastur* not sampled)
Harpiinae[e]	Harpy eagles	*Harpia*, *Morphnus*, *Harpyopsis*, *Pithecophaga* *Harpyhaliaetus* (?)	Extremely large and powerful eagles with unfeathered tarsi, tropical forest predators of medium-sized mammals	*Harpia*, *Morphnus* and *Harpyopsis*
Melieraxinae[e,f]	Chanting goshawks	*Melierax* (*Micronisus*)	Forest accipiters, larger than *Accipiter* species	*Melierax* (*Micronisus*)

[a] Peters (1931).
[b] Gadow (1893).
[c] Brown and Amadon (1968).
[d] Sushkin (1905) in Jollie (1976).
[e] This study.
[f] Alternatively Circinae and Melieraxinae may be united under Accipitrinae with the genus *Accipiter*.

the context of the other primary accipitrid groups using molecular data.

The booted eagles (Aquilinae) are one of the largest accipitrid groups containing 35–36 species in 8–9 genera and are distributed worldwide. The majority of the species fall into three genera, *Aquila*, *Hieraaetus*, and *Spizaetus*, while the remaining five genera are all monotypic. All species have "boots," or feathered tarsi, a trait that separates this group from most other accipitrid taxa. The booted eagles have been considered to be monophyletic (Jollie, 1977b) or polyphyletic (Holdaway, 1994) with morphological data, and only a few species in one genus have been studied phylogenetically with molecular data (cyt-*b*, Seibold et al., 1996; control region, Vali, 2002). Monophyly of the three Aquilinae genera is not well supported with morphological characters, such that the *Hieraaetus* species and some *Spizaetus* species have been placed in the genus *Aquila* by various authors (described by Brown and Amadon, 1968; and Thiollay, 1994). The two molecular studies included about half of the species in the genus *Aquila*, and both found that *A. chrysaetos* was genetically distant from four other *Aquila* species. Sister relationships for *A. clanga* and *A. pomarina*, *A. nipalensis* and *A. heliaca* or *A. heliaca* and *A. adalberti* were also proposed.

The sea eagles (Haliaeetinae) are a much smaller and more easily defined group of large eagles found in coastal and riverine areas worldwide except South America and Antarctica. The two sea eagle genera, *Haliaeetus* and *Ichthyophaga*, share some morphological traits with two genera of kites (*Milvus* and *Haliastur*), suggesting a close relationship (Holdaway, 1994; Jollie, 1977b; Thiollay, 1994). The sea eagles also share some traits with the palmnut vulture (*Gypohierax angolensis*), suggesting a relationship between them and Old World vultures (Brown and Amadon, 1968). Using cyt-*b* sequence data, Seibold and Helbig (1996) studied eight of the nine species of sea eagles in the genus *Haliaeetus*. They supported a clear split between species with temperate versus tropical distributions, and a close relationship between the sea eagles and two *Milvus* kites. The relationship between the two genera of sea eagles has not been investigated with molecular sequence data and the possibility of paraphyly of the genera remains unresolved.

The four species and genera of harpy eagles (Harpiinae) are some of the largest raptors and are found in tropical rain forests in the Americas, the Philippines and New Guinea. This group is generally considered monophyletic due to their large size, lack of feathers on the tarsi, and similarities in behavior (Brown and Amadon, 1968; Thiollay, 1994); however, some have suggested that the Old World species are not sister to the New World species (e.g., Jollie, 1977b). Holdaway (1994) removed one Old World (*Pithecophaga*) and one New World (*Morphnus*) species from the Harpiinae. A close relationship between the booted eagles and the harpy eagles has been proposed but not tested with molecular data.

The 14 species of snake eagles (Circaetinae) in five genera are found only in the Old World. Although usually considered monophyletic (Brown and Amadon, 1968; Friedmann, 1950), the possibility of polyphyletic origins for snake eagles has been raised (Jollie, 1977b, could not identify sister relationships for Eutriorchis and Dryotriorchis; Thiollay, 1994).

The final group we focused on is the Old World vultures, a diverse mix of scavengers including at least one species that uses tools (Egyptian vulture), and potentially including a frugivorous raptor (palmnut vulture). One or two subfamilies have been proposed for the Old World vultures. Three species are highly divergent from the remaining 11 and have been placed by some in a separate family called Gypaetinae (Mundy et al., 1992). The core 11 species are called the Aegypiinae. Seibold and Helbig (1995) used cyt-*b* sequence from 11 Old World vulture species and found evidence of polyphyly for the Old World vultures.

There are no previously published molecular studies that include representatives of all of the Accipitridae subfamilies; however, several molecular studies have used mitochondrial DNA to examine particular Accipitridae subgroups and have found evidence for polyphyly of some traditionally recognized taxa (e.g., polyphyly of the Perninae kites, Riesing et al., 2003; and the genus *Buteo*, Gamauf and Haring, 2004). Relationships among a small set of accipitrids based on mtDNA indicated a closer relationship between a representative sea eagle and kite in the genus *Milvus*, than between the sea eagle and a snake eagle in the genus *Circaetus*. A representative Old World vulture was more closely related to the snake eagle than other accipitrid taxa in the study, including species of *Buteo*, *Haliaeetus*, *Milvus*, *Circus*, *Accipiter*, and *Pernis* (Mindell et al., 1997). Increased sampling of species and molecular characters are needed to improve our understanding of phylogenetic relationships among the Accipitridae.

In this study, we focus on full or nearly complete taxonomic representation of five accipitrid subgroups (sea and fish eagles, harpy eagles, booted eagles, snake eagles, and Old World vultures), corresponding to six potential subfamilies. We use both mitochondrial and nuclear sequences for representatives of 51 out of 65 genera (78%) and just under half of the known Accipitridae species ($n = 111$). At least one representative of each previously proposed subgroup/subfamily within the Accipitridae have been included to help in phylogenetic placement of the focal taxa.

2. Methods

2.1. Taxon sampling

We include at least one representative from all genera and the majority of species of sea and fish eagles (2 genera, 10 species), snake eagles (4 genera, 12 species), harpy eagles (4 genera, 4 species), booted eagles (8 genera, 29 species), and Old World vultures (9 genera, 13 species), based on the taxonomy in Dickinson (2003). In two cases where significant morphological differences among geographical populations have been documented, multiple samples representing different subspecies were included in the analysis. To infer relationships among these subfamilies within the Accipitridae, we also include at least one representative from each primary group or clade within the Accipitridae family as proposed by Gadow (1893), Peters (1931), Brown and Amadon (1968), Jollie (1977b), Stresemann and Amadon (1979), and Holdaway (1994). *Falco longipennis*, *Falco peregrinus*, and *Phalcoboenus megalopterus* (Falconidae) were used as outgroup taxa. Samples used and GenBank Accession numbers are listed in Table 2.

2.2. Sequencing

Genomic DNA was extracted from muscle tissue or blood using proteinase K digestion following the manufacturer's protocols (DNeasy tissue kit, Qiagen), or from the calamus of primary feathers by adding dithiothreitol (30 ml of 100 mg/ml, Cooper, 1994) to the overnight tissue digestion buffer, and then proceeding according to the manufacturer's protocols. For museum skin (toe pad) samples, genomic DNA was extracted from toe pad tissue digested overnight as described for feathers above, with additional washes of 500 μl Salton Wash 1 and Salton Wash 2 (Qbiogene).

All museum toe pad extractions and PCR preparations were conducted in a facility exclusively designated for old/degraded DNA samples at the University of Michigan Museum of Zoology and the Ancient Biomolecules Centre (ABC) at Oxford University. To prevent contamination, no contemporary samples or PCR products are permitted in either facility (Cooper and Poinar, 2000).

We sequenced 1047 bases of mitochondrial NADH dehydrogenase subunit 2 (ND2), 1041 bases of mitochondrial cytochrome-*b* (cyt-*b*) and 1074 bases of nuclear β-fibrinogen intron 7 (BF-I7) in segments of ~250 to 1080 bases in length. Primers used are described in Table 3. PCR products were visualized on a 1% low melting point agarose gel stained with ethidium bromide and gel extracted with a Gel Purification kit (Qiagen). Sequencing was performed on an ABI Model 3730 sequencer. Resulting chromatographs for both strands of DNA were resolved in Sequencher version 4.1.

We took standard precautions against inadvertent amplification of nuclear copies of mitochondrial genes (see Arctander, 1995; Mindell et al., 1997; Sorenson and Fleischer, 1996). In cases where double peaks on chromatographs identified potential multiple copies for ND2, we cloned the PCR products using a TOPO TA Cloning kit (Invitrogen), and sequenced 5 clones to identify separate DNA sequences. Two sequences of identical length lacking internal stop codons were found in multiple clones of the *Morphnus guianensis* PCR product. Both clones were included in the analyses.

Table 2
List of taxa and samples used for dna sequencing

Order Family Subfamily	Species	Locality	Source[a] and voucher #[b]	Tissue ID	GenBank Accession Nos.		
					cyt-*b*	ND2	BF-I7
Falconiformes							
Falconidae	*Falco peregrinus*		Genbank		AF090338	AF090338	
	Falco longipennis	Australia	AM-EBU	10665	AY987229	AY987048	AY987159
	Phalcoboenus megalopterus	South Africa	Captive, WOB, P	WOB-3	AY987230	AY987049	AY987160
Sagiitaridae	*Sagittarius serpentarius*	South Africa	Captive, JBZ, P	JBZ-12	AY987231	AY987050	AY987161
Pandionidae	*Pandion haliaetus*	Michigan	UMMZ 225997	T-264	AY987232	AY987051	AY987162
Accipitridae Elaninae	*Elanus leucurus*	USA	LSUMNS	24997	AY987233	AY987052	AY987163
Polyboroidinae	*Polyboroides typus*	Gambia, Africa	UMMZ 235187	T-1423	AY987234	AY987053	AY987164
Gypaetinae	*Neophron percnopterus*		DWC, Captive, P	DWC-1	AY987235	AY987054	AY987165
	Gypohierax angolensis	Gambia, Africa	UMMZ 235794	A-1232	AY987236	AY987055	AY987166
	Gypaetus barbatus		Captive, SDZ	209	AY987237	AY987056	
	Eutriorchis astur	Madagascar	TPF, Wild, P	123	AY987238	AY987057	
Perninae	*Chondrohierax uncinatus*	Grenada	TPF, Wild, P	147	AY987239	AY987058	AY987167
	Leptodon cayanensis	Paraguay	KUNHM	139	AY987240	AY987059	AY987168
	Elanoides forficatus	Ecuador	LSUMNS	B-12133	AY987241	AY987060	AY987169
	Pernis apivorus		TAU	17	AY987242	AY987061	
	Hamirostra melanosternon	Australia	AM-EBU	1	AY987243	AY987062	AY987170
	Lophoictinia isura	Australia	AM-EBU	0.7591	AY987244	AY987063	
Circaetinae	*Pithecophaga jefferyi*	Philippines	TPEF, captive	227	AY987246	AY987064	AY987171
	Pithecophaga jefferyi	Philippines	TPEF, captive	205	AY987245	AY987065	AY987172
	Pithecophaga jefferyi	Mindanao, Philippines	AMNH 534856	n/a	*	*	
	Terathopius ecaudatus	South Africa	UBP, Captive	UMG-3	AY987248	AY987066	AY987173
	Spilornis elgini	S. Andamens, India	NHM-UK 1885.8.19.1626	n/a	AY987249	AY987067	
	Spilornis holospilus	Mount Calavite, Occ. Mindoro, Philippines	AMNH 784054	n/a	AY987250	AY987068	
	Spilornis cheela burmanicus	Cherrapunji, India	UMMZ 140566	n/a	AY987251	AY987069	
	Spilornis rufipectus	S. Celebes, India	AMNH 536566	n/a	AY987252	AY987070	
	Circaetus pectoralis	South Africa	Captive, PBC, P	PBC-3	AY987253	AY987071	AY987174
	Circaetus gallicus		TAU	363	AY987254	AY987072	AY987175
	Circaetus cinereus	South Africa	Captive, PNZ, P	PNZ-8	AY987255	AY987073	AY987176
	Dryotriorchis spectabilis	Eastern Congo Forest, Africa	AMNH 448333	n/a	AY987256	AY987074	
	Circaetus fasciolatus	South Africa	WOB, Captive, P	WOB-3	AY987257	AY987075	AY987177
	Circaetus cinerascens	Karonga, Nyasaland	NHM-UK 1948.26.1	n/a	AY987258	AY987076	
Aegypiinae	*Necrosyrtes monachus*	Gambia	UMMZ	A1234	AY987259	AY987077	AY987178
	Gyps bengalensis		TPF	138	AY987260	AY987078	AY987179
	Gyps rueppellii	Gambia	UMMZ	A1119	AY987248	AY987079	AY987180
	Gyps fulvus	Gambia	UMMZ 235890	B19181	AY987261	AY987080	
	Gyps coprotheres	South Africa	DWC, Captive, P	DWC-10	AY987262	AY987081	AY987181
	Gyps africanus		TAU	21	AY987263	AY987082	AY987182
	Sarcogyps calvus	South Africa	DWC, Captive, P	DWC-20	AY987264	AY987083	AY987183
	Trigonoceps occipitalis	Senegal	UMMZ 130316	n/a	GI 1050710	AY987084	
	Aegypius monachus		DZ, Captive, P	1903	AY987266	AY987085	AY987184
	Torgos tracheliotus	South Africa	UMMZ 234705	T-2046	AY987267	AY987086	AY987185

(*continued on next page*)

Table 2 (*continued*)

Order Family Subfamily	Species	Locality	Source[a] and voucher #[b]	Tissue ID	GenBank Accession Nos.		
					cyt-*b*	ND2	BF-I7
Harpiinae	*Harpyopsis novaeguineae*	New Guinea, Southern Highlands Province, Piambil Village, Mt.Giluue	UMMZ	238858	AY987268	AY987087	AY987186
	Morphnus guianensis	Peru	HUA, Captive, P	HUA-19	AY987269	AY987088, AY987089	AY987187
	Harpia harpyja	Colombia	Captive, SDZ	402158	AY987270	AY987090	AY987188
Aquilinae	*Spizaetus lanceolatus*	Celebes, Indonesia	NHM-UK 1887.11.1.337	n/a	AY987271	AY987091	
	Spizaetus cirrhatus lineatus	Bamanigaon, Assam, India	UMMZ 140516	n/a	AY987272	AY987092	
	Spizaetus nanus	Lambuk River, Central North Brunei	NHM-UK 1956.60.11	n/a	AY987273	AY987093	
	Spizaetus nipalensis		NBPC, Captive, P	208	AY987274	AY987094	AY987189
	Spizaetus alboniger	Gomantong, North Brunei	NHM-UK 1956.60.9	n/a	AY987275	AY987095	
	Spizaetus tyrannus	Peru	HUA, Captive, P	HUA-25	AY987276	AY987096	AY987190
	Spizastur melanoleucus	Peru	HUA, Captive, P	HUA-28	AY987277	AY987097	AY987191
	Spizaetus ornatus	Darien Province, Panama	LSUMNS	B2267	AY987278	AY987098	AY987192
	Oroaetus isidori	Peru	HUA, Captive, P	HUA-23	AY987279	AY987099	AY987193
	Stephanoaetus coronatus	South Africa	PBC, Captive, P	PBC-9	AY987280	AY987100	
	Hieraaetus kienerii		NHM-UK 1877.85.8.19.1331	n/a	AY754054	AY987101	
	Polemaetus bellicosus	South Africa	EES, Captive, P	EES-1	AY987281	AY987102	AY987195
	Lophaetus occipitalis	South Africa	PBC, Captive, P	PBC-15	AY987282	AY987103	AY987196
	Aquila rapax	South Africa	PBC, Captive, P	PBC-17	AY987283	AY987104	AY987197
	Aquila clanga	Pakistan	UMMZ 78284	n/a	AY987284	AY987105	
	Aquila heliaca		TAU	356	AY987285	AY987106	AY987198
	Aquila pomarina hastata	Bhadwar, India, Himachal Pradesh	UMMZ 78282	n/a	AY987286	AY987107	
	Aquila nipalensis		LSUMNS	B-26977	AY987287	AY987108	AY987199
	Ictinaetus malayensis malayensis		NHM-UK 1932.12.21.-35	n/a	AY754056	AY987109	
	Hieraaetus pennatus	South Africa	WOB, Captive, P	WOB-5	AY987288	AY987110	AY987200
	Hieraaetus pennatus	Punjab, India	UMMZ 75313		AY987289	AY987111	
	Hieraaetus morphnoides morphnoides		UMMZ	T-2796	AY987290	AY987112	AY987201
	Hieraaetus morphnoides morphnoides	Australia	NHM-UK 1969.4.22	n/a	AY754044	*	
	Hieraaetus morphnoides weiskei	New Guinea	AMNH 535061	n/a	AY987291	AY987113	
	Hieraaetus morphnoides weiskei	New Guinea	NHM-UK 1913.3.6.35	n/a	AY754045	*	
	Hieraaetus ayresii	Uganda, Africa	UMMZ 535074	n/a	AY987292	AY987114	
	Aquila wahlbergi		DWC, Captive, P	DWC-21	AY987293	AY987115	AY987202
	Aquila chrysaetos	N. America	UMMZ	238855	AY987294	AY987116	AY987203
	Spizaetus africanus	Liberia	NHM-UK 1977.20.43	n/a	AY987295	AY987117	
	Hieraaetus fasciatus fasciatus	Red Sea, Egypt	UMMZ 224053	n/a	AY987296	AY987118	
	Hieraaetus fasciatus fasciatus	Bhadwar, India	UMMZ 78295	n/a	AY987297	AY987119	
	Hieraaetus fasciatus fasciatus	Parwali, India	UMMZ 78294	n/a	AY987298	AY987120	
	Hieraaetus fasciatus spilogaster	Zimbabwe	TPF, Wild	WOB-13	AY987300	AY987122	AY987205
	Hieraaetus fasciatus spilogaster	South Africa	EES, Captive, P	EES-3	AY987299	AY987121	AY987204
	Hieraaetus fasciatus spilogaster	South Africa	EES, Captive, P	WOB-13	*	*	
	Aquila verreauxii	South Africa	PBC, Captive, P	PBC-8	AY987301	AY987123	AY987206
	Aquila audax	Moomba, South Australia	SAM, SAMAB48364	ABTC-02866	AY987302	AY987124	

	Aquila gurneyi	Halmahera, Indonesia	NHM-UK 1873.5.9.8	n/a	AY987303	AY987125	
Melieraxinae	*Melierax gabar*	Zimbabwe	UMMZ	A765	AY987304	AY987126	AY987207
Circinae	*Circus aeruginosus*		TAU	353	AY987305	AY987127	AY987208
	Circus ranivorus	South Africa	Captive, PBC-6, P	PBC-6	AY987306	AY987128	AY987209
Accipitrinae	*Accipiter bicolor*	Santa Cruz Dept., Bolivia	LSUMNS	B-18875	AY987307	AY987129	AY987210
	Accipiter cooperii	USA	KUNHM	1757	AY987308	AY987130	AY987211
Milvinae	*Haliastur indus girenera*	Brunswick Heads, Australia	AM-EBU, 064910	EBU 11377	AY987309	AY987131	AY987212
	Haliastur sphenurus	Gregory, Northern Territory, Australia	SAM, NTMT651	ABTC-27746	AY987310	AY987132	AY987213
	Milvus migrans parasitus	Cameroon, Africa	AMNH 388140	n/a	AY987311	AY987133	
	Milvus milvus	Rome, Italy	AMNH 531856	n/a	AY987312	AY987134	
Haliaeetinae	*Haliaeetus leucoryphus*	Palasbari, India	UMMZ 142065	n/a	GI 1781269	AY987135	
	Haliaeetus pelagicus		NBPC, Captive, P	JPJ MB 26	AY987314	AY987136	
	Haliaeetus albicilla		TAU	22	AY987315	AY987137	AY987214
	Haliaeetus leucocephalus	N. America	UMRC	N42	AY987316	AY987138	AY987215
	Ichthyophaga humilis	Bhadwar, India	UMMZ 78356	n/a	AY987317	AY987139	
	Ichthyophaga ichthyaetus	Palasbari, India	UMMZ 140540	n/a	AY987318	AY987140	
	Haliaeetus vocifer	Durban, South Africa	UMMZ	A1075	AY987319	AY987141	AY987216
	Haliaeetus vociferoides	Madagascar	R. Tingay	MFE 60 0051	AY987320	AY987142	AY987217
	Haliaeetus leucogaster	Lincoln, South Australia	SAM, SAMAB48773	ABTC 03064	AY987321	AY987143	AY987218
	Haliaeetus sanfordi	Solomon Islands	UMMZ 112326	n/a	GI 1781273	AY987144	AY987216
Buteoninae	*Ictinia plumbea*	Paraguay	KUNHM	2900	AY987322	AY987145	AY987219
	Geranospiza caerulescens	Paraguay	KUNHM	3110	AY987323	AY987146	AY987220
	Rostrhamus sociabilis	Guyana	KUNHM	5852	AY987324	AY987147	AY987221
	Buteogallus urubitinga	Paraguay	UMMZ 227470	SMG 2546	AY987325	AY987148	AY987222
	Buteogallus anthracinus	Panama	LSUMNS	B-28575	AY987326	AY987149	AY987223
	Harpyhaliaetus coronatus	Capitan Bado, Paraguay	UMMZ 101669	n/a	AY987327	AY987150	
	Harpyhaliaetus solitarius	Peru	HUA, Captive, P	HUA-18	AY987328	AY987151	AY987224
	Buteo magnirostris	Loreto Dept., Peru	LSUMNS	B-2862	AY987329	AY987152	AY987225
	Parabuteo unicinctus	Arizona, USA	UMMZ	T-1039	AY987330	AY987153	
	Geranoaetus melanoleucus	Peru	HUA, Captive, P	HUA-03	AY987331	AY987154	AY987226
	Leucopternis albicollis	Tigre Playa, Sucumbios, Ecuador	ZMUC 114919	n/a	AY987332	AY987155	AY987227
	Buteo buteo		Genbank		NP_387496	NP_387486	
	Buteo jamaicensis	N. America	UMMZ	T-2797	AY987334	AY987156	
	Leucopternis kuhli	Loreto Dept., Peru	LSUMNS	B-4598	AY987335	AY987157	
	Leucopternis melanops	Loreto Dept., Peru	LSUMNS	B-7167	AY987336	AY987158	AY987228

[a] AM-EBU, Australian Museum Evolutionary Biology Unit, Sydney, Australia; AMNH, American Museum of Natural History; CRH, Center for Rehabilitation of Wildlife, South Africa; DWC, De Wildt Cheetah and Wildlife Reserve, Pretoria, South Africa; DZ, Detroit Zoo, Detroit, MI; EES, Eagle Encounters at Spier, Stellenbasch, South Africa; HUA, El Huayco, Peru; JBZ, Johannesburg Zoo, South Africa; KUNHM, Kansas University Natural History Museum; LSUMNS, Louisiana State University Museum of Natural Science; NBPC, National Birds of Prey Centre, Newent, England; NHM-UK, The Natural History Museum, Tring, United Kingdom; PBC, Predatory Bird Centre, South Africa; SAM, South Australia Museum, Adelaide, Australia; SDZ, San Diego Zoo, CA; TAU, Tel Aviv University Research Zoo, Israel; TPEF, The Philippine Eagle Foundation, The Philippines; TPF, The Peregrine Fund; UBP, Umgeni Bird Park, South Africa; UMMZ, University of Michigan Museum of Zoology, Ann Arbor, MI; UMRC, University of Minnesota Raptor Center, MN; WOB, World of Birds, Cape Town, South Africa; ZMUC, Zoological Museum, University of Copenhagen.

[b] When a live bird was sampled, a photo was taken at the time of sample collection. Availability of a photo is signified by the letter "P." When a museum skin was sampled only the voucher number is given, no tissue number is given.

[*] These individuals were sequenced for confirmation purposes only, they were not used in the analysis and, therefore, were not submitted to GenBank.

Table 3
Primers used to amplify mitochondrial and nuclear gene regions

Gene and target group	Primer name	Sequence (5′–3′) or reference
ND2 all species	L5143, L5219, H5766, L5758, H6313	Sorenson et al. (1999)
ND2 booted eagle museum skins	H5501.eagle	TGA TAT YTC ATT GGC CDG TRG
	L5367.eagle	CAA CAC BCT YGC YAT CAT CC
	L5418.eagle	CAT YGA RGC YAC WAT CAA RT
	H5755.eagle	ABT TTT CGR AGT TGB GTT TG
	H6044.eagle	TGG ATR AYR AGY CAT TTR GGT A
	L5700.eagle	YCA CTC VCT YAA YCC DAC AYT
	L5971.eagle	TCH CCH HCA CTA AAY GCA AC
ND2 snake eagle museum skins	H5592.snake	TCT GGG AAT CAG AAG TGR AAG
	L5513.snake	GRG AYA TYA CCC AAC TAAC C
	H5906.snake	GGT GAG TTT RGG RYT GTA GA
	L5768.snake	GRT GAA TRG GCC TAA ACC AAA
	H6133.snake	GCG AGR CGG AGG TAG AAG AA
	L6001.snake	GTC CTA CTY TCY CTA GCA GGR CTC
ND2 sea eagle museum skins	H299.cvk	Johnson et al. (2005)
	L247.cvk	Johnson et al. (2005)
	H852	Johnson et al. (2005)
	L768.cvk	Johnson et al. (2005)
Cyt-*b* all species	L14996, H15646, L15560, H16064	Sorenson et al. (1999)
Cyt-*b* booted eagle museum skins	H15334.eagle	GAC TGT DGY CCT CAR AAR G
	L15244.eagle	YAA RGA RAC CTG AAA YACA GGA
	H15588.eagle	TCC YAR RRT RTC TTT TAR GGA GAA
	L15515.eagle	CYT DCA CGA RTC HGG VTC HA
	H15851.eagle	CGR AAD GTT ATT GTD CGY TG
	L15739.eagle	CCT ATT YGC ATA YGC BAT YC
	eagle-cyt-*b*-1f	Bunce et al. (2005)
	eagle-cyt-*b*-3r	Bunce et al. (2005)
Cyt-*b* snake eagle museum skins	H15310.snake	TTG GCC TCA TGG YAG GAC RT
	L15519.snake	CAC GAA WCH RGC TCA AAC AA
	H15587.snake	CCT AGR ATR TCT TTT ARR GAG AA
	H16020.snake	TTC TAG YGC YCC RGY TAG
Cyt-*b* sea eagle museum skins	H15332.cvk	Johnson et al. (2005)
	L15279.cvk	Johnson et al. (2005)
	L15560.cvk	Johnson et al. (2005)
	H15828.cvk	Johnson et al. (2005)
	L15748.cvk	Johnson et al. (2005)

2.3. Dataset construction and analyses

Sequences were aligned in BioEdit v. 7.0.0 (Hall, 1999) by eye. Cyt-*b* did not contain indels and the indels found in ND2 and BF-I7 were easily resolved. Nine vulture species had an insertion of two amino acids immediately preceding the stop codon of ND2 (*Aegypius monachus*, *Torgos tracheliotus*, *Gyps africanus*, *G. bengalensis*, *G. coprotheres*, *G. fulvus*, *G. rueppellii*, *Sarcogyps calvus*, and *Trigonoceps occipitalis*). In BF-I7 the two *Haliastur* species shared an insertion of one base and the two *Accipiter* species shared an insertion of one base and a separate insertion two bases in length. An insertion of 12 bases was found in three *Falco* species (*Falco longipennis*, *F. subniger*, and *F. biarmicus*, though the last species was not included in further analyses) and 11 separate insertions were autapomorphic. The two *Buteogallus* species and *Geranospiza caerulescens* share a deletion of two bases; the two *Buteogallus* species and *Harpyhaliaetus solitarius* share another separate deletion of two bases; the three *Falco* species share a deletion of one base; the two *Harpyhaliaetus* species share a deletion of two bases; *Harpia harpyja*, *Harpyopsis novaeguineae* and *Morphnus guianensis* share a deletion of eight bases; and, *Harpyhaliaetus solitarius*, *Leptodon cayanensis*, and *Rostrhamus sociabilis* share a deletion of two bases.

Phylogenetic analyses were first performed on the individual genes to assess congruence of the phylogenetic signal among genes. Then the data were combined into two data sets for final analyses: one dataset includes 2088 bases of ND2 and cyt-*b* for 113 taxa and is referred to hereafter as the "mt dataset;" the other dataset includes the 2088 bases of mitochondrial data from the

first dataset, plus an additional 1074 bases of nuclear data (BF-I7) for 71 taxa and is hereafter referred to as the "nuc + mt dataset."

Homoplasy and heterogeneity of base composition are two factors that, if not addressed in the phylogenetic model, may confound analyses. We tested our data for saturation at each codon position as a measure of homoplasy. Saturation plots (not shown) were constructed for each gene from the data matrix produced in DAMBE version 4.213 (Xia, 2000) using Tamura-Nei genetic distance and pairwise numbers of transitions and transversions. Saturation of codon position three in both ND2 and cyt-*b* was observed. Codon positions one and two did not show significant saturation in either mitochondrial gene. All base positions were analyzed together for BF-I7 as it does not encode protein, and no evidence of saturation was identified for BF-I7. We also tested for skewness in base composition as implemented in PAUP* 4.0b10 (Swofford, 2004) and found no significant departure from homogeneous base composition in both the mt and nuc + mt datasets.

To reconstruct phylogenies, we used both maximum parsimony (MP) as implemented in PAUP* 4.0b10 (Swofford, 2004) and Bayesian inference using Markov chain Monte Carlo in the program MrBayes 3.01 (Huelsenbeck and Ronquist, 2001). MP analyses were heuristic with starting trees obtained by random addition of taxa with 100 replicate searches and TBR branch swapping. Successive analyses were done with all characters equally weighted, with a transition:transversion ratio of 5:1 for mitochondrial data and 2:1 for the nuclear data. These values were obtained by estimating the transition:transversion ratios from the alignments and from preliminary trees. The data were resampled using 500 bootstrap replicates to determine support at each node.

Models of evolution for parameter estimation and likelihood analysis were determined using the hierarchical log-likelihood ratio tests in the programs MrModelTest (Nylander, 2002) and DT ModSel (Minin et al., 2003). The simplest best-fit model for the two mitochondrial genes (analyzed separately) was GTR + I + G. Therefore, the mt dataset was not partitioned by gene as the model selected independently for both genes was the same. Third codon positions were unlinked from first and second positions to minimize the effect of saturation. We ran four Markov Chains in the program MrBayes for six million generations (mt dataset only), sampling every 500 generations for each dataset.

For the nuclear sequences DTModSel and MrModelTest both identified the GTR + G as the simplest best-fit model. The combined nuc + mt dataset was partitioned for Bayesian analyses so that the best-fit models were applied separately to the mitochondrial and nuclear data, and mitochondrial codon positions were all unlinked from each other. We ran four Markov Chains for four million generations, sampling every 500 generations.

Analyses of both datasets were performed independently three times from random starting points so that convergence of topology and log-likelihood scores could be evaluated. Parameter stationarity was visualized in the program Tracer (Rambaut and Drummond, 2003). All three Bayesian runs of the mt dataset reached stationarity in all substitution model parameters and likelihood scores prior to 400,000 generations and a slightly more conservative burn-in time of 600,000 generations was used. The three Bayesian runs of the nuc + mt dataset reached stationarity in all substitution model parameters and likelihood scores prior to 200,000 generations and a conservative burn-in time of 400,000 generations was used. The tree topologies produced from the three separate runs of each dataset were identical in topology, only varying slightly in support values for nodes (<0.02 difference among Bayesian posterior probabilities).

3. Results

3.1. Gene properties: sequence composition and divergence

We sequenced 1047 bases of ND2 and 1041 bases of cyt-*b* for 110 individuals representing 106–108 species and 1074 bases of BF-I7 for 68 of the same species. ND2 contained the most variable sites, the most parsimony informative sites, the highest transition–transversion ratio and had a higher maximum divergence among species as compared to BF-I7 and cyt-*b* (Table 4). BF-I7 had the lowest percent divergence among taxa and the lowest transition–transversion ratio of the three sequences. Cyt-*b* had the highest G–C content. Consistency and retention indices are reported for each gene although such measures are not predictive of the ability of the gene to infer the correct tree topology (Simmons et al., 2004).

3.2. Phylogenetic analyses

Two to three species of Falconidae were used as outgroup taxa. Some initial analyses were performed using two Musophagiformes as outgroups: *Crinifer piscator* and *Musophaga violacea.* However, this did not alter the results, and trees rooted with Falconidae species are shown here given the existing evidence for a close relationship between the Accipitridae and Falconidae (Mindell et al., 1997; Seibold and Helbig, 1995). We also included the secretarybird (*Sagittarius serpentarius*) and the osprey (*Pandion haliaetus*) to help reduce any long branches between accipitrids and the falconid outgroup.

Both datasets contained at least one representative of every major Accipitridae taxon or clade previously proposed. We used preserved museum skins where fresh tissue, blood or feathers was not available. DNA in museum skins is more degraded than in fresh tissue,

Table 4
Sequence composition and divergence

	Total bases	# Variable sites	% Variable sites	# Parsimony informative sites	% Parsimony informative sites	% G–C	Maximum % divergence between taxa	Minimum % divergence between taxa	Consistency index (combined nuc + mt dataset)	Retention index (combined nuc + mt dataset)	Ti:tv ratio
BF-I7	1035	499	48.2	223	21.5	37.74	26.6	0.20	0.8569	0.8812	1.98
Cyt-*b*	1039	526	50.6	473	45.5	53.83	34.6	5.0	0.303	0.591	5.15
ND2	1059	696	65.7	607	57.3	45.74	76.6	4.9	0.3432	0.5483	5.31

requiring amplification of at least two to four times the number of overlapping regions per gene. Nuclear DNAs are already at a lower concentration than mitochondrial DNAs in bird tissues, increasing the difficulty of amplification of nuclear sequences from museum skins. We attempted to amplify four regions of nuclear BF-I7 for five museum skins of which four amplifications of two regions were successful despite several attempts. Given the lower variability of BF-I7 and the increased amount of work and cost, we did not pursue nuclear sequence for all museum skins but instead focused on representing each major subgroup/subfamily of Accipitridae in both datasets and all species of eagles and vultures in the mt dataset. We also added taxa to our initial analyses to break up long branches among Perninae and Gypaetinae species. While the increased taxon sampling did serve to break up some of those long branches, the longest branches in both analyses, aside from the Elaninae and other families or outgroups, are still found in the early diverging Perninae clade.

Analyses using Bayesian inference of the two different datasets recovered identical tree topologies (Figs. 1 and 2). The tree topology in Fig. 1 was recovered by three independent Bayesian analyses of the mitochondrial dataset and the topology presented in Fig. 2 resulted from three independent Bayesian runs using the nuc + mt dataset. The average Bayesian posterior probability for each node, and bootstrap values for clades corresponding to those recovered in the parsimony analysis are shown on each tree.

The MP analysis of the mitochondrial dataset found three shortest trees, each 18,847 steps in length. There was one polytomy present in the final MP bootstrap tree (not shown). Resolved branching patterns followed the topology recovered in the Bayesian analyses with the following minor discrepancies. First, MP analysis was not able to resolve the branching pattern within the earliest diverging clade of kites and vultures beyond the sister relationships between *Chondrohierax uncinatus* and *Leptodon cayanensis*, *Gypaetus barbatus* and *Neophron percnopterus*, and *Hamirostra melanosternon* and *Lophoictinia isura*. A sister relationship between *Pithecophaga jefferyi* and *Terathopius ecaudatus* was recovered with very low bootstrap support (bs = 52) by MP. In the MP topology, *Necrosyrtes monachus* was not sister to the *Gyps* species, but formed the first branch splitting from the clade containing *Sarcogyps* (bs = 54). Finally, branching patterns within the Buteoninae clade including hawks (*Leucopternis*, *Geranoaetus*, *Buteo*, *Geranospiza*, and *Parabuteo*) and kites (*Ictinia* and *Rostrhamus*) differed slightly in the two analyses, however, both recovered a topology where the two *Harpyhaliaetus* species are nested within a clade of two *Buteogallus* species. The relationships within the Buteoninae aside from the *Harpyhaliaetus* species are not the focus of this paper and will not be addressed further here.

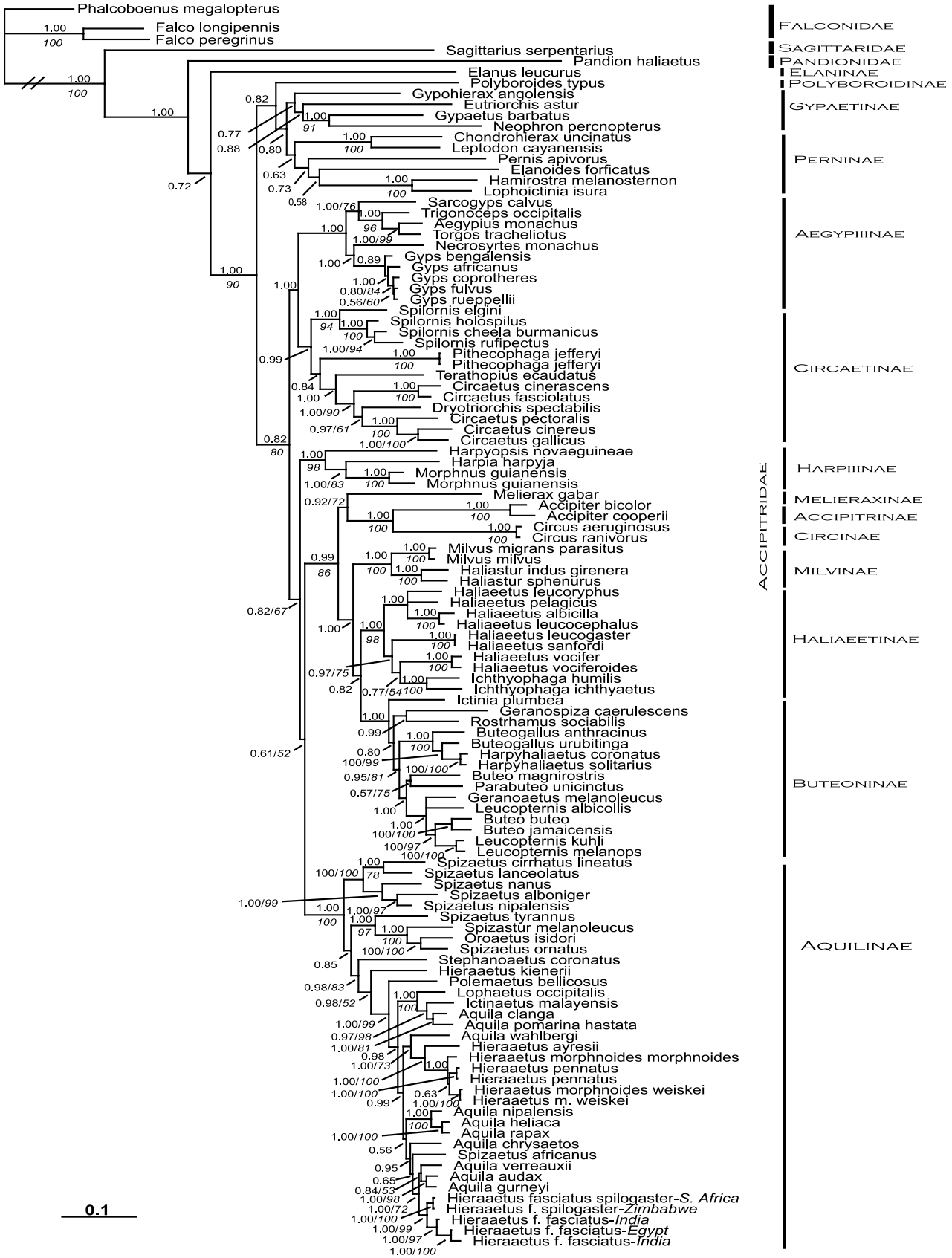

Fig. 1. Phylogeny for Accipitridae taxa inferred from mitochondrial cyt-*b* and ND2 sequences. Topology shown is the Bayesian inference majority rule tree (see text for details). Bayesian posterior probability values are shown above branches and MP bootstrap values (>50%) are shown in italics below the branches.

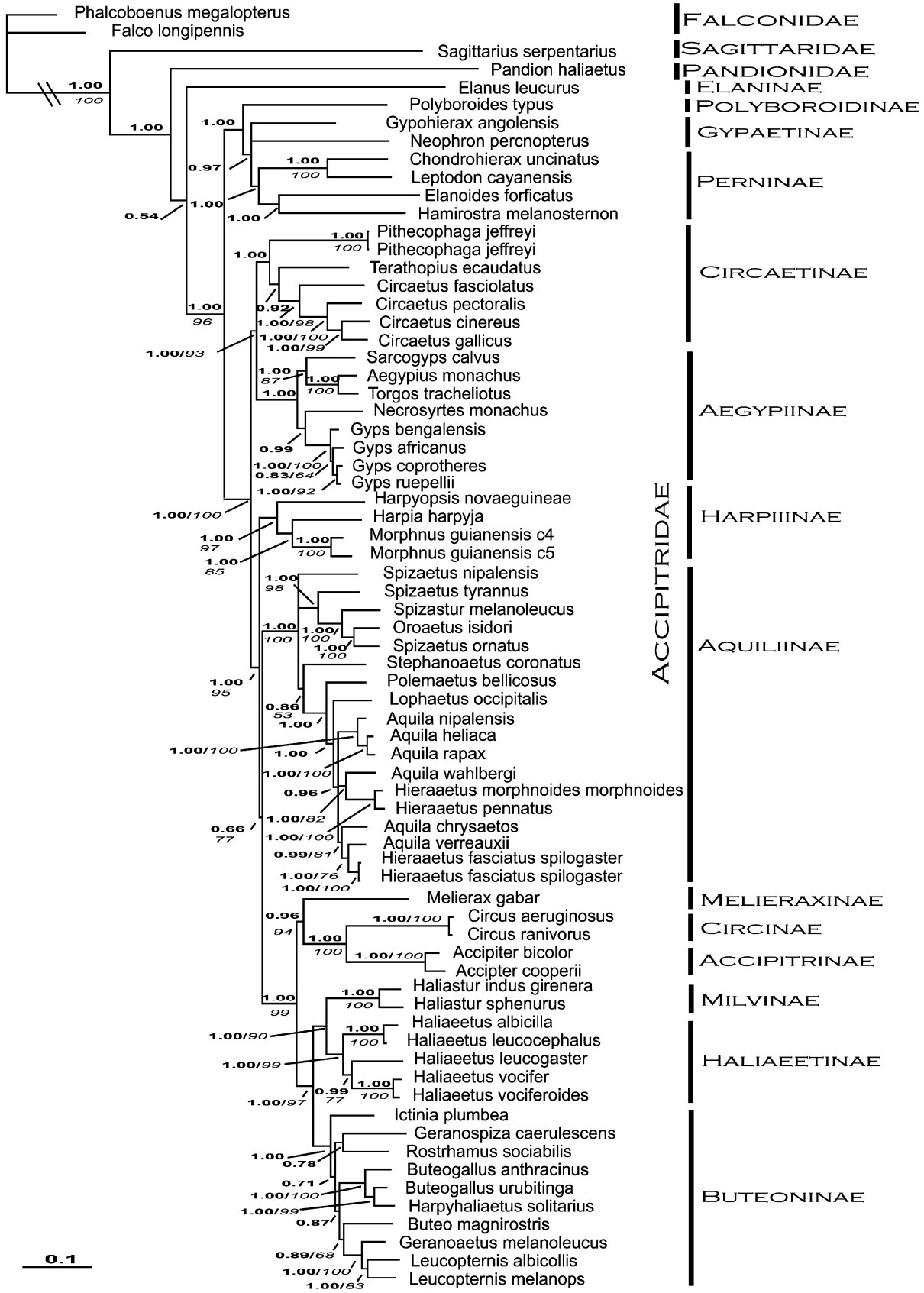

Fig. 2. Phylogeny of the Accipitridae inferred from mitochondrial cyt-*b* and ND2 and nuclear β-fibrinogen intron 7 sequences. Topology shown is the Bayesian inference majority rule tree (see text for details). Bayesian posterior probability values are shown above branches and MP bootstrap values (>50%) are shown in italics below branches.

The MP analysis of the nuc + mt dataset with all characters equally weighted, gaps as a 5th state and a transition–transversion ratio (reflecting their relative frequencies) of 5:1 for mitochondrial genes and 2:1 for BF-I7 found three best trees of length 15,306. Resolved branching patterns followed the topology recovered in the Bayesian analyses and bootstrap values are shown on the Bayesian consensus tree for resolved nodes (Fig. 2). As found in the MP analysis of the mt dataset, in the nuc + mt MP analysis relationships among species in the earliest diverging kite/vulture clade were unresolved, *Pithecophaga jefferyi* and *Terathopius ecaudatus* were sister with low support (bs = 55) and the position for *Necrosyrtes monachus* was unresolved.

3.3. Phylogeny of Accipitridae (Combined results from all datasets and analyses)

Both datasets and all analyses support monophyly for two of the four eagle groups: sea eagles (Haliaeetinae) and booted eagles (Aquilinae). Monophyly of the harpy eagle group (Harpiinae), the snake eagle group (Circaetinae) and the Old World vultures, however, is not supported in any of the analyses. Topologies within these groups are discussed in detail below. Where Bayesian posterior probabilities (PP) and parsimony bootstrap values (bs) are shown in the text, the value from the mitochondrial dataset is listed first followed by the value from the nuclear dataset when available.

Several other Accipitridae genera and subfamilies are also polyphyletic in our analyses. Three separate clades of kite species (Elaninae, Perninae, and Milvinae) proposed by morphological data were identified, however, the Milvinae and Perninae subfamilies are polyphyletic. Two other kite species (*Ictinia plumbea* and *Rostrhamus sociabilis*) were more closely related to buteonine taxa than to other kites and did not fall into any of the traditional kite subfamilies. *Polyboroides typus* and *Geranospiza caerulescens* were not closely related to each other. The genus *Buteo* was polyphyletic with the roadside hawk (*Buteo magnirostris*) not sister to two other *Buteo* species. The genus *Buteogallus* was also polyphyletic, with two *Harpyhaliaetus* species nested within the genus.

3.3.1. Booted eagles (Aquilinae)

While the large booted eagle subfamily forms a well-supported monophyletic group with high Bayesian posterior probability (PP = 1.00, 1.00) and high bootstrap values (bs = 100, 100) with respect to other Accipitridae groups in all analyses, three genera within this group are not monophyletic: *Spizaetus*, *Aquila*, and *Hieraaetus*. Forcing monophyly of the genus *Spizaetus* in the mt dataset adds 226 parsimony steps to the shortest tree of length 18,847 (all such topological constraints in the following text refer to the MP analysis of the mt dataset). Members of the genus *Aquila* are found in three of six main clades in the booted eagle group. To force monophyly of the genus *Aquila* an additional 103 parsimony steps are needed. Species in the genus *Hieraaetus* are placed in the two latest diverging clades of booted eagles and one species forms a separate early diverging clade by itself. Forcing monophyly of the genus *Hieraaetus* requires 84 additional parsimony steps.

Hieraaetus f. fasciatus and *H. f. spilogaster* have been treated variously as separate species (Ferguson-Lees and Christie, 2001; Thiollay, 1994), a superspecies (Stresemann and Amadon, 1979) or subspecies (Sinclair et al., 2002). Here, we sampled two *H. f. fasciatus* individuals from India, one *H. f. spilogaster* individual from Egypt, two *H. f. spilogaster* individuals from South Africa, and one *H. f. spilogaster* from Zimbabwe. The three Indian and Egyptian samples shared identical cyt-*b* sequence except for one Indian sample at one base (sequence identity = 98.9%). The three *H. f. spilogaster* individuals from South Africa and Zimbabwe were different from the three other samples at 16 base positions in cyt-*b* (sequence identity = 90.2%) and another 18 bases in ND2 (sequence identity = 93.2%).

Two individuals of each *Hieraaetus morphnoides* subspecies were sampled: *H. m. morphnoides* and *H. m. weiskei*. Additionally, two *H. pennatus* individuals from disparate locales were sequenced. All *H. pennatus* individuals shared identical ND2 and cyt-*b* sequences. *H. m. weiskei* samples also had identical mitochondrial sequence to each other, but differed from the *H. m. morphnoides* sequences. Parsimony and Bayesian analyses show that *H. m. weiskei* is most closely related to *H. pennatus* with weak support (sequence identity for cyt-*b* = 97.8%, for ND2 = 98.1%; PP = 0.63, bs = 95). *H. m. morphnoides* and *H. m. weiskei* are slightly more divergent: sequence identity for cyt-*b* is 97.3% and for ND2 is 94.2%. Bayesian posterior probability is 1.00 and the bootstrap support is 100 for the node separating *H. m. morphnoides* from *H. m. weiskei* and *H. pennatus*.

3.3.2. Sea eagles (Haliaeetinae)

The sea eagles form a well-supported monophyletic group in the mt dataset consisting of two genera: *Haliaeetus* and *Ichthyophaga*. In this analysis the genus *Haliaeetus* is paraphyletic when the two *Ichthyophaga* species are included. Forcing monophyly of the genus *Haliaeetus*, requires 7 additional steps.

Analyses of the nuc + mt data support a sister relationship between the sea eagles and kites in the genus *Haliastur* (PP = 1.00, bs = 90), however, a sister relationship between the Milvinae and Haliaeetinae was not recovered with the mt dataset.

3.3.3. Harpy eagles (Harpiinae)

Three of four proposed harpy eagles form a clade with high support (PP = 1.00, 1.00, bs = 95, 97): *Harpia*

harpyja, *Morphnus guianensis*, and *Harpyopsis novaeguineae*. These three species are highly similar in sequence (~91% identical). A fourth species typically included in the Harpy eagle group, the Philippine eagle (*Pithecophaga jefferyi*), is sister to a clade of snake eagles (Circaetinae) which is distant from and earlier diverging than the three species found here to belong to the Harpiinae. The support values for these relationships are high in all analyses. We are unaware of any other analysis suggesting a relationship between the Philippine eagle and the Circaetinae. Given this exceptional result in our dataset, we took extra measures to confirm the validity of the sequence and its phylogenetic placement. We sequenced two individual Philippine eagles for all three genes and portions of all three genes for a third individual. All sequences obtained were identical for the three individuals, and uniquely different from other species in the dataset. This novel finding is also corroborated by the distribution of indels noted previously. In particular, the Philippine eagle lacks an eight base deletion in BF-I7 that is shared by the three other traditional members of the harpy eagle group (lack of monophyly for the harpy eagle group species is consistent whether gaps are counted as missing data or as a 5th base state in MP analyses). Forcing monophyly of the traditional harpy eagle group (4 members) would require an additional 51 parsimony steps.

It was proposed that the two species in the genus *Harpyhaliaetus* are members of the harpy eagle group or are closely related (Brown, 1970). In our analyses *Harpyhaliaetus solitarius* and *Harpyhaliaetus coronatus* are placed within the Buteoninae and, more specifically, within a clade of two *Buteogallus* species. Neither *Harpyhaliaetus* species shares the eight base deletion in BF-I7 found in three members of the harpy eagle group. Forcing monophyly of the harpy eagle group including all six potential members increases the tree length by 292 steps.

3.3.4. Circaetinae (snake eagles)

All of the snake eagles form a monophyletic group sister to the Old World vulture group Aegypiinae, except the Madagascar serpent-eagle (*Eutriorchis astur*) which is placed within the Gypaetinae. Forcing monophyly of all snake eagles requires an additional 168 parsimony steps.

The genus *Circaetus* is not monophyletic in these analyses when the West African serpent-eagle (*Dryotriorchis spectabilis*) is included. An additional 7 parsimony steps are needed to force monophyly of the genus *Circaetus*.

The Bayesian posterior probability for the node uniting the Philippine eagle and the African snake eagles (rather than the non-African snake eagles) was high (PP = 0.84, 1.00). The MP analyses recovered a sister relationship between the Philippine eagle and the bateleur albeit with low support (bs = 52, 55).

3.3.5. Old World vultures (Aegypiinae and Gypaetinae)

The Old World vultures also do not form a monophyletic group, but form two separate clades in the analyses (Aegypiinae and Gypaetinae). Each of the Gypaetinae species (*Gypohierax angolensis*, *Eutriorchis astur*, *Neophron percnopterus*, and *Gypaetus barbatus*) are highly divergent from each other genetically (sequence identities ~87%) but are more closely related to each other than to other Accipitridae species (PP = 0.80). Here, we also find that the Madagascar snake eagle is a member of the Gypaetinae, a relationship not proposed before. All remaining Old World vultures form a separate clade (Aegypinae) with a close relationship to other snake eagles (Circaetinae). The relationships within this clade of vultures, the Aegypiinae, are highly concordant in all analyses except the position of *Necrosyrtes monachus*. This species is more closely related to, although highly divergent from, the species of the genus *Gyps* than to the other four monotypic Aegypiinae genera with high support in the Bayesian analyses (PP = 1.00, 0.99), but is sister to the other Aegypiinae taxa in the parsimony analyses (bs = 0.54, unresolved in the nuc + mt dataset). *Necrosyrtes* also lacks the ND2 insertion of two bases that all other Aegypiinae species share.

4. Discussion

We have presented data from both mitochondrial and nuclear sequences for approximately 50% of the recognized species in the Accipitridae, focusing on groups commonly known as eagles and Old World vultures with nearly complete species representation. This is the most complete systematic treatment of the Accipitridae family to date based on molecular data. We found strong evidence for non-monophyly of some existing genera and subfamilies. Although Accipitridae subfamilies are infrequently used in recent classifications we agree with Brown (1976) that subfamilies are useful in clarifying relationships among these diverse birds. Designation of subfamilies is not our primary goal; however, we use and reconfigure the 12 existing subfamilies and recognize two new subfamilies in an effort to make the evolutionary history of the Accipitridae more easily understood (Table 1). Our analyses included representatives of all 14 primary Accipitridae clades that have been recognized by previous researchers. In the following section, we discuss the taxonomic history of the focal subfamilies and several examples of convergences, generally involving traits related to feeding habits, revealed by findings of non-monophyly for traditional taxa.

4.1. Booted eagles (Aquilinae)

We found good support for monophyly of the booted eagles (Figs. 1 and 2), corroborating earlier

morphological assessments. Proposed phylogenetic relationships and taxonomy within the booted eagles, however, have a long history of confusion and revision among authors. Our analyses confirm that the three main genera (*Aquila*, *Hieraaetus*, and *Spizaetus*) are not monophyletic, a result suspected by many morphologists but that has been difficult to resolve with morphological traits.

Our data support an early diverging clade of Asian hawk-eagles (*Spizaetus* species) separate from the New World hawk-eagles (*Spizaetus* spp., *Oroaetus* sp., and *Spizastur* sp.). Brown and Amadon (1968) recognized that the Asian *Spizaetus* species are more similar to each other morphologically than to the other *Spizaetus* species, but did not separate the genus accordingly. Within the Asian hawk-eagle clade we find support for sister relationships between *S. cirrhatus* and *S. lanceolatus*, and *S. alboniger* and *S. nipalensis*. Within each pairing, the two species have largely overlapping ranges and are similar morphologically (Ferguson-Lees and Christie, 2001).

The New World hawk-eagles comprise three genera (including *Spizaetus*) and are all each others closest relatives, forming a separate clade within the booted eagles that is not sister to the Old World hawk-eagles. These species have largely overlapping ranges within the New World but are found in vastly different habitat types ranging from open areas (*Spizaetus tyrannus*) to heavily forested regions at higher altitude (*Oroaetus isidori*).

Three Old World species each branch off separately within the Aquilinae and are shown not to have any close relationships with other species: the crowned hawk-eagle (*Stephanoaetus coronatus*), the rufous-bellied eagle (*Hieraaetus kienerii*), and the Martial eagle (*Polemaetus bellicosus*). Both the crowned hawk-eagle and the martial eagle have been placed in monotypic genera because of their divergent morphology. Genetically these birds are also highly divergent from other booted eagles in our dataset. Recently, the rufous-bellied eagle was recognized as a member of the genus *Hieraaetus* (Dickinson, 2003), although monophyly of the genus *Hieraaetus* has been questioned (Brown and Amadon, 1968). The rufous-bellied eagle is a morphologically specialized bird having long toes, a crest, and adult plumage that is dissimilar from the other booted eagles. Here, it is shown that it is genetically distant from other extant booted eagles, and phylogenetically distinct from its current congeners in *Hieraaetus*.

The well-supported clade including the Asian black eagle (*Ictinaetus malayensis*), the long-crested eagle (*Lophaetus occipitalis*) of Africa and two species in the genus *Aquila* has not been proposed before. The species of these two monotypic genera are highly unique in morphology. The long-crested eagle is an African woodland species found in moist savannahs and riverine strips feeding on rodents, while the Asian black eagle is a resident of mountain woodlands with morphological traits that accompany its feeding specialization on bird's eggs and young. The other two species in this clade, the lesser spotted eagle (*Aquila pomarina*) and the greater spotted eagle (*Aquila clanga*) are difficult to separate morphologically and hybrids of the two species have been documented (Vali and Lohmus, 2004). The two specimens we sampled were significantly different genetically, but clearly more closely related to each other than any of the other accipitrid taxa in the study.

The next three diverging Aquilinae clades include species from the genera *Hieraaetus* and *Aquila*, and one species currently in the genus *Spizaetus*. Most of these species have been recognized as members of different genera in the past. Brown and Amadon (1968) separate *Hieraaetus* species from those in the genus *Aquila* by morphological traits. *Aquila* species appear generally smaller than eagles in the genus *Hieraaetus*, with a smaller bill, longer and more slender legs, and deeper emargination on primaries; however, these characters do not hold for all species in these genera. In our analyses, we find members of these two genera intermixed with each other and with Cassin's hawk-eagle (*Spizaetus africanus*) such that, again, none of these genera are monophyletic. One of these clades includes six closely related species: *A. chrysaetos*, *Spizaetus africanus*, *H. fasciatus*, *A. verreauxii*, *A. audax*, and *A. gurneyi*. A close relationship among *A. gurneyi*, *A. chrysaetos*, *A. audax*, and *A. verreauxii* has been proposed based on morphological data (Brown and Amadon, 1968). Cassin's hawk-eagle is morphologically divergent from these four *Aquila* species so it was not previously included in that group. This species has been placed in the genus *Hieraaetus* (Thiollay, 1994) and a monotypic genus (Cassin, 1865), but has not been a member of the genus *Aquila*. The remaining two species (*H. fasciatus* and *H. spilogaster*) in this clade have sometimes been considered as conspecific subspecies (see below).

Three species currently placed in the genus *Aquila* (*A. nipalensis*, *A. rapax*, and *A. heliaca*) form a monophyletic group whereas seven other *Aquila* species are separated from these three and, variously, from each other (Fig. 1). The close relationship of these species relative to each other rather than the remaining booted eagle species is clear from morphological data, our analysis and some previously published genetic data (Vali, 2002). The placement of these three species separate from the other seven congeners in this study supports the need for taxonomic revision of the genus *Aquila*, so that it designates a monophyletic group.

The final clade of booted eagles in our analyses includes four currently recognized species with wide distributions and habitat preferences: *Aquila wahlbergi*, *Hieraaetus ayresii*, *H. morphnoides*, and *H. pennatus*. All but one of these species is in the genus *Hieraaetus*. This outlying species, Wahlberg's eagle (*A. wahlbergi*) is an Afrotropical species of wooded savannah or

bushveld. It has been placed in the genus *Hieraaetus* as well as the genus *Spizaetus* before.

Two booted eagle clades identified in our analyses correspond closely to the geographical distribution of species: Indomalayan hawk-eagles of the genus *Spizaetus* form a clade separate from the New World hawk-eagles (*Spizaetus*, *Spizastur*, and *Oroaetus*). The remaining booted eagle clades show evidence of only one other (apparent) colonization of booted eagles in the New World, which is by the golden eagle (*Aquila chrysaetos*), a species that is found in both the Old and New Worlds.

4.1.1. Aquilinae subspecies

While our study has focused on recognized eagle species, we realize that some taxa currently classified as subspecies might be better elevated to species. The results of such analyses could have important implications for conservation, as many Accipitridae species are declining or endangered.

In the first case, we sampled multiple representatives of the two known subspecies of *Hieraaetus morphnoides* (*H. m. morphnoides* and *H. m. weiskei*) which do not overlap in range. *H. m. morphnoides* is found only in Australia whereas *H. m. weiskei* is found only in New Guinea. Furthermore, *H. m. weiskei* is both smaller in size and darker in color than *H. m. morphnoides*. Although Brown and Amadon (1968) reported the differences between these two subspecies and a close relationship between the two species *H. pennatus* and *H. morphnoides* they maintained subspecies status for these birds. Other authors have elevated the two to species status (in Brown and Amadon, 1968). While sister relationships among these three *Hieraaetus* taxa is not entirely resolved here, the amount of sequence variation between *H. m. morphnoides* and *H. morphnoides weiskei* is as much as is found between species in this analysis. This result is based on the sampling of multiple individuals of each species/subspecies in our analysis and a previous study (Bunce et al., 2005) and supports the phylogenetic distinctiveness and recognition of *H. m. weiskei* and *H. m. morphnoides* as separate species (*H. weiskei* and *H. morphnoides*, respectively).

In the second case, we sampled multiple individuals of *Hieraaetus fasciatus*. The distribution of *H. fasciatus* is disjunct in that birds that reside year-round in southern Africa are separated from migratory birds found in Europe, northern Africa, Asia, and India. Some of the more northern birds migrate to spend the winter in southern Africa, but do not remain to breed. South African birds are also smaller in size with recognizable plumage differences. The taxonomy of *H. fasciatus* has long been debated. Brown and Amadon (1968) recognized one species *H. fasciatus* with two subspecies noting the distinct appearance but similar habits of the South African representatives (*H. f. spilogaster*). Two *H. f. spilogaster* individuals that are residents of South Africa and a third from Zimbabwe form a distinct lineage separate from the *H. f. fasciatus* individuals in our analyses with high Bayesian posterior probability. Genetic distances between the *H. f. spilogaster* individuals and the *H. f. fasciatus* individuals are slightly greater than that of other sister species pairings in booted eagles, such as *H. morphnoides* and *H. pennatus*, and *Aquila audax* and *A. gurneyi* (95, 98, and 97% sequence similarity, respectively). Our findings suggest that further study with greater sampling of individuals is warranted in order to determine if *H. f. spilogaster* should be elevated to species status (*H. spilogaster*) and, if so, where the limits of its geographic distribution lie.

4.2. Sea eagles (Haliaeetinae)

Sea eagles have long been considered to be a monophyletic group with a close relationship to the Milvinae kites. This relationship is largely based on the shared trait of basal fusion of the second and third phalanges found in all sea eagles and the Milvinae kites (Holdaway, 1994), but not in other accipitrid taxa. Previous molecular studies supported monophyly of the sea eagles in the genus *Haliaeetus* (Seibold and Helbig, 1996), and indicated a close relationship between single species representatives of the two groups (Mindell et al., 1997). Here, with more comprehensive sampling, we support monophyly of the subfamily Haliaeetinae, but not of the genus *Haliaeetus* when the other sea eagle genus, *Ichthyophaga*, is included in analyses. We also support a sister relationship between the Milvinae kites (species in the genera *Milvus* and *Haliastur* only) and the sea eagles with the nuc + mt dataset.

The monotypic palmnut vulture is one of the few frugivorous Accipitridae species, eating palm fruits, and occasionally fish, crabs, snails, and other small animals. Behavioral and morphological traits, such as rounding of the underside of the talons, suggest a relationship between the sea eagles and the palmnut vulture. Brown and Amadon (1968), and Jollie (1977a,b) note that it resembles the Egyptian vulture. In a phylogenetic analysis of osteological characters, Holdaway (1994) found support for a monophyletic group of vultures with the palmnut vulture as the earliest diverging lineage. Here, we present the first molecular evidence that the palmnut vulture is an early diverging Old World vulture species more closely related to the lammergeyer (*Gypaetus barbatus*), the Madagascar snake eagle and the Egyptian vulture. Thus, the similarities between the sea eagles and the palmnut vulture are clearly convergent in nature.

The sea eagles of the genus *Haliaeetus* are neatly divided by a split between species with northern distributions (*H. albicilla*, *H. leucocephalus*, and *H. pelagicus*) and species of tropical distributions (*H. vocifer*, *H. vociferoides*, *H. leucogaster*, and *H. sanfordi*). *H. leucoryphus* is a year-round resident of the tropics, however, this species

also breeds in the northern temperate region. Here, we find that *H. leucoryphus* clusters with the northern species. These results are similar to those found by Seibold and Helbig (1996). We also included three taxa not represented in previous studies: *Ichthyophaga ichthyaetus*, *I. humilis*, and *H. vociferoides*. These three species further support the tropical—temperate split, as all of these species have tropical distributions and are found to be members of the tropical clade. *H. vociferoides*, the Madagascar sea eagle and *H. vocifer*, the African sea eagle are sister species, a relationship also supported by their unique reddish plumage and complex, melodious vocalizations.

4.3. Harpy eagles (Harpiinae)

The members of the harpy eagle group (as defined by Brown and Amadon, 1968) are easily distinguished from other Accipitridae eagles by traits such as their extremely large size, with female wing-spans ranging from 1.76 to 2.01 m in length and female body weights ranging from 6 to 9 kg in *Pithecophaga* and *Harpia* (Ferguson-Lees and Christie, 2001). All of the traditional harpy eagle group members live in primary tropical forest, preying on medium-sized mammals (e.g., monkeys, sloths, and tree kangaroos). There are two Old World species, the Philippine eagle (*Pithecophaga jefferyi*) and the New Guinea harpy eagle (*Harpyopsis novaeguineae*), and two New World harpy eagles, the harpy eagle (*Harpia harpyja*) and the crested eagle (*Morphnus guianensis*). Brown and Amadon (1968) suggested that specialization in tropical forests and on a diet of mammals may have led to convergent characters such that the Old World species are not closely related to the New World species. Our data partially agree with this hypothesis as we found strong support for one of the Old World species, the Philippine eagle, being more closely related to the snake eagles (Circaetinae) than to the three others species in the traditional harpy eagle group. Therefore, we do not include the Philippine eagle in the Harpiinae.

The two *Harpyhaliaetus* species are not members of the Harpy eagle group, but are more closely related to the two *Buteogallus* species, a relationship proposed by Brown and Amadon (1968). The genus *Buteogallus* is paraphyletic in our analyses when the *Harpyhaliaetus* species are included.

4.4. Snake eagles (Circaetinae)

Genetic data have previously been published for only two of the snake eagles, and morphologists have had difficulty identifying species that are closely related to the snake eagle group. Here, we present strong evidence that, when the Madagascar serpent-eagle is excluded, the snake eagles form a monophyletic subfamily (Circaetinae) that is most closely related to some Old World vultures (Aegypiinae) and the Philippine eagle than to other Accipitridae. We are the first to propose that the Madagascar serpent-eagle (*Eutriorchis astur*) is not a member of the clade including the other snake eagles. For instance, Brown and Amadon (1968) suggested that *Eutriorchis* and *Dryotriorchis* should be united in one genus, or, based on the shape of the crown feathers, that the Madagascar serpent-eagle is more closely related to the *Spilornis* species. In our analyses the Madagascar serpent-eagle clusters with three Old World vultures in the subfamily Gypaetinae.

Another surprising finding is the placement of the Philippine eagle (*Pithecophaga jefferyi*) within the snake eagle clade (see Section 4.3).

The *Spilornis* species are extremely rare and generally island endemics in the Indomalayan region, a region of high species loss and conservation importance (Collar et al., 2001; Mooers and Atkins, 2003). Jepson et al. (2001) suggest that Indonesia's lowland forests will entirely vanish by 2006 and the outlook for Malaysian forests is similarly poor. This situation highlights the importance of assessing the phylogenetic history and genetic distinctiveness of the Indonesian *Spilornis* species. Here, we included four *Spilornis* species, Brown and Amadon (1968) proposed five, Sibley and Monroe (1990) recognized six, and (Ferguson-Lees and Christie, 2001) identified 13 *Spilornis* species. We suggest relationships of all of the snake eagle species and subspecies should be further explored with increased sampling to inform attempts to conserve these unique and relatively little known taxa.

Snake eagles are found only in the Old World and mainly in the Indomalayan and the Afrotropical regions. One species (*Circaetus gallicus*) is found in the western part of the Palearctic region. The deepest split within the snake eagles corresponds largely to their geographic distribution where Indomalayan species form a clade separate from the Afrotropical species. Only the Philippine eagle does not follow this pattern as it is more closely related to the African snake eagles. The West African serpent-eagle (*Dryotriorchis spectabilis*) falls within a clade of species in the genus *Circaetus*, suggesting that the taxonomy of these two genera should be revised.

4.5. Old World vultures (Aegypiinae and Gypaetinae)

Old World vultures have been proposed to be monophyletic (Brown and Amadon, 1968; Thiollay, 1994) or polyphyletic with *Gyophierax*, *Neophron*, and *Gypaetus* forming one or more groups separate from the others (Jollie, 1977b; Mundy et al., 1992; Seibold and Helbig, 1995). Sister groups had not been identified for the Old World vultures, although the palmnut vulture (see Section 4.2) was proposed to represent the "transition" from vultures to sea eagles (Brown and Amadon, 1968). Here, we find clear support for two separate subfamilies of different evolutionary origin. The Gypaetinae is the

earlier diverging group, and its constituent taxa (including *Gypohierax*, *Gypaetus*, *Eutriorchis*, and *Neophron*) are relatively divergent genetically as well as morphologically. The remaining vultures form a monophyletic group, the Aegypiinae, sister to the Circaetinae snake eagles. The phylogenetic position of *Necrosyrtes* within the Aegypiinae remains uncertain.

4.6. Kites (Milvinae, Perninae, and Elaninae)

Friedmann (1950) described three kite subfamilies (Milvinae, Perninae, and Elaninae) without identifying sister relationships among them, and considered them to be early diverging Accipitridae taxa. Brown and Amadon (1968) considered kites to be the most "primitive" Accipitridae group due to their specialization on insects (e.g., bee and wasp larvae) or snails. Here, we provide evidence of at least four distinct clades, including the three traditional kite subfamilies with some novel hypotheses of relationships, and two non-sister lineages within the Buteoninae. The non-sister relationship for *Ictinia* and *Rostrhamus* requires further analysis as nodal support values are relatively low and taxonomic representation of kites and Buteoninae is limited.

4.7. Convergent evolution in Polyboroides and Geranospiza

The gymnogene (*Polyboroides typus*) and the crane hawk (*Geranospiza caerulescens*) are specialized birds that have developed a series of morphological characteristics related to capturing birds in cavity nests or other small animals in holes or crevices. These traits include an extended circular range of motion for the tarsus, a short outer toe and a relatively weak bill. Based on these traits a close relationship between the two species has been proposed (Friedmann, 1950). Some morphological differences between these two species, including differences in extent of tarsus rotation, suggest that two different evolutionary paths led to the traits allowing exploitation of cavity nesting species (Burton, 1978). These two species are not closely related in our analyses, denoting a clear example of convergent evolution in specialized morphology in the Accipitridae.

5. Conclusions

This study takes a large step toward resolving the uncertain relationships among birds in the Accipitridae. Our analyses include over 3000 bases of nuclear and mitochondrial DNA and a sampling of almost half of the known Accipitridae species, with nearly complete species sampling for eagles and Old World vultures. We find support for a set of phylogenetic relationships among Accipitridae taxa that differ from previous hypotheses based on morphological data. Fourteen subfamilies, of which two are new, are discussed here in order to represent the diversity and evolutionary history of Accipitridae taxa in a manner reflecting our findings. If taxonomy is to reflect phylogeny, revisions are warranted within the booted eagle (Aquilinae), snake eagle (Circaetinae), sea eagle (Haliaeetinae), and harpy eagle (Harpiinae) groups. We report significant genetic differentiation among several sets of subspecies investigated, suggesting that further analyses, particularly of booted eagles, should include multiple samples of species across their ranges or representing described subspecies. The rarity and threatened status of many of the Accipitridae species make such investigations of imminent importance to conservation.

Acknowledgments

We are very grateful to the many institutions and individuals that contributed samples for this study (see Table 1) as well as those who helped in aspects of data collection: Mike Bunce and others at the ABC, Joshua Rest, Wendy Grus, Jeff Johnson, Jared Rubenstein, Matthew C. Klaver, Jill Ceitlin, Christie Chapin, Soochin Cho, Michael Lerner, Walter Boles, David Allen, Alistair McInnes, Ben Hoffman, Sarah Chabangu, Andrew Jenkins, Gerwin van Deventer, Kerri Wolters, Jeanne-Marie Pittman, Hendrik Louw, Clair Louw, Craig Copeland, Shawn Williamson, Pam Struttenburg, Dora Susanibar, Oscar Beingolea, Hector Miranda, Russell Thorstrom, Lindsay Oaks, and Martin Gilbert. Many individuals at various governmental and private organizations assisted us in obtaining the appropriate permits for collection and shipment of the samples, including: Ellen Paul (The Ornithological Council), The Peregrine Fund, Deon Hignett (Western Cape Nature Conservation Board, South Africa), Dora Susanibar (Museo de Historia Natural, Peru), Peter Thomas (USFWS), Mike Carpenter (USFWS), Paul Scobie (ISIS), Department of Wildlife and National Parks for the Gambia, Hector Miranda, and Andrea Gaski (USFWS). We thank Jeff Johnson and Bob Payne for valuable comments on manuscript drafts. This research was supported by grants from the University of Michigan Rackham Graduate School, Latin American and Caribbean Studies Program, International Institute, Department of Ecology and Evolutionary Biology, University of Michigan, Museum of Zoology, the National Institutes of Health (graduate student support for HL) and the National Science Foundation.

Author's note

After this article was accepted for publication a treatment of a subset of booted eagles (Aquilinae) using

cyt-*b* and additional nuclear sequences was published by Helbig et al. (2005). The findings of Helbig et al. are concordant with our study, as are the cyt-*b* sequences with the notable exception of *Aquila pomarina*. While both analyses place *A. pomarina* as sister to *A. clanga* with high support, the cyt-*b* sequences are relatively dissimilar (87.4% similarity index). *Aquila pomarina* has a disjunct population distribution with separate Indian and European populations; the Indian population is morphologically distinct and denoted by the subspecies *A. p. hastata*. The specimen used in our study is from the Indian population/subspecies, while Helbig et al. used a specimen of European origin. This large sequence divergence between two specimens from separate populations of the same species suggests that further study of the populations of this species is warranted. The placement of the crowned hawk-eagle (*Stephanoaetus coronatus*) also differs between the two studies. We find the crowned hawk-eagle to be the first diverging species after the Old and New World hawk-eagles, while Helbig et al. support a sister relationship between Old World hawk-eagles and the crowned hawk-eagle. This difference is likely a result of the larger and slightly different taxon set used in our study, as we do not find a sister relationship between the crowned hawk-eagle and the Old World hawk-eagles even when we analyze our cyt-*b* sequences separately from the other sequences in our study.

References

Arctander, P., 1995. Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. Proc. R. Soc. Lond. B Biol. Sci. 262, 13–19.

Brown, L.H., 1970. Eagles. Arco Publishing, New York.

Brown, L.H., 1976. Birds of Prey Their Biology and Ecology. A & W, New York.

Brown, L.H., Amadon, D., 1968. Eagles, Hawks and Falcons of the World. Country Life Books, London.

Bunce, M., Szulkin, M., Lerner, H.R.L., Barnes, I., Shapiro, B., Cooper, A., Holdaway, R.N., 2005. The evolutionary history of New Zealand's extinct giant eagle revealed by ancient DNA. Public Library Sci. Biol. 3, e(9).

Burton, P.J.K., 1978. The intertarsal joint of the Harrier-hawks *Polyboroides* spp. and the Crane hawk *Geranospiza caerulescens*. Ibis 120, 171–177.

Cassin, J., 1865. Proc. Acad. Nat. Sci. Philadelphia, 4.

CITES-Secretariat 2003. Update to Appendices I, II, III. Convention on International Trade in Endangered Species of Wild Fauna and Flora.

Collar, N.J., Andreev, A.V., Chan, S., Crosby, M.J., Subramanya, S., Tobias, J.A., 2001. Threatened Birds of Asia: The Birdlife International Red Data Book. BirdLife International. Cambridge, UK.

Cooper, R.A., 1994. DNA from museum specimens. In: Herrmann, B., Herrmann, S. (Eds.), Ancient DNA: Recovery and Analysis of Genetic Material from Paleontological, Archaeological, Museum, Medical, and Forensic Specimens. Springer, New York, pp. 149–165.

Cooper, R.A., Poinar, H.N., 2000. Ancient DNA: do it right or not at all. Science 289, 1139.

Dickinson, E.C., 2003. The Howard and Moore Complete Checklist of the Birds of the World. Christopher Helm, London.

Ferguson-Lees, J., Christie, D.A., 2001. Raptors of the World. Christopher Helm, London.

Friedmann, H., 1950. The Birds of North and Middle America. US Government Printing Office, Washington, DC.

Gadow, H., 1893. Vogel. In: Bronns, H.G. (Ed.), Klassen und Ordnungen des Thier-Reichs, wissenschaftlich dargestellt in Wort und Bild. Sechster Band. C.F. Winter'sche, Leipzig und Heidelberg.

Gamauf, A., Haring, E., 2004. Molecular phylogeny and biogeography of honey-buzzards (genera *Pernis* and *Henicopernis*). J. Zool. Syst. Evol. Res. 42, 145–153.

Hall, TA., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Helbig, A.J., Kocum, A., Seibold, I., Braun, M.J., 2005. A multi-gene phylogeny of aquiline eagles (Aves: Accipitriformes) reveals extensive paraphyly at the genus level. Mol. Phylogenet. Evol. 35, 147–164.

Holdaway, R.N., 1994. An exploratory phylogenetic analysis of the genera of the Accipitridae, with notes on the biogeography of the family. In: Meyburg, B.-U., Chancellor, R.D. (Eds.), Raptor Conservation Today. World Working Group on Birds of Prey and Owls. London, pp. 601–649.

Huelsenbeck, J.P., Ronquist, F., 2001. MR BAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Jepson, P., Jarvie, J.K., MacKinnon, K., Monk, K.A., 2001. The end for Indonesia's lowland forests?. Science 292 859–861.

Johnson, J.A., Watson, R.T., Mindell, D.P., 2005. Prioritizing species conservation: does the Cape Verde kite exist? Proc. R. Soc. Lond. B. Biol. Sci.

Jollie, M., 1976. A contribution to the morphology and phylogeny of the Falconiformes, Part 1. Evol. Theory 1, 285–298.

Jollie, M., 1977a. A contribution to the morphology and phylogeny of the Falconiformes, Part 2. Evol. Theory 2, 285–298.

Jollie, M., 1977b. A contribution to the morphology and phylogeny of the Falconiformes, Part 3. Evol. Theory 2, 209–300.

Minin, V., Abdo, Z., Joyce, P., Sullivan, J., 2003. Performance-based selection of likelihood models for phylogeny estimation. Syst. Biol. 52, 674–683.

Mindell, D.P., Sorenson, M.D., Huddleston, C.J., Miranda, H.C.J., Knight, A., Sawchuck, S.J., Yuri, T., 1997. Phylogenetic relationships among and within select avian orders based on mitochondrial DNA. In: Mindell, D.P. (Ed.), Avian Molecular Evolution and Systematics. Academic Press, London, pp. 211–245.

Mooers, A.O., Atkins, R.A., 2003. Indonesia's threatened birds: over 500 million years of evolutionary heritage at risk. Anim. Conservat. 6, 183–188.

Mundy, P., Butchart, D., Ledger, J., Piper, S., 1992. The Vultures of Africa. Academic Press, London.

Nylander, J.A.A., 2002. MrModeltest. Program distributed by the author, Uppsala University Department of Systematic Zoology.

Peters, J.L., 1931. Check-list of Birds of the World. Harvard University Press, Cambridge.

Rambaut, A., Drummond, A.J., 2003. Tracer, Oxford. Available from: <http://evolve.zoo.ox.ac.uk/>.

Riesing, M.J., Kruckenhauser, L., Gamauf, A., Haring, E., 2003. Molecular phylogeny of the genus *Buteo* (Aves: Accipitridae) based on mitochondrial marker sequences. Mol. Phylogenet. Evol. 27, 328–342.

Seibold, I., Helbig, A.J., 1995. Evolutionary history of New and Old World vultures inferred from nucleotide sequences of the mitochondrial cytochrome *b* gene. Philos. Trans. R. Soc. Lon. Series B-Biol. Sci. 350, 163–178.

Seibold, I., Helbig, A.J., 1996. Phylogenetic relationships of the sea eagles (genus *Haliaeetus*): reconstructions based on morphology, allozymes and mitochondrial DNA sequences. J. Zool. Syst. Evol. Res. 34, 103–112.

Seibold, I., Helbig, A.J., Meyburg, B.U., Negro, J.J., Wink, M., 1996. Genetic differentiation and molecular phylogeny of European *aquila* eagles according to cytochrome *b* nucleotide sequences. In: Meyburg, B.U., Chancellor, R.D. (Eds.), Eagle Studies. World Working Group on Birds of Prey (WWGBP), London.

Sibley, C.G., Monroe, B.L., 1990. Distribution and Taxonomy of Birds of the World. Yale University Press, New Haven.

Simmons, M.P., Reeves, A., Davis, J.I., 2004. Character-state space versus rate of evolution in phylogenetic inference. Cladist. Int. J. Willi Hennig Soc. 20, 191–204.

Sinclair, I., Hockey, P., Tarboton, W., 2002. Birds of Southern Africa. Struik Publishers, Cape Town.

Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T., Mindell, D.P., 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol. Phylogenet. Evol. 12, 105–114.

Sorenson, M.D., Fleischer, R.C., 1996. Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. Proc. Nat. Acad. Sci. USA 93, 15239–15243.

Stresemann, E., Amadon, D., 1979. Order Falconiformes. In: Peters, J.L. (Ed.), Check-list of the Birds of the World. Harvard University Press, Cambridge, MA, pp. 271–425.

Sushkin, P.P., 1905. Zur Morphologie des Vogelskelets. Vergleichende Osteologie der Normalen Tagraubvogel (accipitres) und die Fragen der Classification. Tel I. Grundeintelung der Accipitries Teil II. Falken undihrenachsten Verwandten Nouv. Mem. Soc. Imp. Natural. Moscou, 16: iv-247.

Swofford, D.L., 2004. PAUP*: phylogenetic analysis using parsimony (* and other methods). Sinauer, Sunderland, MA.

Thiollay, J.M., 1994. Family Accipitridae (Hawks and Eagles). In: del Hoyo, J., Elliott, A., Sargatal, J. (Eds.), Handbook of the Birds of the World. Lynx Ediciones, Barcelona, pp. 52–205.

Vali, C., Lohmus, A., 2004. Nestling characteristics and identification of the lesser spotted eagle *Aquila pomarina*, greater spotted eagle *A. clanga*, and their hybrids. J. Ornithol. 145, 256–263.

Vali, U., 2002. Mitochondrial pseudo-control region in old world eagles (genus *Aquila*). Mol. Ecol. 11, 2189–2194.

Xia, X., 2000. Data Analysis in Molecular Biology and Evolution. Kluwer Academic Publishers, Boston.