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Convergent evolution and paraphyly of the hawk-eagles of the genus *Spizaetus* (Aves, Accipitridae) – phylogenetic analyses based on mitochondrial markers

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Abstract

The phylogenetic relationships within the New and Old World hawk-eagle assemblage (genus *Spizaetus*; Aves: Accipitridae) were studied using mitochondrial DNA sequences (*cytochrome b*, control region). Eighty-four specimens representing all *Spizaetus* species and almost all currently distinguished subspecies as well as 11 other booted and non-booted 'eagle' genera from the Neotropics, Africa, Eurasia, South Asia and Australasia (*Oroaetus*, *Harpia*, *Morphnus*, *Lophaetus*, *Stephanoaetus*, *Hieraaetus*, *Aquila*, *Ictinaetus*, *Spilornis*, *Pithecophaga*, *Harpyopsis*) were investigated. Although the basal branching could not be resolved, our investigations clearly indicate that hawk-eagles represent a paraphyletic assemblage and thus their external similarities have to be ascribed to convergent evolution. The New World taxa of *Spizaetus* cluster together, but the South American species *Oroaetus isidori* appears embedded within this clade. The taxa from Southeast to East Asia form a clearly separated monophyletic group. It is further divided into two subgroups, which are also characterized by distinct juvenile plumage patterns. *Spizaetus africanus*, the only African representative of the genus, is found in a mixed cluster consisting of members of the genera *Aquila* and *Hieraaetus*. These findings are in accordance with previous studies of other authors based on various molecular markers and different sets of taxa, but disagree with current taxonomy. Therefore, we suggest assigning the species of the genus *Spizaetus* to three different genera: (1) *Spizaetus* (including *Oroaetus isidori*) in Central and South America and (2) *Nisaetus* for the Southeast to East Asian group. (3) The African taxon (*Spizaetus africanus*) is discussed to be included into the genus *Aquila*. Furthermore, we propose to use the former genus name *Lophotriorchis* Sharpe, 1874, for the monotypic species *Hieraaetus kienerii*, which has an isolated phylogenetic position.

Key words: *Spizaetus* – hawk-eagles – molecular phylogeny – taxonomy – resurrected name *Lophotriorchis*

Introduction

Although many biological aspects in birds of prey are well known, the phylogenetic relationships among the various genera are only incompletely understood. One example is a group comprising 59 species commonly subsumed under the name 'eagles' in the broadest sense: sea-eagles, snake-eagles, buteonine eagles and booted (true) eagles (Brown 1976). The latter group, which is the largest, has its highest diversity in the tropics and subtropics. It includes several monotypic genera (e.g. *Stephanoaetus*, *Lophaetus*, *Oroaetus*, *Spizastur*). Among the polytypic genera of the booted eagles, the genus *Spizaetus*, on which the present study is focused, is the most diverse comprising 22 taxa (12 species, modified after Thiollay 1994; Ferguson-Lees and Christie 2001; Gjershaug et al. 2004a; Gamauf et al. 2005a). Its members are distributed over the tropical rain forests of both the New and the Old World, where they live as predators hunting mainly arboreal agile prey (birds, mammals and reptiles). Most representatives of the genus are only little known (Amadon 1953, 1982), and detailed studies concerning their biology, ecology and taxonomy are scarce and have been carried out only on a few species (e.g. Lyon and Kuhnigk 1985; Klein et al. 1988; van Balen and Meyburg 1994; Sözer and Nijman 1995; Gamauf et al. 1998, 2005a; Inoue 1998; Yamazaki 1998; Gjershaug et al. 2004a,b). The majority of the species are medium-sized (1000–1600 g), but some exceptions exist in form of small (510–610 g, *Spizaetus nanus*) or rather large (2500–3500 g, *Spizaetus nipalensis*) taxa (Thiollay 1994; Ferguson-Lees and Christie 2001). Current taxonomy is based on comparative external morphology and plumage pattern. Characteristics shared by all species of the genus are similar body proportions, slender appearance, short rounded wings, long tail and long feathered legs. Although plumage coloration is highly variable, specific colour barring on belly or at least on thighs, legs and under tail coverts is found in varying markedness in the adults of all species. The immature plumage differs strikingly from that of adults, except in the melanistic form of *Spizaetus cirrhatus limnaeetus* and the white ventral side of *Spizaetus floris*. A long, pointed crest is found in most taxa. Only a few are crestless with elongated neck feathers (some *S. cirrhatus* ssp.) and only one species (*Spizaetus tyrannus*) has a short bushy crest. Until now, concerning the taxonomic classification within the genus *Spizaetus*, biogeographical circumstances of these pantropically distributed eagles have been widely ignored.

The genus name *Spizaetus* was used for the first time by Vieillot in 1816, who described the South American *Spizaetus ornatus*. In the Neotropics, this species together with *Spizaetus tyrannus* are the only representatives (Brown and Amadon 1968; Weick 1980; Ferguson-Lees and Christie 2001). Each of the two species is divided into two subspecies. In Africa, the only species of the genus *Spizaetus* is the monotypic *Spizaetus africanus*. The highest species diversity is found in the region of Southeast Asia. Among the Asian species, all but three are monotypic: *S. cirrhatus*, *S. nipalensis*, and *Spizaetus nanus* are polytypic, reflecting their wider distribution range. In the past, three monotypic genera were thought to be close but more specialized relatives of the genus *Spizaetus* (Brown and Amadon 1968): the African genera *Stephanoaetus* (*St. coronatus*) and *Lophaetus* (*L. occipitalis*) as well as the South American *Oroaetus* (*O. isidori*). In the past, *Stephanoaetus* (Swann, 1926) and *Lophaetus* (Brown, 1976) have been even included into the genus *Spizaetus*. On the other hand, Brown and Amadon (1968) stated that *S. africanus*, *S. ornatus* and *S. tyrannus* are 'not obviously tied' with any of the other species of the genus *Spizaetus* (i.e. the Asian representatives), but they did not draw any taxonomical consequences from that.

No clarification of the phylogenetic relationships of this group of booted eagles was achieved by DNA-DNA-hybridization (Sibley and Monroe 1990), by comparing syrinx anatomy (in particular the variation in the intrinsic musculature; Griffiths 1994), and by osteological investigations (Holdaway 1994). Up to now, molecular studies based on three mitochondrial (mt) and four nuclear marker sequences included only a part of the representatives of the genus *Spizaetus*. One of our molecular studies based on mtDNA sequences included only some Asiatic members of this genus and mainly concentrated on intraspecific relationships of *S. cirrhatus* and its supposed relatives (Gamauf et al. 2005a). Several more recent studies on aquiline eagles were published based on mt sequences (Bunce et al. 2004) or a combination of mt and nuclear genes (Helbig et al. 2005; Lerner and Mindell 2005). Those analyses, although they included different taxonomic subsets of the genus *Spizaetus*, showed that *Spizaetus* is paraphyletic: Representatives of the Asian, African as well as South American hawk-eagles cluster together according to their geography, but in each case with members of other genera. Moreover, in those studies, the genera *Hieraaetus* and *Aquila* are paraphyletic groups, and in two of them even the South American *Spizaetus* assemblage appears paraphyletic (Helbig et al. 2005; Lerner and Mindell 2005) due to the positions of other genera (*Oroaetus* and/or *Spizastur*).

In the present comprehensive molecular analysis, we investigated the phylogenetic relationships of hawk-eagles comprising the genus *Spizaetus* including all species and all but one subspecies (*S. nanus stresemanni*). For better perceivability and to enable comparisons with previous studies, we retained the classical taxonomy used by Ferguson-Lees and Christie (2001) and Dickinson (2003). However, in the discussion, we will interpret our results together with other recently published data (Bunce et al. 2004; Gamauf et al. 2005a,b; Helbig et al. 2005; Lerner and Mindell 2005) and compile our views about taxonomic consequences. As in our previous study (Gamauf et al. 2005a), we analysed two mt sequences, the non-coding control region (CR) for low taxonomic levels and the *cytochrome b* (*cytb*) slower evolving. We included those taxa that were formerly considered congeneric with *Spizaetus* (*Stephanoaetus*, *Lophaetus*, *Oroaetus*) as well as representatives of sea-eagles (*Haliaeetus*), snake-eagles (*Circaetus*, *Spilornis*), 'buteonine eagles' (*Harpia*, *Morphnus*, *Pithecophaga*, *Harpyopsis*) and the second species-rich group of 'booted eagles' (genera *Aquila* and *Hieraaetus*). As a relative of unbooted eagles, we included the Common Buzzard *Buteo buteo*, a member of a genus that is widely distributed in the New and Old World.

We addressed the following questions: Which species are the closest relatives of 'hawk-eagles'? Do the Asian hawk-eagles form a monophyletic group or are their similarities in morphology the result of convergent evolution? What are the inter- and intraspecific relationships within the pantropically distributed group of hawk-eagles, and are their genetic data in accordance with current taxonomy and previous phylogenetic interpretations based on morphology?

Materials and Methods

Samples

Representatives of all currently recognized and one invalid taxon (*S. nipalensis* '*fokiensis*') of the genus *Spizaetus* were analysed (Table 1). In addition, almost all remaining genera of booted eagles (except *Polemaetus* and *Spizastur*) as well as representatives of non-booted eagles were included. Previously published sequences included in the analyses are listed in Table 1. Altogether, sequences of 87 individuals representing 45 taxa were included. Fresh samples (mostly feathers) were available only for a few taxa. Most of them were represented by museum specimens (skin of foot pad). Consequently, only short DNA fragments could be amplified. The analyses were performed in two different laboratories: the Laboratory of Molecular Systematics of the Museum of Natural History (NMW),Vienna, and the Norwegian Institute for Nature Research (NINA), Trondheim. Thus, two protocols for DNA extraction, PCR and sequencing were used.

DNA extraction

Protocol 1 (NMW): Incubation of tissues in a 10% Chelex (Biorad, Hercules, CA, USA) solution containing proteinase K (0.5 mg ml^{-1}) for 4 h at 50°C (with agitation); subsequently extractions were heated to 95°C for 5 min and centrifuged for 1 min. The supernatant was purified using the QIA Quick PCR Purification Kit (QIAGEN, Hilden, Germany) with a final volume of 30–70 µl elution buffer. Protocol 2 (NINA): DNA extraction according to Taberlet and Bouvet (1991) was performed except that a Microcon YM-50 filter (Amicon, Billerica, MASS, USA) was used instead of a Centricon 30 (Amicon) to desalt and concentrate the sample. If 10 µl of TE-buffer (1 mM EDTA, 10 mM Tris–HCl, pH 8.0) was added to the Microcon YM-50 filter (Amicon) and left for 10 min before centrifugation was performed again. Control extractions with pure extraction buffer (without tissue) were for PCR experiments.

PCR amplification and marker sequences

Protocol 1 (NMW): PCR (polymerase chain reaction) was carried out in a volume of 25 µl containing 1 unit Dynazyme DNA polymerase (Finnzymes Oy, Finland), 0.5 µM of each primer, and 0.2 mM of each dNTP. Initial denaturation (95°C, 2 min) was followed by 30 reaction cycles: 95°C (10 s), annealing temperature (10 s), 72°C (30 s); final extension at 72°C (5 min). Protocol 2 (NINA): PCR was performed in a 25 µl reaction mix containing 15 pmol of each primer, 2.0 mM $MgCl_2$, 0.2 mMol each dNTP, 1 µl 10 × PCR-buffer II and 1–1.25 U AmpliTaq Gold polymerase (Applied Biosystems) or HotStar Taq (QIAGEN). After denaturation and activation of AmpliTaq Gold (10 min at 95°C) or HotStar Taq (15 min at 95°C), 40 cycles of 30 s at 94°C, 45 s at 50°C, and 90 s at 72°C were performed on a 2600 or 2700 thermocycler (Applied Biosystems).

Optimal amounts of template DNA extracted from museum material were determined empirically (2–10 µl of the DNA solution) or dilutions from 1 to 50 fold. If necessary, re-amplifications were performed with 1–2 µl template. Negative controls were carried out to detect contaminated reagents: (1) control extractions (without DNA) instead of template; (2) reaction with H_2O instead of template. Since a major part of the study was based on tissue of museum specimens, the expected maximum length of PCR fragments was mostly <400 bp. The two primers used for both PCR amplification and direct sequencing of the *cytb* gene were named mt-A and mt-I. Primer mt-A (L-14970) (5′-CAA CAT CTC AGC ATG ATG AAA CTT CG-3′) was based on the *cytb* sequence by Kocher et al. (1989) modified by Wink (1998). Primer mt-I (H-15350) (5′-TGC TGA GAA TAG GTT GGT GAT GAC-3′) was designed based on *cytb* sequences of five *Aquila* species (Seibold et al. 1996) and optimized at NINA, Trondheim. Of the 381 bp PCR-product obtained, 264 bp corresponding to positions 15034–15297 of the *Gallus gallus* f. *dom.* mt genome (Desjardins and Morais 1990) were used for sequence comparisons. For the control region, two primer pairs were used: CR5+: 5′-CCC CCC CTT CCC CCC C-3′, CR7-: 5′-GAC CGA CTA AGA GAT AAC CTA-3′ (annealing temperature 50°C). For specimens where no PCR product could be obtained with the primer pair CR5+/CR7–, two nested primers were employed (CR1+: 5′-ATG TAC TAT TGT ACA TTA AAC-3′, CR2–: 5′-CAA GTT ATG ACC TGC TACC-3′; annealing temperature 50°C).

Table 1. Sample list ($n = 87$) of 45 raptor taxa included in the genetic analysis

English name	Scientific name	Code	Locality, year	Origin of source	Accession number	
					cytb	**CR**
Ornate Hawk Eagle	*Spizaetus o. ornatus* (Daudin, 1800)	Sornorn-2	Suriname, 1965	RMNH 36449 (II/12)	EF459641	EF459594
	S. o. vicarius Friedmann, 1935	Sornvic-1	Captivity, 1999	Zoo Berlin Friedrichsfelde	EF459640	EF459593
Black Hawk Eagle	*S. t. tyrannus* (Wied, 1820)	Styrtyr-3	Captivity, 1999	Zoo Berlin Friedrichsfelde	EF459639	EF459596
Cassin's Hawk Eagle	*S. africanus* (Cassin, 1865)	Safr-3	Liberia, 1968	BMNH 1977.20.43	EF459637	EF459577
		Safr-4	Cameroon, 1902	BMNH 1902.12.5.1	–	EF459578
Sulawesi Hawk Eagle	*S. lanceolatus* Temminck & Schlegel, 1844	Slan-1	Indonesia, Sulawesi, 1931	ZMB 33.114	AY701129[1]	AY701095[1]
		Slan-3	Indonesia, Sulawesi, 1887	BMNH 1887.11.1.337 (22)	AY701130[1]	AY701096[1]
Pinsker's Hawk Eagle	*S. pinskeri* Preleuthner & Gamauf, 1998	Sphipin-8	Philippines, Negros, 2003	J. B. Serrano	AY701125[1]	AY701091[1]
		Sphipin-9	Philippines, S-Negros, 2003	J. B. Serrano	AY701126[1]	AY701092[1]
Philippine Hawk Eagle	*S. philippensis* Gurney in Gould, 1863	Sphiphi-2	Philippines, Luzon, 1895	BMNH 1897.5.13.311 (24)	AY701124[1]	AY701090[1]
		Sphiphi-6a	Philippines, Luzon, 1985	J. A. James	AY701123[1]	–
Changeable Hawk Eagle	*S. c. cirrhatus* (Gmelin, 1788)	Scircir-3	India, Bombay, 1874	BMNH 1887.11.1.342	AY701197[1]	AY701067[1]
	S. c. ceylanensis (Gmelin, 1788)	Scircey-5	Sri Lanka	RMNH 5210 (35)	AY701198[1]	AY701069[1]
	S. c. limnaeetus (Horsfield, 1821)	Scirlim-1	Indonesia, Java, 1931	NMW 322	AY701101[1]	AY701071[1]
	S. c. vanheurni Junge, 1936	Scirvan-1	Indonesia, Sinalur Is. (= Simeulue), 1913	RMNH 87258 syntype	AY701114[1]	AY701081[1]
	S. c. andamanensis Tytler, 1865	Scirand-1	India, Andaman Is., 1874	BMNH 1885.8.19.14-68	AY701113[1]	AY701079[1]
	S. c. floris (Hartert, 1898)	Scirflo-1[2]	Indonesia, S Flores	AMNH 534.895	AY701118[1]	AY701085[1]
Mountain Hawk Eagle	*S. n. nipalensis* (Hodgson, 1836)	Snipnip-4	Sikkim, 1876	BMNH 1885.8.18.1389	EF459652	EF459557
		Snipnip-8	Taiwan	K.-Y. Huang	EF459650	EF459559
		Snipnip-9	Taiwan	K.-Y. Huang	EF459660	EF459560
		Snipnip-11	Taiwan	K.-Y. Huang	EF459661	EF459561
	S. n. fokiensis W. L. Sclater, 1919	Snipfok-1	China, Fohkien, 1899	BMNH 1914.5.1.86 (12)	EF459649	EF459558
	S. n. orientalis Temminck & Schlegel, 1844	Snipori-4	Japan, Shiga	T. Yamazaki, 9701	EF459662	EF459562
		Snipori-4a	Japan, Hokkaido, Asahikawa	K. Saito	EF459666	–
		Snipori-5	Japan, Shiga,	T. Yamazaki, 9203	EF459663	EF459563
		Snipori-5a	Japan, Hokkaido, Asahikawa	K. Saito	EF459667	–
		Snipori-6	Japan, Shiga	T. Yamazaki, 9102	EF459664	–
		Snipori-6a	Japan, Hokkaido, Asahikawa	K. Saito	EF459668	EF459564
		Snipori-7a	Japan, Honshu, Kyoto	K. Saito	EF459651	EF459565
		Snipori-8	Russia, Primorye	V. A. Nechaev	EF459659	EF459566
		Snipori-9	Japan, 1868	RMNH 1 (39)	EF459665	EF459567
		Snipori-12	Russia, Primorye	A. Kryukov, 802	–	EF459568
	S. n. kelaarti Legge, 1878	Skel-2[3]	India, Myanall, Travancore, 1876	BMNH 1885.8. 19.1407 (17)	EF459647	EF459570
		Skel-3[3]	Sri Lanka	BMNH 1955.6.N.20.388	EF459648	EF459569
Blyth's Hawk Eagle	*S. alboniger* Blyth, 1845	Salb-1	Malaysia, Borneo, Sarawak, Patah	AMNH 648.605	EF459653	EF459555
		Salb-2	Malaysia, Borneo, Sarawak, Patah	AMNH 534.873	EF459655	–
		Salb-4	Malaysia, Perak, Moywell Hills	RMNH 28.121(31)	EF459654	EF459556
Javan Hawk Eagle	*S. bartelsi* Stresemann, 1924	Sbar-1	Indonesia, Sumatra, 1920	RMNH 14.277	EF459658	EF459549
		Sbar-3	Indonesia, Java, Pangerango, 1925	RMNH B14660 (6)	EF459656	EF459551
		Sbar-4	Indonesia, Java, 1928	RMNH (29)	EF459657	EF459550
Wallace's Hawk Eagle	*S. n. nanus* Wallace, 1868	Snan-1	Indonesia, Java	AMNH 447.454	EF459644	–
		Snan-2	Indonesia, Kotanuringan, SW Borneo	AMNH 447.255	EF459646	–
		Snan-5	–	BMNH 1888.8.13.7(18)	EF459643	EF459552
		Snan-6	Malaysia, Sarawak, N Borneo	RMNH 5245 (30)	EF459642	EF459553
		Snan-7	Indonesia, Sumatra, Palembang, 1926	RMNH 4777 (II/1)	EF459645	EF459554
Isidor's Hawk Eagle	*Oroaetus isidori* (Des Murs, 1845)	Oisi-1a	Bolivia, Puria, Iripana River, 1951	ZFMK 52.147	EF459638	EF459595

Table 1. Continued

English name	Scientific name	Code	Locality, year	Origin of source	Accession number *cytb*	Accession number CR
Crowned Eagle	*Stephanoaetus coronatus* (Linnaeus, 1766)	Scor-1	Liberia, 1906	RMNH cat. 6 (II/717)	EF459615	EF459573
		Scor-1a	Congo, 1944	NMW 45.113	EF459614	EF459574
		Scor-2	Tansznia, 1965	RMNH 37249 (II/18)	EF459613	EF459575
		Scor-3	captivity	NMW 72.926	EF459618	EF459576
Long-crested Eagle	*Lophaetus occipitalis* (Daudin, 1800)	Locc-2	East Africa	RMNH cat. 4 (II/19)	EF459610	EF459590
Bonelli's Eagle	*Hieraaetus f. fasciatus* (Vieillot, 1822)	Hfasfas-1	–	RMNH 9 (III/5)	EF459630	EF459588
		Hfasfas-1a	Italy, Sicily, 2000	H. Frey	EF459631	EF459585
	H. f. renschi Stresemann, 1932	Hfasren-1	Indonesia, Flores, Rureng	RMNH 66076 (III/3)	EF459628	EF459586
		Hfasren-2	Indonesia, Flores	RMNH 81113 (III/4)	EF459629	EF459587
Booted Eagle	*H. pennatus* (Gmelin, 1788)	Hpen-2	France, Massif Central	Th. Noré	EF459635	–
Ayres's Hawk Eagle	*H. ayresii* (Gurney, 1862)	Hayr-1	Ghana, Accra, 1973	BMNH 1973.1.1	EF459634	EF459591
		Hayr-2	Liberia, Mt Nimba, 1968	BMNH 1977.20.44	EF459635	EF459592
Rufous-bellied Hawk Eagle	*H. kienerii formosus* Stresemann, 1924	Hkiefor-1	Indonesia, Java	RMNH 4833 (III/8)	EF459632	–
		Hkiefor-2	Indonesia, Java	RMNH 2588 (III/9)	EF459633	–
Golden Eagle	*Aquila c. chrysaetos* (Linnaeus, 1758)	AchrGB	Europe	Seibold and Helbig 1995	X86740	–
		Achrchr1	Russia, St Petersburg, 2003	R. Probst	–	EF459579
Wedge-tailed Eagle	*A. a. audax* (Latham, 1801)	Aaudaud-2	Australia	RMNH 8311 (II/14)	EF459626	EF459581
		Aaudaud-3	Australia, Victoria	NMW 49.649	EF459625	EF459580
	A. a. fleayi Condon & Amadon, 1954	Aaudfle-2	Australia, Tasmania	BMNH 1912.7.20.1 (28)	EF459624	–
Gurney's Eagle	*A. gurneyi* G. R. Gray, 1860	Agur-1	Indonesia, Aru Is.	NMW 84.999	EF459619	EF459582
		Agur-2	New Guinea	NMW 46.102	EF459620	EF459583
		Agur-4	Indonesia, Halmahera	RMNH 4 (36)	EF459621	–
		Agur-5	Indonesia, Bacan 1867	RMNH cat. 2 (II/7)	EF459622	–
		Agur-6	New Guinea	BMNH1955.6. N.20.433 (25)	EF459623	EF459584
Lesser Spotted Eagle	*A. pomarina* C. L. Brehm, 1831	Apom-1	Slovakia, 1998	J. Chavko	EF459616	EF459589
Indian Tawny Eagle	*A. rapax vindhiana* Franklin, 1831	Arapvin-1	India, Khandeish, Ohulia, 1884	NMNH 1925.12.23.31	EF459627	–
Indian Black Eagle	*Ictinaetus m. malayensis* (Temminck, 1822)	Imal-1	Indonesia, Java	RMNH 15391 (III/10)	EF459611	EF459571
		Imal-2	Indonesia, Sumatra	RMNH 28190 (III/11)	EF459612	EF459572
Harpy Eagle	*Harpia harpyja* (Linnaeus, 1758)	Hhar-2	Venezuela, Terr. Amazonas, 1997	A. Gamauf	EF459603	EF459599
Crested Eagle	*Morphnus guianensis* (Daudin, 1800)	Mgui-1	British Guiana, Bartica Grove, 1880	BMNH 1887.5.1.619	EF459617	–
Short-toed Snake- eagle	*Circaetus g. gallicus* (Gmelin, 1788)	Cgalgal-1	France, Correze	Th. Noré	EF459604	EF459598
White-tailed Eagle	*Haliaeetus a. albicilla* (Linnaeus, 1758)	Halb-4	Russia, Rybinsk, 2003	R. Probst	–	EF459600
		Halb-GB	Eurasia	Seibold and Helbig 1995	X86753	–
Crested Serpent- eagle	*Spilornis cheela burmanicus* Swann, 1920	Schebur-1	Thailand, Khang ang rue rai, 1996	W. Sontag	EF459607	–
Philippine Serpent- eagle	*S. holospilus* (Vigors, 1831)	Shol-3	Philippines, Negros, 1994	C. Escario	EF459609	EF459597
		Shol-4	Philippines, Negros, 1994	C. Escario	EF459608	–
Philippine Eagle	*Pithecophaga jefferyi* Ogilvie-Grant, 1896	Pjef-1	Philippines, Mindanao	ZFMK 63464	EF459601	–
		Pjef-2	Philippines, Mindanao, 1993	C. Escario	EF459602	–
New Guinea Eagle	*Harpyopsis novaeguineae* Salvadori, 1875	Hnov-1	Indonesia, New Guinea, Arfak, 1997	J.-O. Gjershaug	EF459606	–
		Hnov-1a	Papua New Guinea, Owen-Stanley Mts	NMW 73.000	EF459605	–
Bearded Vulture	*Gypaetus b. barbatus* (Linnaeus, 1758)	Gbar-1GB	Eurasia	Seibold and Helbig 1995	X86749	–
		Gbar-2GB	–	Roques et al. 2004	–	AY542900
Common Buzzard	*Buteo b. buteo* (Linnaeus, 1758)	BbutGB	Austria, Haringsee, 1997	Haring et al. 2001	AF380305	AF380305

Scientific names refer to classical taxonomic view, while proposed taxonomic changes are discussed in the text and presented in Figs 1 and 2. Labcodes are derived from taxonomic names (first letter: genus name, letters 2–4: species, letters 5–7: subspecies) and are the same as in our previous publication.
[1]Sequences we published previously (Gamauf et al. 2005a).
[2]*N. floris* after Gjershaug et al. (2004).
[3]*N. kelaarti* after Gjershaug et al. (in prep.).

Cloning and sequencing

PCR products were extracted from agarose gels using the QIAquick Gel Extraction Kit (QIAGEN) and cloned (TOPO TA Cloning Kit®, Invitrogen (Carlsbad, CA, USA)). For the NMW, sequencing of cloned PCR products (both directions) was performed by MWG-Biotech (Ebersberg, Germany). Protocol 2 (NINA): Successful amplification and approximate quantification of PCR products were checked by running 1/5 of the PCR samples on a 2% agarose gel. PCR products were purified using the QIAquick-spin PCR purification kit (QIAGEN). Yields of PCR products after purification were estimated by agarose gel electrophoresis. Direct sequencing of PCR products was performed (both strands). Purified PCR products were sequenced on an Applied Biosystems 310 DNA sequencer (Foster City, CA, USA) with Taq DNA polymerase and Dye Terminators or Big Dye Terminators (Applied Biosystems) according to the manufacturer's instructions. PCR conditions for sequencing of PCR products were 30 cycles with 30 s at 96°C, 15 s at 50°C, and 4 min at 60°C. The sequence extension products were purified by ethanol precipitation according to manufacturer's instruction (Applied Biosystems) except that ethanol was not chilled and the procedure was performed at room temperature.

Sequence analysis

Alignments were produced manually. The reading frames of all *cytb* sequences proved to be intact corroborating that the sequences are derived from functional mt genes. Nevertheless, examination of the reading frame cannot be the sole control of authenticity (the fragments are short and the CR is non-coding). The assumed mt origin is supported by several additional arguments: (1) The data obtained from different laboratories, partly with different primers, yielded consistent results. (2) No double bands were observed in the sequencing reactions of PCR products. (3) Consistency between sections of *cytb* used the present work and published sequences. Distance (neighbour-joining algorithm; NJ: Saitou and Nei 1987), maximum parsimony (MP) and maximum likelihood (ML) methods were used to infer phylogenetic relationships. All dendrograms were calculated with the software package PAUP (test version 4b6–10; Swofford 2002). For NJ trees, uncorrected distances (p-distances) were used. Distances were calculated with PAUP using the pairwise-deletion mode (the distance for each pair of sequences is computed by ignoring only those gaps that are involved in comparison). Average p-distances between clades and subclades were calculated with the software PHYLTEST (Kumar 1996). MP trees were generated with heuristic search using the TBR (tree bisection reconnection) algorithm and a random taxon addition sequence (1000 replicates). For MP analysis of the CR data set, gaps were treated as fifth character state. For the MP analysis of the *cytb* data set, we tested differential weighting of the three codon positions. Because this did not improve the resolution of the tree, all characters were weighted equally. Bootstrap analyses were performed with 1000 replicates (10 random addition replicates) and delayed character transformation (DELTRAN). The optimal substitution model for the ML analysis was selected using MODELTEST (version 3.06; Posada and Crandall 1998). The following model and parameters were used: CR: HKY85 + Γ with estimated base frequencies (A = 0.293, C = 0.218, G = 0.167, T = 0.322), a transition/transversion ratio of 3.2218, four rate categories, and a gamma distribution shape parameter of 0.6385; *cytb*: TVM + Γ + I with estimated base frequencies (A = 0.300, C = 0.413, G = 0.125, T = 0.162), six rate categories, a gamma distribution shape parameter of 0.6385, and a proportion of invariable sites of 0.5443. The ML tree was calculated with heuristic search and TBR branch swapping using a NJ starting tree. Bootstrap support values for ML trees were calculated from 100 replicates with NNI branch swapping.

Sequences accession numbers are included in Table 1.

Results

We analysed a partial section of the mt *cytb* gene (length of alignment 264 bp) and the CR (length of alignment 269 bp). Among the 87 specimens analysed, 45 specimens represented the genus *Spizaetus*, while the remaining were members of other eagle genera. The CR sequence could not be obtained from all specimens, although several primer combinations were tested. Thus, the data set for CR was somewhat smaller. Together with some sequences obtained from GenBank, the CR alignment comprised 65 sequences (38 taxa in 27–29 species), while the *cytb* alignment contained 84 sequences (47 taxa in 34–36 species). We performed NJ, MP and ML analyses of both *cytb* and CR data set as well as of a combined data set (from 62 specimens both sequences could be obtained). Comparing the trees calculated with these three data sets, the following general conclusions can be drawn: (1) With both marker sequences, several of the nodes are not resolved, and *cytb* has lower resolution power for deeper nodes than CR. All trees contain several nodes with bootstrap support $< 50\%$. (2) In general, the NJ tree from CR sequences has the highest bootstrap support as compared to *cytb* and other tree building methods (Fig. 1). (3) In the trees based on combined data set, most nodes have even lower bootstrap support than in those derived from CR sequences only (data not shown). (5) Some groups in the trees are in accordance with geographic affiliations (Asia) and several clades contradict current taxonomy.

Trees based on CR data set

The NJ tree based on CR sequences is presented in Fig. 1. In this tree, bootstrap values from other analyses (ML, MP) are included. As outgroup species, *G. barbatus* was used. Among the ingroup taxa, a clade formed by *B. buteo* and *H. albicilla* splits off from the basal node, followed by a clade composed of *Sp. holospilus*, *C. gallicus* and *H. harpyja*. The remaining sequences are divided into two clades (nodes 1 and 2 in Fig. 1), each subdivided into further subclades.

Clade 1 – the Asian clade: Clade 1 contains all Asian representatives of the genus *Spizaetus* as well as *Ictinaetus malayensis*, which splits off from the basal node of this clade. Distances between sequences of the Asian clade are given in Table 2. The *Spizaetus* species are distributed in two subclades consisting of *S. cirrhatus*, *S. philippensis*, *S. pinskeri* and *S. lanceolatus* on the one hand, and *S. nipalensis*, *S. nanus*, *S. alboniger* and *S. bartelsi* on the other hand. All Asian species form monophyletic groups, which are highly supported except *S. cirrhatus*. According to the two prominent polytypic species in the two subclades, we designated them *S. cirrhatus* group and *S. nipalensis* group, respectively (see Fig. 1). The average p-distance between the two groups is 10.8%. In the *S. cirrhatus* group, the basal split separates *S. lanceolatus* from the rest. The subclade containing the two Philippine species *S. philippensis* and *S. pinskeri* is the sister group of *S. cirrhatus*. The *S. cirrhatus* group has been investigated more detailed in a separate study (Gamauf et al. 2005a). Therefore, we included only one representative of each subspecies, whereas of the *S. nipalensis* group, a larger number of samples (22) was included covering the whole distribution area. Here, a well supported subclade formed by *S. alboniger* and *S. bartelsi* splits off from the basal node. The average p-distance between the two species is 6.7%. The clustering of the remaining two subclades, comprising *S. nanus* and *S. nipalensis*, respectively, is supported only weakly. Thus, the relationships within the *S. nipalensis* group can be considered an unresolved trichotomy. Between these subclades, average p-distances are 7.7% (*S. alboniger* + *S. bartelsi*/*S. nipalensis*), 7.7% (*S. alboni-*

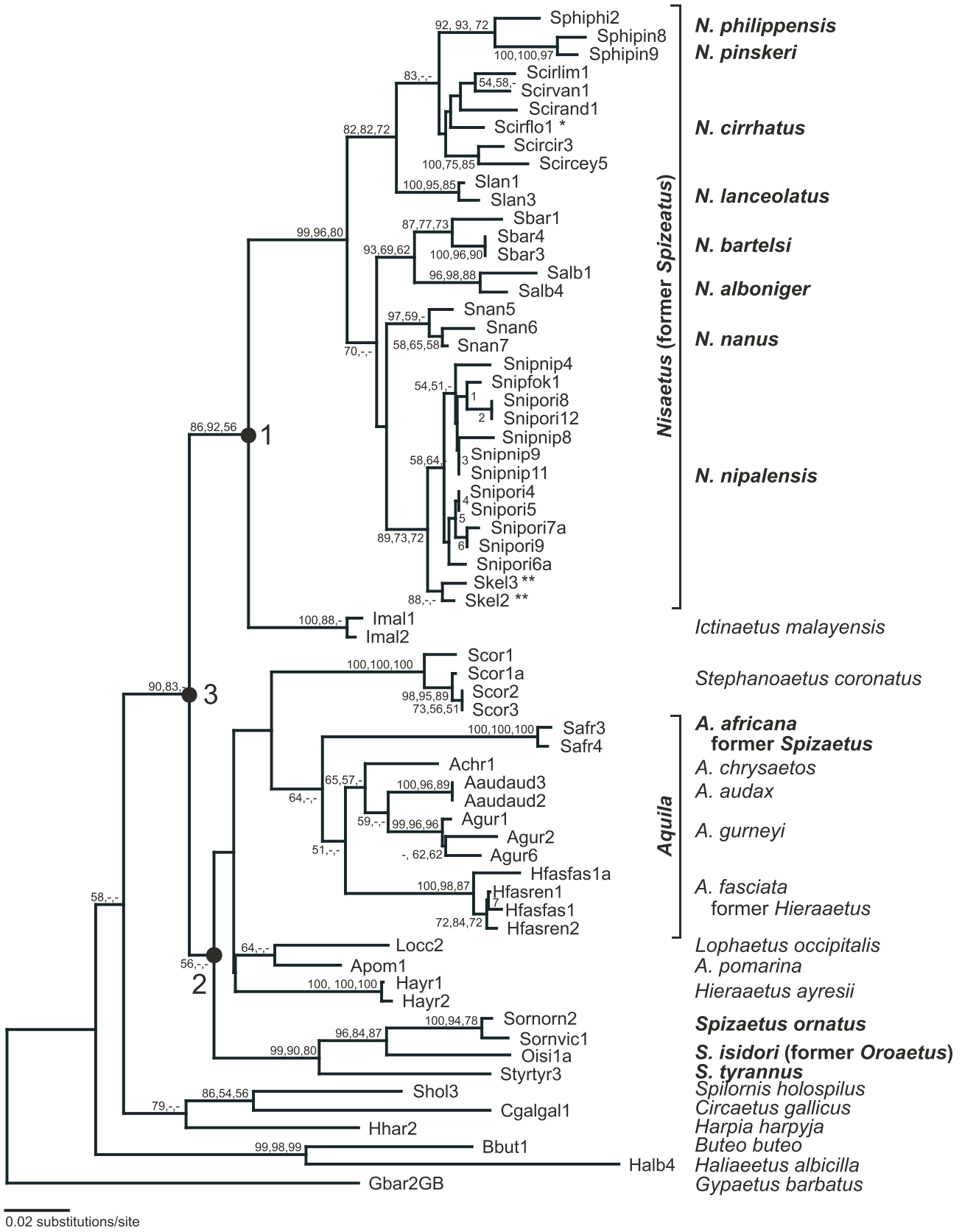

Fig. 1. NJ tree based on CR sequences. Abbreviations of specimens are according to Table 1. Taxonomic names on the right summarize the revised taxonomic view. Former and current *Spizaetus* taxa are indicated in bold letters. Major clades 1, 2 and 3 (mentioned in the text are indicated). Bootstrap values of NJ (left), MP (middle) and ML (right) analyses are indicated. The following bootstrap values (nodes 1–7 in the tree) could not be depicted because of limited space: 1: 57, 53, -; 2: 99, 100, 73; 3: 69, -, -; 4: 64, -, -; 5: 52, 51, -; 6: 56, -, -; 7: 75, 62, 61. * = *N. floris* after Gjershaug et al. (2004a); ** = *N. kelaarti* after Gjershaug et al. (in prep.)

Table 2. Average p-distances between subclades of the Asian hawk eagles

	cir-m	cir-I	phi	pin	lan	alb	bar	nip-m	nip-j	kel	nan
cir-m	—	0.9 ± 0.4	3.0 ± 1.1	4.6 ± 1.3	4.2 ± 1.2	7.7 ± 1.6	7.6 ± 1.6	9.0 ± 1.7	8.4 ± 1.7	7.2 ± 1.6	11.0 ± 1.9
cir-i	4.7 ± 1.1	—	3.1 ± 1.0	5.4 ± 1.3	4.5 ± 1.2	7.8 ± 1.6	7.7 ± 1.6	8.5 ± 1.7	8.0 ± 1.6	7.3 ± 1.6	11.3 ± 1.9
phi	5.5 ± 1.4	5.6 ± 1.3	—	3.4 ± 1.1	3.8 ± 1.2	5.8 ± 1.4	5.7 ± 1.4	7.1 ± 1.6	6.5 ± 1.5	5.1 ± 1.3	9.9 ± 1.8
pin	8.2 ± 1.7	8.0 ± 1.6	4.6 ± 1.3	—	4.2 ± 1.2	7.3 ± 1.6	7.2 ± 1.6	7.1 ± 1.6	6.9 ± 1.5	5.1 ± 1.3	8.3 ± 1.7
lan	7.0 ± 1.6	7.7 ± 1.6	7.6 ± 1.7	8.2 ± 1.7	—	5.8 ± 1.4	6.4 ± 1.5	7.1 ± 1.6	6.5 ± 1.5	5.5 ± 1.4	8.3 ± 1.7
alb	12.8 ± 2.0	11.6 ± 1.9	12.0 ± 2.0	13.3 ± 2.1	10.8 ± 1.9	—	1.6 ± 0.8	4.7 ± 1.3	4.7 ± 1.3	4.5 ± 1.3	8.6 ± 1.7
bar	11.5 ± 1.9	11.2 ± 1.9	12.2 ± 2.1	13.8 ± 2.1	8.9 ± 1.7	6.7 ± 1.4	—	4.6 ± 1.3	4.6 ± 1.3	4.4 ± 1.2	8.7 ± 1.7
nip-m	10.3 ± 1.9	10.2 ± 1.8	11.9 ± 2.1	13.1 ± 2.1	9.1 ± 1.8	8.4 ± 1.7	7.3 ± 1.5	—	1.1 ± 0.6	4.4 ± 1.2	8.2 ± 1.7
nip-j	9.4 ± 1.8	9.4 ± 1.7	10.9 ± 2.0	12.2 ± 2.1	8.1 ± 1.7	9.2 ± 1.8	7.5 ± 1.6	2.0 ± 0.7	—	4.4 ± 1.2	9.3 ± 1.8
kel	10.9 ± 2.0	9.4 ± 1.7	11.2 ± 2.0	12.0 ± 2.0	8.7 ± 1.8	7.4 ± 1.6	6.7 ± 1.5	3.2 ± 0.9	2.5 ± 0.9	—	7.8 ± 1.6
nan	10.8 ± 1.9	10.3 ± 1.8	11.8 ± 2.1	12.5 ± 2.1	8.9 ± 1.8	7.8 ± 1.6	7.7 ± 1.6	5.6 ± 1.3	5.5 ± 1.4	5.3 ± 1.3	—

Above diagonal: *cytb*; below: CR. The species are abbreviated by three letters. phi *S. philippensis*; pin, *S. pinskeri*; lan, *S. lanceolatus*; alb, *S. alboniger*; bar, *S. bartelsi*; nan, *S. nanus*. *S. cirrhatus* was subdivided into a mainland (cir-m) and an island (cir-i) group; *S. nipalensis* was subdivided into three groups: mainland (nip-m), Japan (nip-j), *S. n. kelaarti* (kel).

ger + *S. bartelsi*/*S. nanus*) and 5.5% (*S. nipalensis*/*S. nanus*). The *S. nipalensis* individuals are divided into three groups. The two individuals of south-west Indian *S. n. kelaarti* (Snipkel-2, 3) branch off from the basal node in this subclade; individuals in the two other groups cluster according their geographic origins: one group contains all individuals from Japan (subspecies *orientalis*), whereas the other one comprises all mainland individuals: subspecies *nipalensis* (Snipnip-4 to- 11, '*fokiensis*' (Snipfok-1) from northern India and China, and *orientalis* (Snipori-8, 12) from south-eastern Russia. Average distances (p-distances) between the three groups are 3.2% (*S. n. kelaarti*/mainland), 2.5% (*S. n. kelaarti*/Japan) and 2.0% (Japan/mainland). In Table 2, the average distances among subclades of the Asian representatives of *Spizaetus* are given.

Clade 2 – American and African hawk-eagles: The three members of the genus *Spizaetus* occurring outside Asia, namely the South American species *S. ornatus* and *S. tyrannus* as well as the African *S. africanus*, are found in the second main clade (node 2 in Fig. 1), which is not well supported (bootstrap value 50%). Within this clade, a group comprising the South American species splits off from the basal node. Besides *S. ornatus* and *S. tyrannus*, it contains *O. isidori*, which is the sister group to *S. ornatus*. Their genetic divergences are remarkably high: 8.3% (*S. ornatus*/*O. isidori*), 13.9% (*S. ornatus*/*S. tyrannus*) and 14.1% (*O. isidori*/*S. tyrannus*). The remaining taxa of clade 2 comprise five genera: besides *S. africanus*, it includes species of the genera *Aquila*, *Hieraaetus*, *Lophaetus*, and *Stephanoaetus*. *S. africanus* is the sister group of a subclade containing *H. fasciatus*, *A. chrysaetos*, *A. audax* and *A. gurneyi* (i.e. *Aquila-Hieraaetus* subclade), whereas *A. pomarina* + *L. occipitalis*, *H. ayresii* and *St. coronatus* split off from more basal nodes, thus rendering the genera *Hieraaetus* and *Aquila* paraphyletic. The average distance between *S. africanus* and *Aquila-Hieraaetus* subclade is 13.5% (between the *Aquila* and *Hieraaetus* representatives of this subclade: 10%).

Both the MP and ML analyses resulted in trees with topologies very similar to the NJ tree. In MP analysis (171 variable characters, 127 MP informative), 264 most parsimonious trees were obtained (not shown; TL = 576, CI = 0.444, RI = 0.795, RC = 0.353). The two main clades are found in all trees (bootstrap value for clade 1 = 95%, for clade 2 < 50%). Major differences between the MP strict consensus tree and the NJ tree are not well supported: (1) *S. nanus* splits off from the basal node of the Asian clade (bootstrap value 65%); (2) *S. bartelsi*/*S. alboniger* subclade clusters with the *S. cirrhatus* group and not with the *S. nipalensis* group (bootstrap value < 50%); and (3) *S. africanus* clusters with *St. coronatus* (bootstrap value < 50%). All nodes recovered in the 50% bootstrap consensus tree are also found in the NJ tree (Fig. 1). The ML analysis resulted in a tree with a topology very similar to the NJ tree. Some differences are found with respect to the South American species and within the Asian clade, but these nodes were not supported in the bootstrap analysis (values < 50%): (1) The subclade containing *S. ornatus*, *S. tyrannus* and *O. isidori* clusters with *St. coronatus*. (2) *S. nanus* is the sister group of all remaining Asian *Spizaetus* representatives. Bootstrap values of MP and ML analyses are included in Fig. 1.

Trees based on the *cytb* data set

The NJ tree based on *cytb* sequences contains representatives of several other genera (Fig. 2) and is rooted with *G. barbatus*. *H. albicilla* besides additional genera included in the *cytb* analysis split off from basal nodes: *P. jefferyi*, *H. novaeguineae* as well as the snake-eagles *Sp. holospilus*, *Sp. cheela* and *C. gallicus*. *B. buteo* is found within the ingroup, clustering with the South American *H. harpyja*. With the exception of *Sp. holospilus*, *Sp. cheela* and *C. gallicus*, which form a highly supported group, relationships of all those branches are ambiguous. In general, the number of nodes with weak bootstrap support (< 50%) is considerably higher than in the CR trees. Despite this unsatisfying result, several clusters in the *cytb* tree are concordant with the CR tree: (1) The clustering of the South American species *S. tyrannus* (Styrtyr3), *S. ornatus* (Sornorn2, Sornvic1) and *O. isidori* (Oisi1a), the latter two species being more closely related (p-distances: 5.1% and 8.4%, respectively), while the distance between them and *S. tyrannus* is slightly higher, ranging from 7.1% to 9.2%. (2) *S. africanus* clusters with a group formed by *H. fasciatus*, *A. audax* and *A. gurneyi* (average p-distance: 3.7%). But, in contrast to the CR tree, *A. chrysaetos* lies outside this subclade. (3) The genera *Hieraaetus* and *Aquila* are paraphyletic. None of the four representatives of the genus *Hieraaetus* cluster together (in addition, in the *cytb* data set, *H. kienerii* and *H. pennatus* were included). With regard to *Aquila*, only two species (*A. audax* and *A. gurneyi*) cluster, whereas *A. pomarina*, *A. chrysaetos* and *A. rapax* appear on three distinct

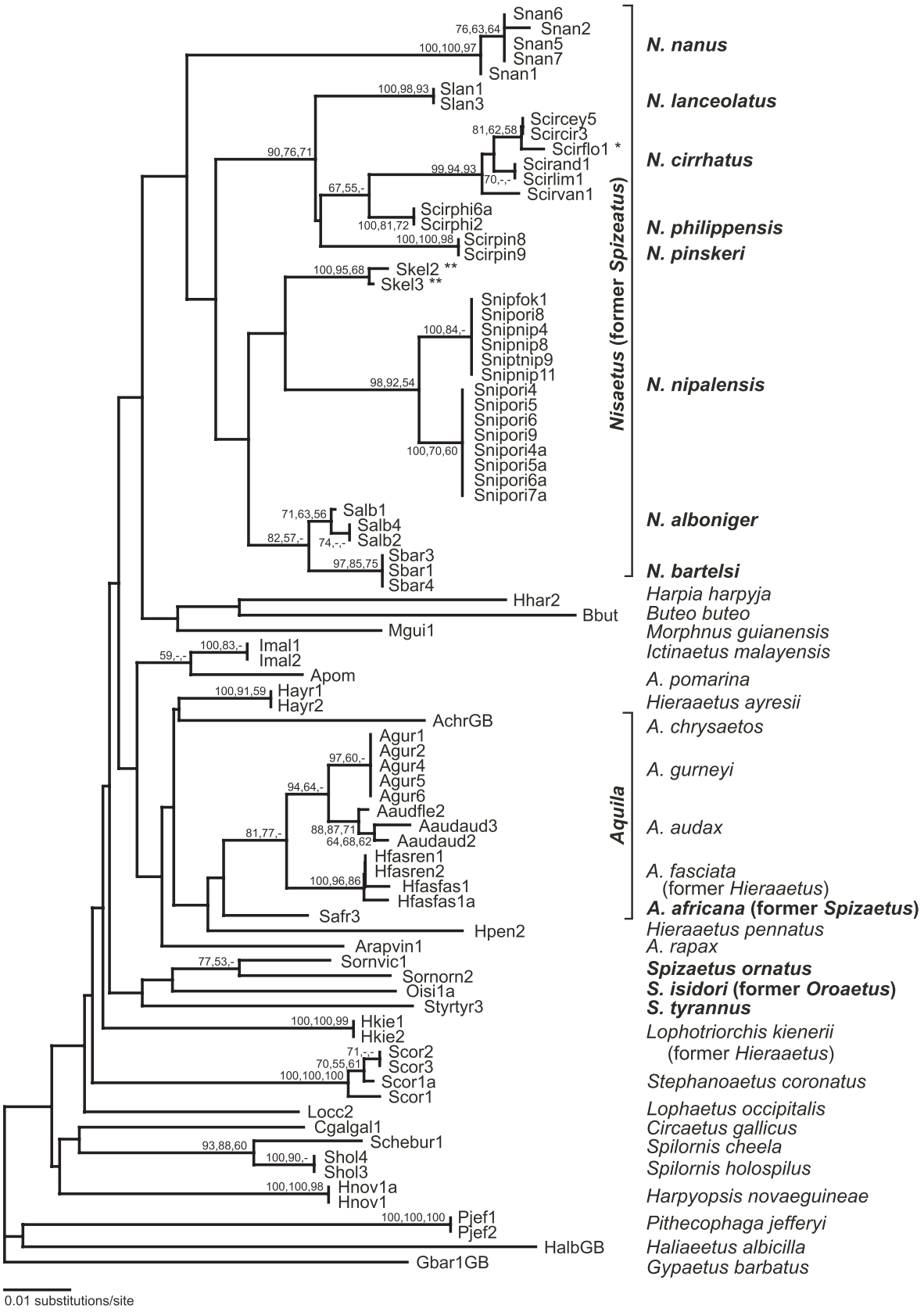

Fig. 2. NJ tree based on *cytb* sequences. Abbreviations of specimens are according to Table 1. Taxonomic names on the right summarize the current state of scientific view. Former and current *Spizaetus* taxa are indicated in bold letters. Bootstrap values of NJ (left), MP (middle) and ML (right) analyses are indicated. * = *N. floris* after Gjershaug et al. (2004a); ** = *N. kelaarti* after Gjershaug et al. (in prep.)

branches. (4) The members of the Asian hawk-eagles form one clade. The topology within the Asian group is identical to the NJ tree based on CR sequences with two exceptions: *S. philippensis* and *S. pinskeri* do not cluster. Moreover, although the same two groups (*S. cirrhatus* and *S. nipalensis*) are found, the position of *S. nanus* is different. In the NJ tree of the CR sequences, *S. nanus* clusters with the species of *S. nipalensis* group, whereas in the *cytb* tree it splits off from the most basal node of the Asian clade with a rather high average distance (9.2%) to the other Asian representatives of the genus *Spizaetus* (for comparison: 7.3% between the *cirrhatus* and *nipalensis* species groups). Comparisons of average p-distances between *cytb* and CR sequences are presented in Table 2. A comparison of CR and *cytb* distances including all taxa investigated reveals that the latter marker reaches saturation of substitutions at considerably lower distances than the CR (data not shown).

The divergent position of *S. nanus* in the *cytb* tree raised doubts whether these sequences are of mitochondrial origin. Nevertheless, no double bands were observed in the electropherograms and the sequences contain no stop codons or insertions/deletions. Except three positions, all substitutions are synonymous with respect to the other members of the Asian clade. The amino acid sequences are identical throughout the Asian clade except three autapomorphic replacements in three sequences. All sequences of *S. nanus* share one amino acid change (methionine–threonine), a replacement that is also found in *A. gurneyi*. Another replacement (Snan-2, -5, -6, -7: isoleucine–valine) is found in four of the *S. nanus* sequences and one in a single sequence (Snan-2: alanine–threonine). We repeated the sequencing of the respective samples in both laboratories. From one of the samples (Snan-1), two PCR reactions performed with different primers yielded the same sequence. Thus, we concluded that these sequences represent functional mt genes rather than nuclear copies (numts).

In the MP analysis, 98 most parsimonious trees were obtained (94 from 107 variable characters were MP informative; TL = 0.389, CI = 0.388, RI = 0.798, RC = .310). Bootstrap support in the MP analysis was lower than in the NJ tree (included in Fig. 2). Only a few clades or subclades comprising more than one species obtained bootstrap values > 50%: (1) the *S. cirrhatus* group, (2) *S. alboniger* + *S. bartelsi*, (3) *H. fasciatus* + (*A. gurneyi* + *A. audax*) and (4) *Sp. holospilus* + *Sp. cheela*. Similarly, in the tree obtained from ML analysis, all nodes are only poorly supported: the only clades with support > 50% were (1) the *S. cirrhatus* group and (2) *P. jefferyi* + (*Sp. holospilus* + *Sp. cheela*).

Discussion

Marker sequences

In general, comparing bootstrap support in the various trees obtained from the two mt sections, the CR appears as more informative marker sequence, even for deeper nodes. Besides the fact that the genus *Spizaetus* is not monophyletic in our trees, the molecular phylogenies based on both the CR and *cytb* data revealed several other paraphylies. Many groupings reflect the geographic affiliations of species. In most respects, our data are in accordance with those obtained with other marker sequences (Bunce et al. 2004; Helbig et al. 2005; Lerner and Mindell 2005). Nevertheless, there are some discrepancies (discussed below), especially in the *cytb* trees. We compared our (shorter) *cytb* sequences with those published by Helbig et al. (2005) and found no discrepancies. All taxa present in both studies proved to yield identical or almost identical sequences. Thus, different clustering may be the consequence of the shortness of our sequences showing the limitations of molecular studies based on museum material. From some museum specimens, often very small quantities of tissue are available for investigation. In such cases, only a limited number of extractions (often only one) and PCR amplifications can be performed, and it is not possible to obtain longer sequences combining sequences of smaller, overlapping fragments. The consequence is that aDNA (ancient DNA) based studies, especially when they include a huge sample, unavoidably are based on short sequences: the samples of bad quality are the limiting factor. On the other hand, an advantage of analysing museum material is that individuals from various geographic populations, from which fresh material cannot be obtained, may be investigated, thus providing comprehensive views on a species. Nevertheless, the limitations in resolution observed in our trees are probably not only due to the limited phylogenetic information obtained from short sequences but may also be a consequence of saturation especially for the *cytb* gene. For deeper nodes, the investigation of nuclear genes undoubtedly provides better resolution (Bunce et al. 2004; Helbig et al. 2005; Lerner and Mindell 2005).

'*Spizaetus* hawk-eagles' – a result of convergent evolution

Spizaetus hawk-eagles have a pantropical distribution. With the exception of Pliocene depositions in Florida (Emslie 1992), no fossil remains of *Spizaetus* have been discovered so far. From the present state of knowledge, it is not possible to locate the geographic origin of the genus. Nevertheless, although bootstrap support in our trees is rather low, especially for the deeper nodes, the following facts regarding the subdivision of the genus *Spizaetus* emerge from the present study: (1) A well supported group (node 3) in the tree comprises several other genera (*Aquila*, *Hieraaetus*, *Ictinaetus*, *Lophaetus*, *Stephanoaetus*), which are interspersed among representatives of *Spizaetus* rendering the genus paraphyletic. (2) The Asian group is unambiguously a monophylum. (3) The South American group appears only distantly related to the Asian *Spizaetus*. (4) *O. isidori* is placed within a well supported South American subclade, and thus the South American *Spizaetus* group too becomes paraphyletic. (5) Obviously, the African *S. africanus* belongs neither to the Asian nor the South American group, irrespective of the fact that its relationships to other eagle genera (e.g. *Aquila*, *Hieraaetus*) are not clearly resolved. These results are in accordance with several other molecular analyses performed recently, indicating that the current genus *Spizaetus* is not of monophyletic origin (Bunce et al. 2004; Helbig et al. 2005; Lerner and Mindell 2005). Thus, the paraphyletic group of the *Spizaetus* eagles appears as another example of convergent evolution. It can be assumed that in these birds several common characters originated independently under similar environmental and social behaviour conditions (Ax 1988).

Jollie (1977) was the only author who assumed that the genus *Spizaetus* consists of two (Asian and South American) groups resembling each other 'as much through convergence, as through common ancestry'. Adaptation to rain forest habitat at the three continents led to independent development of morphological characters (e.g. short rounded wings, long

tails). Hunting behaviour on similar prey species may have caused the development of long and feathered legs with sturdy claws, large powerful bills and the corresponding body size. These highly territorial species developed optical signals for intraspecific recognition, such as contrasting barred ventral body side, and a long and pointed crest in most taxa (Butcher and Rohwer 1989; C. Edelstam in Ferguson-Lees and Christie 2001).

Despite the paraphyly of *Spizaetus* hawk-eagles, the group of booted eagles forms a monophylum (node 3 in Fig. 1) in our trees (CR and *cytb*), which is in accordance with the data presented by other authors (Helbig et al. 2005; Lerner and Mindell 2005). In contrast, the buteonine eagles, the South American species *H. harpyja* and *M. guianensis* as well as *P. jefferyi* (Philippines) and *H. novaeguineae* (New Guinea), as defined by Brown (1976), appear paraphyletic in our *cytb* tree. Moreover, although the resolution is rather low, none of these unbooted eagles is closely related to one of the groups of hawk-eagles. The same is the case with the group of snake eagles (*C. gallicus*, *Sp. holospilus* and *Sp. cheela*).

Which species are the closest relatives of hawk-eagles?

The division of the genus *Spizaetus* into three groups (Asian, African and South American) found in our CR tree is in accordance with the trees based on other sequences (Lerner and Mindell 2005) with respect to the order of splits: The split of the Asian lineage occurred prior to the separation of the South American and African lineages. In our *cytb* tree, this topology is not found, instead the South American taxa split first. Nevertheless, the deeper phylogenetic relationships in the *cytb* tree cannot be regarded as resolved as none of the nodes obtained bootstrap values $>50\%$.

Interestingly, in our CR trees, the quite distantly related sister group of the Asian lineage is *I. malayensis*. Bootstrap support of this node in the ML analysis is rather weak, although this clustering is well supported in the NJ and MP bootstrap analyses. Nevertheless, this grouping is neither found in our *cytb* tree nor in the trees published by Bunce et al. (2004) and Lerner and Mindell (2005). In both the papers, a cluster consisting of *I. malayensis* and *A. pomarina* is highly supported. In Lerner and Mindell (2005), *L. occipitalis* also belongs to that clade. In the tree of Helbig et al. (2005), a group comprising *L. occipitalis*, *A. pomarina*, and *A. clanga* was found, but *I. malayensis* was not included in that study. Considering the relationships found by Lerner and Mindell (2005) to be correct and taking into account that Helbig et al. (2005) suggested to include *A. pomarina* and *A. clanga* into *Lophaetus*, *I. malayensis* should consequently be part of that genus. Nevertheless, it would be worthwhile to analyse this enigmatic species in more detail using all currently employed markers (nuclear and mitochondrial) and more samples of the taxa under discussion.

The Asian hawk-eagles obviously represent an old distinct lineage with no close relatives, which arose and radiated exclusively in Asia without any recent offshoots in other regions. One could hypothesize that the strict morphological adaptation to rain forest habitat restricted the dispersal abilities of the Asian hawk-eagles. In contrast, the second clade to which all remaining members of the booted eagles belong is by far more heterogeneous with respect to geographic distribution and habitat preferences of its members. Therefore, the morphological differences are more pronounced (Parry 2001).

S. africanus, the only African representative, is most closely related to the group containing *H. fasciatus* as well as members of the genus *Aquila*, although this relationship is only poorly supported. Nevertheless, this result is in concordance with the well supported topology in the tree of Lerner and Mindell (2005) based on *cytb* and ND2. The weak bootstrap support for this grouping in our trees could also be due to the small sample of African eagle taxa, e.g., several *Aquila* species or *H. spilogaster* proved to be closely related to *H. fasciatus* according to Wink and Sauer-Gürth (2000). While the exact position of *S. africanus* in the tree is not elucidated with the marker sequences analysed so far, it obviously belongs neither to the Asian nor the South American group of hawk-eagles.

The sister group relationship between the '*Aquila-Hieraaetus*' group and *St. coronatus* as presented in our CR tree (but bootstrap support for this grouping is $<50\%$) was not found in phylogenies previously published. In the tree of Lerner and Mindell (2005), *St. coronatus* is also a member of a group corresponding to our clade 2, but splits off from a more basal node. In contrast, in the tree of Helbig et al. (2005), it is the sister group of the Asian clade. Despite these unresolved relationships concerning *St. coronatus*, the position of *S. africanus* is concordant among our trees (CR and *cytb*) and that of Lerner and Mindell (2005) (*cytb*, ND2). Nevertheless, final conclusions could be made only after a more detailed study including larger samples from all relevant taxa. Conspicuous similarities supporting the molecular data are found between *S. africanus* and *H. fasciatus*/*H. spilogaster* in adult as well as juvenile plumage colour and pattern. These traits might be interesting for taxonomic considerations. Nevertheless, these similarities are not shared with the genus *Aquila*.

The South American representatives of *Spizaetus* (*S. ornatus*, *S. tyrannus*) are not monophyletic in our trees, due to the clustering of *S. ornatus* with *O. isidori*. In the trees published by Helbig et al. (2005) and Lerner and Mindell (2005), *Spizastur melanoleucus* is also a member of this group, being the sister group of *S. ornatus* and *O. isidori*. Thus, with respect to the *Spizaetus* hawk-eagles of the neotropical region, the molecular results are in disagreement with the classical taxonomic view (e.g. Stresemann and Amadon 1979, Thiollay 1994; Ferguson-Lees and Christie 2001). Similarities in juvenile plumage, which often indicate phylogenetic relationships (e.g. Brown 1976), support the molecular results. However, in the South American group, only juvenile *S. ornatus* and *O. isidori* share some similarities in colour and pattern. The finding of Griffiths (1994) that the neotropical *S. ornatus* clusters with Old World *Circaetus*, standing in the vicinity of *Spilornis* based on an investigation of syrinx anatomy, is not at all corroborated by our results. *Spilornis* is clearly not a member of the clade comprising the booted eagles. The molecular data imply that the next relatives of the South American hawk-eagles in a broader sense (including *O. isidori* and *S. melanoleucus*) are no New World raptors but are found among the Old World Aquilinae raptors.

Inter- and intraspecific relationships within the Asian hawk-eagles

The phylogenetic relationships among Asian hawk-eagles were seen controversially in the past. Based on colour and plumage pattern of adult birds, several authors (Thiollay 1994; Ferguson-Lees and Christie 2001) included all but *S. cirrhatus* into

one superspecies, which was largely in concordance with Stresemann (1924), who differentiated only *S. cirrhatus* and *S. nipalensis*. In the latter species, he included all the remaining taxa. However, Amadon (1953) arrived at the conclusion that *S. nanus* is a subspecies of *S. alboniger* and is more distantly related to *S. cirrhatus* and *S. nipalensis*. Ferguson-Lees and Christie (2005) even regard some subspecies of *S. cirrhatus* (*limnaeetus*, *vanheurni*, *andamanensis*) as distinct species, without any supporting data.

Our data provide strong support for the monophyly of the Asian group of hawk-eagles, in which the highest radiation took place. We do not attempt to apply a molecular clock to date the phylogenetic splits since there is no possibility for calibration. Nevertheless, the distances between species belonging to this group (3.0–5.4% in *cytb*) imply that the radiation started probably in the late Pliocene. According to CR data, Asian hawk-eagles are divided into two groups (*S. cirrhatus* group and *S. nipalensis* group). In the *cytb* tree, the same subdivision is found with the exception of *S. nanus*, which splits from the basal node of the Asian group. Nevertheless, this clustering has bootstrap support $< 50\%$, and we assume that the CR tree more accurately reflects the true phylogenetic relationships within the Asian group. This view is also corroborated by the results of Lerner and Mindell (2005), although in that paper only five taxa of this group were included. The members of the *S. cirrhatus* and *S. nipalensis* groups differ also by juvenile plumage characteristics: The *S. nipalensis* group is characterized by rufous-buff to creamy non-patterned ventral side, whereas juveniles of the *S. cirrhatus* group are ventrally pure white.

Interspecific relationships, taxonomy and phylogeographic scenarios within the *S. cirrhatus* group have been discussed in detail previously (Gjershaug et al. 2004a; Gamauf et al. 2005b). A new result arising from the current data is the basal split of *S. lanceolatus* within this group, which is found in both data sets, although bootstrap support is not very good. With respect to the *S. nipalensis* group, this paper provides the first molecular analysis carried out so far. Interspecific divergences of CR sequences within this group are similar to those found within the *S. cirrhatus* group (Table 2), and the more or less trichotomic branching pattern suggests similar time levels when the group split into three branches: *S. nanus*, *S. alboniger* + *S. bartelsi*, and *S. nipalensis*. Thus, the sister group relationship between these branches remains ambiguous. In contrast, the sister group relationship between *S. alboniger* and *S. bartelsi* is well supported in accordance with their geographic proximity. The current subspecific division of *S. nipalensis* is reflected by mt data, assuming that the two mt groups represent *S. n. nipalenis* and *S. n. orientalis*, respectively. Consequently, the individuals from Primorye should be regarded as *S. n. nipalenis* rather than *S. n. orientalis* to which they have been assigned because of their phenotypic appearance (Nechaev et al. 1999). The individuals representing *S. n.* '*fokiensis*' (southern China) and Taiwanese birds are not differentiated genetically in the *cytb* tree, while in the CR tree their distances are in the same magnitude as found among the other members of this group. The differentiations in our trees are observed only between mainland populations (subspecies *nipalenis*, '*fokiensis*', *orientalis*) and Japan (*orientalis*) as well as Southwest India + Sri Lanka (*kelaarti*). The distribution gap in mainland populations between south-eastern Asia and Russian Far East (Primorye Region) is probably a consequence of habitat loss. The genetic similarity between individuals from both regions indicates a former continuous range.

The distinct position of *S. n. kelaarti* is found in the CR tree (average distance to the remaining sequences of *S. nipalensis*: 3.1%) as well as the *cytb* tree, where the distance is even higher (4.4%). Thus, in both *S. n. kelaarti* and *S. nanus*, genetic differentiation seems to be more extreme in *cytb* as compared to CR (Table 2). Assuming that both originated from small populations that might have undergone genetic bottlenecks in small relic rainforest habitats during colder periods of the Pleistocene, founder effects could be a possible explanation for the exaggerated rates of *cytb* in these two lineages. *S. n. kelaarti* will be described as a distinct species based on morphological and vocal differences (Gjershaug *et al.* in prep.).

Taxonomic consequences

In the following, we provide a few suggestions on how to deal with taxonomic inconsistencies resulting from molecular investigations performed so far (Bunce et al. 2004; Helbig at al. 2005; Lerner and Mindell 2005; this study). Although the various trees do not resolve the cladogenesis of booted eagles completely, there are several relationships that are concordant in all analyses. We treat only those cases that are conspicuous and require a taxonomic solution.

The genetic distances between *Hieraaetus kienerii* and other members of *Hieraaetus* (e.g. *H. fasciatus*, *H. pennatus*) are in a similar range as in other monotypic genera, such as *Stephanoaetus* or *Polemaetus*. Despite the fact that its sister group has not been identified so far, it is advisable to separate it from the genus *Hieraaetus* and treat it as a different genus. The use of the genus name *Astur* with the first description of this taxon by Saint-Hilaire in 1835 (but see Dickinson 2005) was not possible because it was preoccupied. Brehm had already used it for the genus *Accipiter* in 1831. Therefore, we propose to give the monotypic species *Hieraaetus kienerii* the resurrected name *Lophotriorchis* Sharpe, 1874.

The division of the genus *Spizaetus* into three groups (Asian, African and South American), as revealed by molecular data, is not in accordance with classical taxonomic classifications based on external morphology and plumage characters. Therefore, taxonomy has to be changed in several points. According to the priority rule of the International Commission on Zoological Nomenclature (ICZN 1999), only the South American taxa can carry the genus name *Spizaetus*, because Vieillot (1816) used it the first time for the taxon formerly described as *Falco ornatus* (Daudin 1800). Two previous classifications would not be in conflict with the phylogenetic relationships deduced from sequence data: Kaup (1850) created an own subgenus *Ptenura* for *S. tyrannus*, whereas Amadon (1982) synonymized *Oroaetus* with *Spizaetus*. It could be proposed to split the South American taxa into four genera: *Spizaetus* (*ornatus*), *Oroaetus* (*isidori*), *Spizastur* (*melanoleucus*) and *Ptenura* (*tyrannus*). But, we tend to follow the conservative opinion of Helbig et al. (2005) retaining the genus name *Spizaetus* for all four taxa.

The genus names of the African *S. africanus* had repeatedly changed in the past, expressing different taxonomical interpretations. The first alternative name was *Limnaetus* after Cassin (1865). Afterwards, Ridgway (cit. in Amadon 1953) renamed it to *Phoeoaetus* since *Limnaetus* was preoccupied (*L. cirrhatus*). Later on, the genus was designated *Cassinaetus*

by Sclater (1922). Most recently, *Hieraaetus* was treated as a synonym of *Aquila* by Dementiev et al. (1966). Considering the molecular trees (Gamauf et al. 2005b; Lerner and Mindell 2005), the phylogenetic relationships of *S. africanus* suggest the following solution: The species clusters with one of several distinct *Aquila* subclades. This group includes the *fasciatus-spilogaster* group, which until recently belonged to the genus *Hieraaetus*. However, because of the priority rule (ICZN 1999), these *Hieraaetus* taxa should be transferred into the genus *Aquila* (Helbig et al. 2005), and consequently *S. africanus* should be included into that genus. Accordingly, its name should be *Aquila africana*.

The new designation of the Southeast Asian taxa is straightforward. As already proposed by Gamauf et al. (2005b) and Helbig et al. (2005), the Asian representatives of the genus *Spizaetus* have to be renamed to *Nisaetus*, since Hodgson in 1836 (cit. in Amadon 1953) has used this genus name first for the description of *Nisaetus nipalensis*.

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Zusammenfassung

Konvergente Evolution und Paraphylie der Haubenadler aus der Gattung Spizaetus: eine phylogenetische Studie auf Basis mitochondrialer Marker

Die phylogenetischen Verwandtschaftsbeziehungen innerhalb der alt- und neuweltlichen Haubenadler (Gattung *Spizaetus*, Aves: Accipitridae) wurden mit Hilfe mitochondrialer DNA-Sequenzen (*Cytochrom b*, Kontrollregion) untersucht. Diese auf 84 Individuen basierende Studie repräsentiert sämtliche *Spizaetus* – Arten mit fast allen gegenwärtig unterschiedenen Unterarten sowie 11 weitere "Adler"-Gattungen mit befiederten und unbefiederten Läufen aus der Neotropis, Aethiopis, Eurasien, Südasien und Australasien (*Oroaetus*, *Harpia*, *Morphnus*, *Lophaetus*, *Stephanoaetus*, *Hieraaetus*, *Aquila*, *Ictinaetus*, *Spilornis*, *Pithecophaga*, *Harpyopsis*). Obwohl die basalen Verbindungen nicht eindeutig geklärt werden konnten, zeigt die vorliegende Untersuchung, dass die Haubenadler eine paraphyletische Gruppe darstellen und daher die phänotypischen Ähnlichkeiten konvergent entstanden sind. Die neuweltlichen *Spizaetus*-Taxa clustern gemeinsam, jedoch liegt die südamerikanische Art *Oroaetus isidori* innerhalb der Klade. Im Gegensatz dazu bilden die südostasiatischen-Taxa eine klar abgetrennte, monophyletische Gruppe. Diese ist in zwei Untergruppen unterteilt, die auch durch verschiedene Jugendgefieder charakterisiert sind. *Spizaetus africanus*, der einzige afrikanische Vertreter dieser Gattung, liegt in einem gemischten Cluster bestehend aus einigen *Aquila*- und *Hieraaetus*-Arten. Diese Ergebnisse stimmen mit anderen Studien, welche auf unterschiedlichen mitochondrialen Markern basieren und jeweils andere Arten umfassen, überein, stehen aber im Widerspruch zur gegenwärtigen Taxonomie. Deshalb schlagen wir vor, die Arten der Gattung *Spizaetus* auf drei Gattungen aufzuteilen: (1) *Spizaetus* (inklusive *Oroaetus isidori*) für die Arten aus Mittel- und Südamerika und (2) *Nisaetus* für die südost-ostasiatische Gruppe. (3) Bezüglich des afrikanischen Taxons (*Spizaetus africanus*) wird eine Zuordnung in die Gattung *Aquila* diskutiert. Weiters empfehlen wir, für die monotypische Art *Hieraaetus kienerii* aufgrund ihrer isolierten Stellung den ehemals verwendeten Gattungsnamen *Lophotriorchis* Sharpe, 1874, wieder einzuführen.

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