J. Zool. Syst. Evol. Research **42** (2004) 145–153
© 2004 Blackwell Verlag, Berlin
ISSN 0947–5745

Received on 27 May 2003

Museum of Natural History Vienna, Vienna, Austria

Molecular phylogeny and biogeography of Honey-buzzards (genera *Pernis* and *Henicopernis*)

A. Gamauf and E. Haring

Abstract

A partial sequence of the *cytb* gene (382 bp) was amplified and sequenced from 35 individuals (mainly museum specimens) of the genus *Pernis* representing all valid taxa (10) and two taxa (*P. p. gurneyi*, *P. p. japonicus*) with questionable validity as well as representatives of the Old World Perninae, namely *Henicopernis* and *Aviceda*, to assess their relationships to the genus *Pernis*. Furthermore, *Gypaetus barbatus*, *Neophron percnopterus*, and *Buteo buteo* were included as outgroup taxa. In the trees derived from the sequence data, *Aviceda* represents the sister group of the genus *Pernis*. The genus *Henicopernis* and the Old World vultures *Gypaetus* and*Neophron* appear rather distantly related to *Pernis*. Within the genus *Pernis*, two of the described species (*Pernis apivorus*, *Pernis ptilorhyncus*) form monophyletic groups, whereas the relationships of the two clades representing three subspecies of *Pernis celebensis* are still uncertain. Although this study is based on comparatively short DNA-sections, the trees deduced from these sequences can be considered as a first approach for inferring the phylogenetic relationships of the genus *Pernis* and related genera and for addressing questions concerning the evolutionary history, biogeography, and systematics of this group.

Key words: Molecular phylogeny – Accipitridae – *Pernis* – *Henicopernis* – biogeography

Introduction

Since the description by Linnaeus (1758), the Honey-buzzards (genus *Pernis*) always had a special position within the family Accipitridae. Among birds of prey they possess some characteristic morphological peculiarities, e.g. a small, pigeon-like head, densely feathered lores with short imbricated feathers, a slight bill with long cere, very short and stout tarsi, long and thin claws. Since birds of prey were considered to have evolved from less predatory birds, e.g. taxa which subsist largely on insects, Peters (1931) recognized only one species (*apivorus* – with five subspeciess) and placed the genus *Pernis*, together with the genera *Aviceda*, *Henicopernis*, *Leptodon*,*Chondrohierax*, and *Elanoides*, into the new subfamily Perninae. All these 'kites' lack the os supraorbitale, a bony shield projecting above the eye that is present in hawks, and therefore this group was assumed to branch off at a basal position within the Accipitridae (Brown 1976).

Because of some phenotypic similarities (Brown and Amadon 1968) the closest relative of the genus *Pernis* was supposed to be the genus *Henicopernis*. Both genera comprise only a few species. Since Stresemann (1940) the genus *Pernis* consists of three species subdivided into 10 subspecies (Vaurie and Amadon 1962), whereas *Henicopernis* consists of two species only (Ferguson-Lees and Christie 2001). For taxonomists the species of the genus *Pernis* are difficult to classify because of some morphometric differences, their plumage polymorphism (colour and pattern), and the presence or absence of a crest. Nevertheless, the systematics of this genus is based mainly on these characters, which have been interpreted controversially in previous studies (e.g. Stresemann 1940; Weick 1980). As a basis for the present work (i.e. classification of samples prior to the analysis), we followed the currently used taxonomy of Thiollay (del Hoyo et al. 1994) and Ferguson-Lees and Christie (2001).

The geographic distribution of the *Pernis* taxa is depicted in Fig. 1. The monotypic Eurasian Honey-buzzard *Pernis apivorus* has the widest geographic distribution, breeding in boreal and temperate open forests and woodlands of western Eurasia. It is highly migratory and winters in afro-tropical and subtropical rain forests as well as wooded savannahs. The Eastern Honey-buzzard *Pernis ptilorhyncus* is usually divided into six subspecies. Only one of them, *P. p. orientalis*, is a migrant which breeds in the eastern Palearctic and winters in south-eastern Asia. All the other taxa are basically sedentary: *P. p. ruficollis* at the Indian subcontinent, Sri Lanka, Indo-China, *P. p. torquatus* in Indo-Malaya (including Sumatra and Borneo), *P. p. ptilorhyncus*as an endemic on Java, *P. p. palawanensis* on Palawan, and *P. p. philippensis* on the largest Philippine Islands. The non-migratory Barred Honey-buzzard *Pernis celebensis* occurs in forested habitats of Sulawesi (nominate form *P. c. celebensis*) and the Philippines (*P. c. winkleri* on Luzon, *P. c. steerei* on the southern Islands). The Long-tailed Honey-buzzard *Henicopernis longicauda* is restricted to the tropical rain forests of New Guinea and adjacent islands, and the Black Honey-buzzard *Henicopernis infuscata* to New Britain.

No fossils exist to provide any direct evidence on the evolutionary age of the Honey-buzzards. Since the oldest fossils from an archaeological site in south-western Bulgaria (Boev 1996) are dated only 9000 BP, they are too young to provide any information about ancestral taxa. The first systematical investigations were performed by Jollie (1977) using ptilodiaomography (plumage development), anatomical and osteological characters, and by Holdaway (1994) who analysed skeletons and feather lice (*Degeeriella*, *Mallophaga*) (Clay in Holdaway 1994). According to these authors, *Pernis* is considered as a basal lineage of the Accipitridae and clusters with two of the Old World vultures, the bearded vulture *Gypaetus barbatus* and the Egyptian vulture *Neophron percnopterus*. Based on karyological analyses also DeBoer and Sinoo (1984) proposed a relationship between *Pernis* and *Gypaetus*. This assumption as well as the basal position of *Pernis* within the accipitrid tree was later corroborated in a molecular study by Wink (1995) and Wink and Sauer-Gürth (2000) using the mitochondrial (mt) cytochrome b (*cytb*) gene as a marker. The only *Pernis* taxon included in molecular studies of birds of prey so far was *P. apivorus* (Seibold and Helbig 1995; Wink 1995; Wink and Sauer-Gürth 2000), but the intrageneric

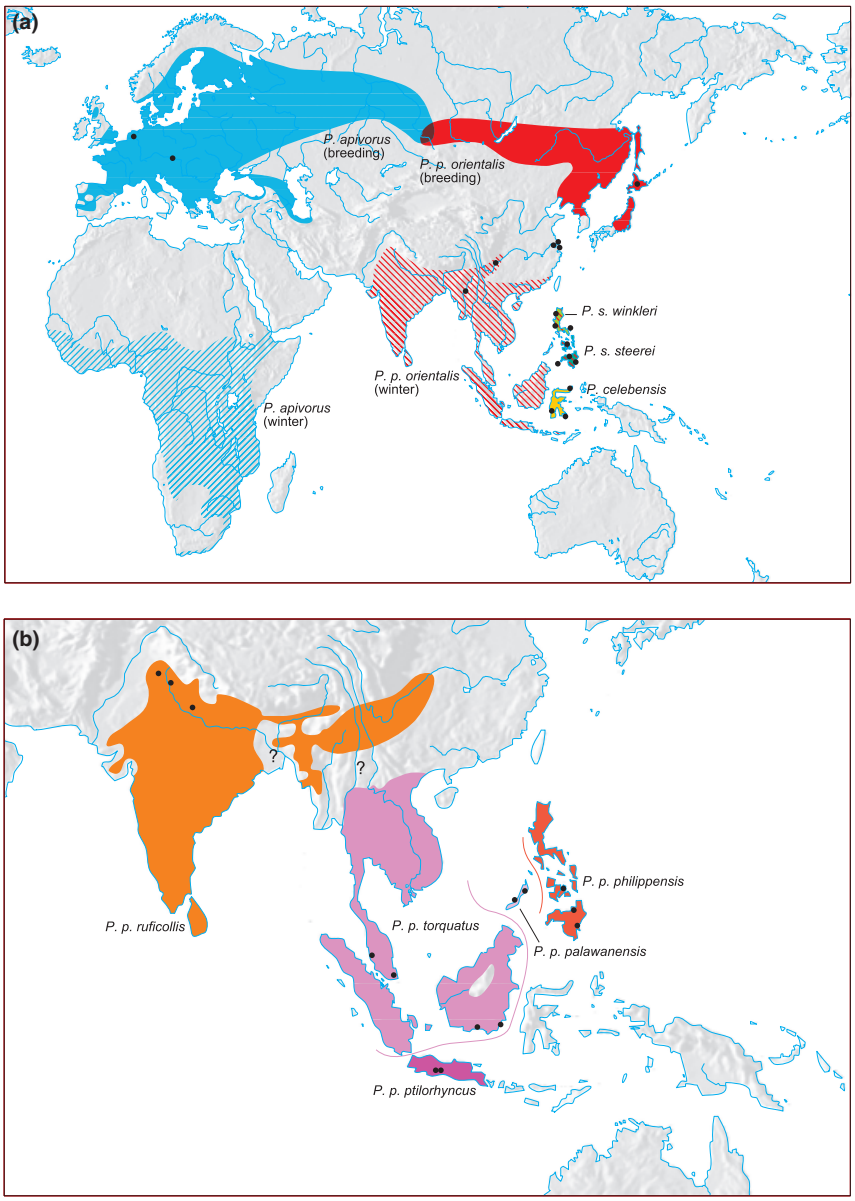

Fig. 1. Distribution of taxa of the genus *Pernis*. Species and subspecies names are according to our proposed taxonomy: (A) *P. apivorus*, *P. p. orientalis*, *P. steerei*, *P. celebensis*. (B) *P. ptilorhyncus* (remaining ssp.)

relationships within the genus *Pernis* have not yet been analysed at the molecular level. In the present study we tried to infer the phylogeny of the Honey-buzzards on the basis of a mitochondrial marker sequence, the *cytb* gene. We included all valid taxa as well as two taxa with questionable validity (*P. p. gurneyi* from Myanmar; Stresemann 1940; *P. p. japonicus* from Japan, Vaurie and Amadon 1962) of the genus *Pernis*. The taxon name *gurney* was included in Brown and Amadon (1968), but since then it was not used. The measurement differences of *japonicus* are not sufficiently well differentiated for its recognition to be warranted (Vaurie and Amadon 1962). Furthermore, we analysed representatives of the Old World Perninae, namely *Henicopernis* and *Aviceda* to assess their relationships to the genus *Pernis*. The following questions will be addressed: (1) Can the currently recognized *Pernis* species/subspecies be differentiated using mtDNA sequences? (2) What are the phylogenetic relationships among species within the genus *Pernis*? (3) Can we draw any conclusions concerning their phylogeography? (4) Where is the position of *P. apivorus* in the phylogenetic tree? (5) Is the genus *Pernis*, as currently defined, a monophyletic group? (6) How closely related is *Pernis* to the genera *Henicopernis* and *Aviceda*?

Materials and methods

Samples

Based on the biogeographical distribution we believe that the genera *Leptodon*,*Chondrohierax*, and *Elanoides*, which occur almost exclusively in the neotropical region, are not closely related to *Pernis*. Therefore we included in our study only the Old World genera *Aviceda* and *Henicopernis* as possible relatives to the genus *Pernis*. Samples from 35 individuals of known origin (see Table 1) of the genera *Pernis*, *Henicopernis*, and *Aviceda* were analysed.

Since it was not possible to obtain fresh tissue from most of the relevant taxa included in this study, we had to rely mainly on museum material (study skins). Fresh material (blood, moulted feathers) was available of *P. apivorus* from its European breeding grounds (Austria, The Netherlands), of *P. p. orientalis* from its Japanese breeding range and overwintering areas in south-eastern China, as well as of *P. p. torquatus* from Malaysia. Tissues from all the remaining taxa, altogether 28 samples, were taken from museum specimens. In three cases (*P. c. winkleri*, *P. p. palawanensis*, *P. p. gurneyi*) we could even

Table 1. Sample list (taxonomy according to our proposed changes)

Taxon	Code of sample	Tissue	Locality, year	Source, voucher	GenBank
Buteo b. buteo	Bbutbut 2	bl	Austria, Haringsee, 1997	H.Frey	AF380305
Henicopernis longicauda	Hlon 2	ba	SE New Guinea, 18?	USNM 89898	AY424399
Gypaetus b. barbatus	Gbarbar GB	bl	Eurasia	Seibold and Helbig (1995)	X86749
Neophron percnopterus ginginianus	Npergin GB	bl	India	Seibold and Helbig (1995)	X86757
Aviceda cuculoides verreauxi	Acucver 1	ba	Central Africa, Matengo Plateau, 1934	NMW 23433	AY424398
Pernis apivorus	Papi 1	bl	Austria, Orth, 1998	H. Frey	AY424396
	Papi 5	bl	The Netherlands, Njimegen, 2000	R. G. Bijlsma	AY424397
	Papi GB	bl	Central Europe	Seibold and Helbig (1995)	X86758
Pernis s. steerei	Pcelste 2	ba	Philippines, Mindanao, Bukidnon, 1951	UMZC 940	AY424386
	Pcelste 3	ba	Philippines, Samar, San Isidoro, 1896	AMNH 247372	AY424387
	Pcelste 7	ba	Philippines, Mindanao, Mt Matutum, 1966	USNM 578095	AY424388
	Pcelste 9	ba	Philippines, Basilan	AMNH 531836	AY424389
Pernis s. winkleri	Pcelwin 1	ba	Philippines, Luzon, Bataan, 1881	ZMB 25.464, holotype	AY424390
	Pcelwin 2	ba	Philippines, NW Luzon, Benguet Dist., 1894	BMNH 1897.6.14.12	AY424391
	Pcelwin 3	ba	Philippines, Pollilo Is., 1960	AMNH 782.342	AY424392
Pernis celebensis	Pcelcel 2	ba	Indonesia, S Sulawesi, Lombasang 1931	ZMB 33.118	AY424393
	Pcelcel 5	ba	Indonesia, N Sulawesi, Bolaeng, 1917	L 40.721 (14)	AY424394
	Pcelcel 6	ba	Indonesia, Muna Is., Labasa, 1948	L 40.960	AY424395
Pernis ptilorhyncus orientalis	Pptiori 4	fe	China, Mt Omei, 1923	USNM 297743	AY424380
	Pptiori 10	fe	SE China, Shanghai, 2000	T. Yamasaki	AY424381
	Pptiori 11	fe	SE China, Shanghai, 2000	T. Yamasaki	AY424382
	Pptiori 12	fe	SE China, Shanghai, 2000	T. Yamasaki	AY424383
	Pptiori 14	fe	Japan, Hokkaido, 2001	K. Saito, 'japonicus'	AY424384
	Pptiori 15	ba	Myanmar, Mandalay, 1938	ZMB 39.733, '*gurneyi*'-holotype	AY424385
	Pptiori 16		Philippines, Negros	AMNH 18.019	AY424376
Pernis p. philippensis	Pptiphi 2	ba	Philippines, Mindanao, Davao, 1910	BMNH 1913.9.8.14	AY424377
	Pptiphi 3	ba	Philippines, Cebu, Toledo, 1892	USNM 314856	AY424378
	Pptiphi 4	ba	Philippines, Mindanao, Inahanan, 1965	USNM 578098	AY424379
Pernis p. ruficollis	Pptiruf 2	ba	India, Himalaya, 1834	NMW 44.145	AY424373
	Pptiruf 3	ba	N India, Punjab 1923	BMNH 1949.Whi.1.92	AY424374
	Pptiruf 6	ba	India, Saharanpur, 1878	BMNH 885.8.19.2006	AY424375
Pernis p. torquatus	Pptitor 1	ba	Indonesia, SE Borneo, Klumpeng Bay, 1908	USNM 181443	AY424365
	Pptitor 3	ba	Malaysia, Trengganu, 1958	USNM 470559	AY424366
	Pptitor 6	ba	Indonesia, SE Borneo, Rantan, 1916	L 4617 (10)	AY424367
	Pptitor 7	fe	Malaysia, Kuala Lumpur, 2001	L. Poh, M. Chong	AY424368
Pernis p. ptilorhyncus	Pptipti 2	ba	Indonesia, Java, Tinggardjaja, 1928	L 98.855 (9)	AY424371
	Pptipti 3	ba	Indonesia, Java, Tinggardjaja, 1931	L 98.856 (10)	AY424372
Pernis p. palawanensis	Pptipal 1	ba	Philippines, Palawan, 1887	AMNH 531.839	AY424369
	Pptipal 2	ba	Philippines, Palawan, Puerto Princesa, 1887	Mus. Tierkunde Dresden 9369, holotype	AY424370

investigate samples of the holotypes. To assess genetic variability within the numerous Southeast Asian islands, up to seven samples per taxon were analysed (e.g. *P. p. torquatus, P. p. philippensis, P. c. steerei, P. c. winkleri*). Since some of the SE Asian taxa are sometimes difficult to distinguish we used only material of nestlings and adults collected during their breeding season. Previously published sequences of the following taxa were used: *P. apivorus* (X86758, Seibold and Helbig 1995), *Gypaetus b. barbatus* (X86749, Seibold and Helbig 1995), *Neophron p. ginginianus* (X86757, Seibold and Helbig 1995), *Buteo b. buteo* (AF380305, Haring et al. 2001). The sequences of four Central European individuals of *P. apivorus* published by Seibold and Helbig (1995) from Germany (2), Austria (1), and Switzerland (1) are identical and therefore only one sequence (X86749) was included.

DNA extraction

DNA extractions from museum material (skin from the foot pads of study skins) were performed in a 10% Chelex (Biorad) solution containing proteinase K (0.5 mg ml^{-1}). After incubation (4 h, 50°C, with agitation) solutions were heated to 95°C for 5 min and centrifuged for 1 min. For purification and to remove short fragments of degraded DNA the supernatant was purified using the QIA Quick PCR Purification Kit (QIAGEN, Venlo, NL) with a final volume of 30–70 μl elution buffer. DNA from fresh tissue was extracted by overnight incubation at 37°C in extraction buffer (10 mM Tris–HCl, pH 8.0, 10 mM ethylene diaminetetraacetic acid (EDTA), 50 mM NaCl, 40 mM dithiothreitol, 1% sodium dodecyl sulphate (SDS), 0.5 mg ml^{-1} proteinase K). DNA was purified by two PCI (phenol/chloroform/isoamylalcohol, 25 : 24 : 1) and one CI (chloroform/isoamylalcohol, 24 : 1) extractions followed by precipitation with 1/10 volume 3 M NaAc, 3× volume EtOH 96%. Control extractions with pure extraction buffer (without tissue) were prepared for the polymerase chain reaction (PCR) experiments.

PCR amplification

PCR was carried out with an Eppendorf thermocycler in a volume of 25 μl, containing 1 unit Dynazyme DNA polymerase (Finnzymes OY), 0.5 μM of each primer, and 0.2 mM of each dNTP. The solutions were heated to 95°C (2 min) and then put through 30 reaction cycles: 95°C (10 s), 59°C (10 s), 72°C (30 s), followed by a final extension at 72°C (5 min). Since the major part of the study was based on tissue of museum specimens, the expected maximum length of PCR fragments is <400 bp. The following PCR primers were used: cytb1+: 5'-CAA

Table 2. Uncorrected distances within and between clades (1–5, corresponding to clades in Figs 1 and 2) and lineages representing other genera

	Clade 5 pti/pal/tor	Clade 4 phi/ori/ruf	Clade 3 cel	Clade 2 ste/win	Clade 1 api	A. cuc	Gyp/Neo	H. lon
Clade 5	*0.72*	–	–	–	–	–	–	–
	0–1.51							
Clade 4	2.68	*0.76*	–	–	–	–	–	–
	2.11–3.92	*0–1.81*						
Clade 3	4.66	4.71	*0.20*	–	–	–	–	–
	3.92–5.42	4.22–5.72	*0–0.30*					
Clade 2	4.85	4.22	3.33	*0.43*	–	–	–	–
	3.92–5.42	3.61–5.12	3.01–3.92	*0–0.90*				
Clade 1	5.91	5.82	6.12	4.43	*0.00*	–	–	–
	5.42–6.33	5.42–6.63	6.02–6.33	4.22–4.82	*0.00*			
A. cuc	9.37	9.94	9.14	8.65	10.24	–	–	–
	9.04–9.94	9.64–10.54	9.04–9.34	8.43–9.04	–			
Gyp/Neo	10.79	11.47	10.74	10.31	11.45	11.47	–	–
	9.94–11.45	10.84–12.35	10.24–11.45	9.64–11.15	10.24–12.65	11.45–12.05		
H. lon	12.16	12.70	12.15	11.96	12.35	12.95	11.45	–
	11.75–12.35	12.35–13.25	12.05–12.35	11.75–12.35	–	–	11.15–11.75	
B. but	11.90	11.52	13.35	12.26	12.05	15.06	13.86	12.952
	11.45–12.05	11.15–12.35	13.25–13.55	12.05–12.65	–	–	13.55–14.16	–

A. cuc, *Aviceda cuculoides*; Gyp/Neo, *Gypaetus barbatus*/*Neophron percnopterus*; H. lon, *Henicopernis longicauda*; B. but, *Buteo buteo*. Ranges of distances are given below average distances. Distances within the five clades of *Pernis* are in italics.

CATCTCAGCATGATGAAACTTCG-3', cytb2-: 5'-TGCTGAGAA TAGGTTGGTGATGAC-3' which amplify a 382 bp section of the *cytb* gene. For PCR reactions with DNA from fresh tissue 50–200 ng were used as template DNA. Optimal amounts of template DNA of Chelex extractions were determined empirically (2–10 μl of the DNA solution). If necessary, reamplifications were performed with 1–2 μl template. Negative controls for PCR reactions were performed to screen for contaminated reagents: (1) control extractions (without DNA) instead of template; (2) reaction with H_2O instead of template.

Cloning and sequencing

PCR products were extracted from agarose gels using the QIA Quick Gel Extraction Kit (QIAGEN) and cloned (TOPO TA Cloning Kit®, Invitrogen, Carlsbaad, USA). Sequencing (both directions) was performed by MWG-Biotech (Ebersberg, Germany). The sequences determined in the course of the present study are registered under the GenBank accession numbers included in Table 1.

Sequence analysis

Alignments were produced manually. The alignment (including also the GenBank sequences from *P. apivorus*, *G. barbatus*, *N. percnopterus*, and *B. buteo*) has a length of 332 sites. The reading frames of all sequences proved to be intact suggesting that the sequences are derived from functional mitochondrial genes. Both distance (neighbour-joining algorithm, NJ; Saitou and Nei 1987) and maximum parsimony (MP) methods were used to infer the 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. Using other models for the computation of distances did not alter the topologies of the trees. MP trees were generated with heuristic search using the TBR (tree bisection reconnection) algorithm and a random taxon addition sequence (1000 replicates). All characters were weighted equally.

Results

The partial sequence of the *cytb* gene was amplified and sequenced from 35 specimens representing 12 taxa. Based on the alignment, which included the published sequences of *P. apivorus*, *G. barbatus*, *N. percnopterus*, and *B. buteo*, ranges of distances and average distances between and within taxa (i.e. groups) were calculated (Table 2).

Distances and outgroup taxa

Initially we planned to use *B. buteo* as an outgroup to infer the relationships between *Pernis* and the genera *Gypaetus*, *Neophron*, *Henicopernis*, and *Aviceda*. Yet, the distances found between *H. longicauda* and the sequences of the taxa representing the genera *Gypaetus*, *Neophron*, *Aviceda*, and *Pernis* are considerably high (11.2–13.0%) similar to those between *B. buteo* and the ingroup taxa ranging from 11.2–14.2% and that between *H. longicauda* and *B. buteo* (13%). Thus, no closer relationship between *H. longicauda* and the genera *Pernis* or *Aviceda* can be deduced from our data. Consequently, any of these four taxa (representing *Buteo*, *Gypaetus*, *Neophron*, *Henicopernis*) could be used as an outgroup species, which becomes also apparent when midpoint rooting is performed (both in MP as well as NJ trees).

Phylogenetic analysis

The MP analysis resulted in a single most parsimonious tree (Fig. 2) in which *Aviceda* is the sister genus to a cluster composed of all *Pernis* sequences. Within *Pernis*, *P. apivorus* stands basal (clade 1 in Fig. 2) followed by a clade (2) representing seven specimens of *P. celebensis* (subspecies *steerei* and *winkleri*). The three sequences of nominate *P. c. celebensis* are found in a separate clade (3). Clade 3 is the sister group to the *P. ptilorhyncus* clade which is further divided into two distinct groups: one containing the subspecies *orientalis*, *philippensis*, and *ruficollis* (clade 4), the other consisting of the subspecies *ptilorhyncus*, *torquatus*, and *palawanensis* (clade 5).

With one exception the MP tree has the same topology as the corresponding NJ dendrogram (not shown). While in the MP tree, *P. apivorus* stands basal to the remaining *Pernis* taxa, it clusters with the Philippine *P. c. steerei–winkleri* clade II in the

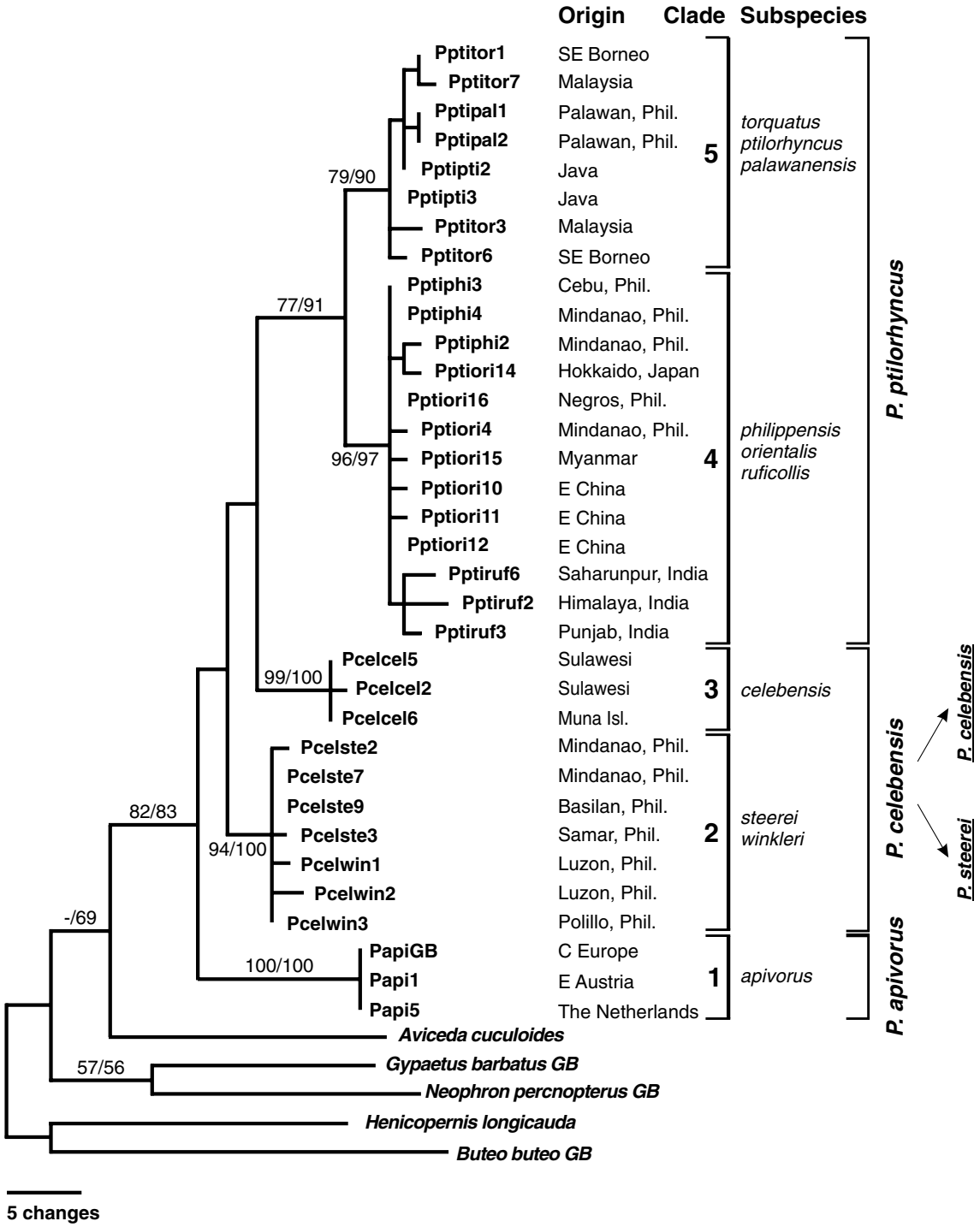

Fig. 2. Molecular phylogeny of the genus *Pernis*. MP tree based on a partial *cytb* sequence (outgroup: *Henicopernis*, *Buteo*). TL = 0.184, CI = 0.717, RI = 0.825, RC = 0.594. Clades 1–5 are described in the text. Bootstrap values (1000 replicates) > 50% are given at the nodes (left: MP, right: NJ). Assignment according to current taxonomy as well as our proposed taxonomical change (underlined) are included

NJ tree. Thus, *P. celebensis* appears paraphyletic in both trees. The incongruence between the two trees is also reflected in the results of the bootstrap analyses. Using both algorithms (MP, NJ) the topology of a bootstrap consensus tree (70% majority rule) is the same. When only *Aviceda* is used as outgroup and the other (more distinct) taxa are excluded from the analyses, the clustering of *P. apivorus* with the *P. c. steerei–winkleri* clade is observed in both the MP and the NJ tree. Nevertheless, this grouping is not supported in the bootstrap analyses (values below 50%). Fig. 3 shows the bootstrap consensus tree (70% majority rule) of these analyses. Whereas high bootstrap values are observed for each of the five clades as well as for the clustering of clades 4 and 5 (corresponding to the species *P. ptilorhyncus*), the clustering among the remaining clades is not supported by the bootstrap analyses (nodes with values < 70% collapsed).

With respect to haplotype diversity within the five clades *P. apivorus* is homogenous (no differences among six

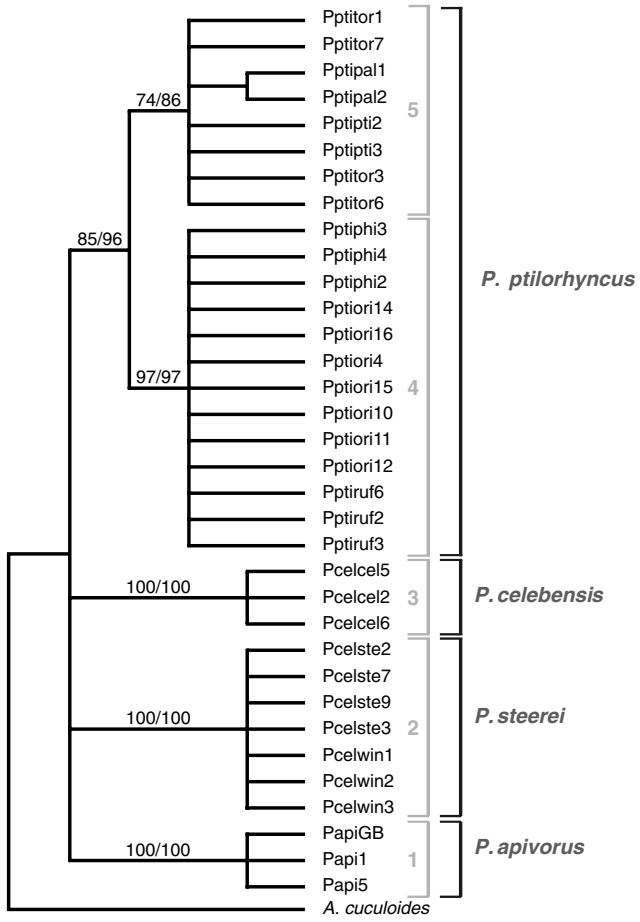

Fig. 3. Bootstrap consensus tree (70% majority rule) depicting the five lineages found within *Pernis*. *A. cuculoides* was used as outgroup. Bootstrap values (1000 replicates) are given above branches (left: MP, right: NJ). Clades 1–5 are described in the text. Assignment according to our proposed taxonomic change

sequences), while most of the other taxa show some sequence variability. Among the seven Philippine representatives of the Barred Honey-buzzard (*P. c. steerei–winkleri* clade 2) each of the samples represents a distinct haplotype. Within the nominate form *P. c. celebensis*, which is represented by three specimens (clade 3), two haplotypes are found, one from South Sulawesi and the other from the birds from North Sulawesi and Muna Island. Within clades 4 and 5, 16 of the 21 specimens possess a unique haplotype. The migratory *P. p. orientalis* is represented by seven samples, each displaying a different haplotype. The three specimens of *P. p. ruficollis* from the Indian subcontinent form a cluster of three haplotypes. Clade 4 contains the sedentary taxa of the region extending from India to Indo-Malaya, the Greater Sundas, and the Philippines. Within this clade only the two specimens of *P. p. palawanensis*, which is endemic to Palawan and Calamias Islands, show the same haplotype. The other specimens representing the subspecies *torquatus* (mainland, Sumatra, Borneo) and *ptilorhyncus* (Java) have different haplotypes.

Discussion

The molecular phylogeny presented in this paper can be considered as a first approach for inferring the phylogenetic relationships of the genus *Pernis* and related genera and for addressing questions concerning the evolutionary history, biogeography, and systematics of this group. We are aware that some unresolved relationships might result from saturation (basal splits) and the fact that the data are based on short DNA sections only. Nevertheless, this study gives first estimations on intra- and interspecific diversity as well as on the magnitude of divergence between *Pernis*, *Aviceda*, and *Henicopernis*, and thus might provide the basis for further investigations. Especially for assessing the more basal relationships other marker sequences (e.g. the more slowly evolving mitochondrial 12S or 16S rRNA genes) could be more promising. Which of the questions raised in the introduction can be answered on the basis of our data?

Relationships to other genera

The two Old World vultures *Gypaetus* and *Neophron* were included into the study because they appeared as most closely related to *Pernis* in molecular trees of accipitrids published so far (Seibold and Helbig 1995; Wink 1995). A closer relationship between *Gypaetus* and*Neophron* and *Pernis* is not found in our trees. In addition, *Henicopernis* does not cluster with *Gypaetus* and *Neophron*, but rather may belong to an old endemic Australasiatic lineage (Thiollay in del Hoyo et al. 1994; Olsen 1995) standing somewhere between the *Gypaetus*/*Neophron* lineage and *Buteo*. Thus, it seems likely that the three genera *Pernis*, *Aviceda*, *Henicopernis* as well as *Gypaetus*/*Neophron* (Wink 1995) might represent old phylogenetic lineages of raptors. Their phylogenetic relationships should be investigated with a more conserved (e.g. nuclear) marker sequence. Furthermore, additional genera should be included. The special position of *Pernis* within the Accipitridae has been also demonstrated by histological studies of egg shells. The Accipitridae differ from the Falconidae in the ratio between total nitrogen and soluble nitrogen of the shell, but *Pernis* and *Pandion* (which has a special position within Accipitridae too) are intermediate (Tyler 1966).

The fact that, due to the separated position of *Henicopernis*, the representatives of Perninae do not form a monophyletic group might also be explained by sequence saturation within the *cytb* gene observed among the distantly related taxa of our sample. In any case, *Henicopernis* and *Pernis* are only distantly related which is also in accordance with the findings of Griffiths (1994). The morphological similarities between them may be explained by convergent evolution of specific characters in adaptation to similar functions (e.g. hymenoptera larvae as main prey) under similar environmental conditions (Coates 1985). Among the Old World genera of the subfamily Perninae (Peters 1931) *Aviceda* seems to be the closest relative to *Pernis*, as already proposed by Jollie (1977). The typical display behaviour of *Aviceda* (*A. cuculoides* in Brown et al. 1982; *A. subcristata* in Coates 1985, Marchant and Higgins 1993), which resembles very closely that of *Pernis*, but is quite different from that of *Henicopernis* (*H. longicauda* in Coates 1985), supports this relationship. Furthermore, Brown et al. (1982) mention the similarity of the vocalization of the two genera.

The genus *Pernis*

The genus *Pernis* clearly appears as a monophyletic group in our trees. Within *Pernis*, two of the described species

Table 3. Proposed classification of Honey-buzzards

Genus
Pernis
Species
Pernis apivorus
Pernis steerei (with ssp. *steerei, winkleri*)
Pernis celebensis
Pernis ptilorhyncus (with ssp. *orientalis, ruficollis, philippensis, torquatus, ptilorhyncus*, *palawanensis*)

(*P. apivorus*, *P. ptilorhyncus*) are monophyletic, whereas the relationships of the two clades representing *P. c. celebensis*/*steerei* are still uncertain. The genetic division (average distance 3.3%), together with morphological differences (shape, plumage characters, presence or absence of a crest), between these two groups might be considered as an argument for splitting *P. celebensis* into two rainforest species: *P. celebensis*, endemic to Sulawesi, and *P. steerei* in the Philippines. Compared to sequence divergences between other raptor species the distances found between clades 2, 3, and 4/5 are in the range of that observed between 'good species' (e.g. *Haliaeetus albicilla*/*Haliaeetus leucocephalus*, 2.5%; *Falco eleonorae*/*Falco concolor*, 2.7%; *Milvus milvus*/*Milvus migrans* 1.7%; Helbig et al. 1995).

Similar arguments may be put forward to split *P. ptilorhyncus* into two species. Average distances between the two clades of *P. ptilorhyncus* are comparatively high (clade 4 versus 5; 2.7%). Nevertheless, a splitting into two species might be premature because are lacking so far unequivocal morphological differences characteristic for each of the two groups. Table 3 summarizes our suggestions for a reasonable classification of the genus *Pernis* based on this investigation.

Concerning earlier proposed taxonomic alterations, the sequence data clearly show that *P. ptilorhyncus* represents a well-separated species which is neither conspecific with *P. apivorus* (Hartert 1914; Swann 1926; Peters 1931; Vaurie and Amadon 1962; Stresemann and Amadon 1979; Weick 1980) nor forms a superspecies with *P. apivorus* (Thiollay in del Hoyo et al. 1994). This interpretation is also corroborated by the fact that the two species do not hybridize in the overlapping parts of their distribution ranges in central Siberia (Mosquitin 1973; Kislenko 1974; Stepanyan 1983), although this had been assumed in the past (Johansen 1957). *Pernis ptilorhyncus* is not even the closest relative of *P. apivorus* as has been assumed for a long time (Stresemann 1940). Nevertheless, the position of *P. apivorus* at the base of representatives of the SE Asian *Pernis* radiation, as suggested by the MP analysis, still needs confirmation (i.e. using another/longer marker sequence). Average distances between *P. apivorus* and any of the five clades are higher than those in comparisons among the other clades suggesting that in the cladogenesis of this genus *P. apivorus* split off first.

Within each of the clades 1–5 (i.e. groups of subspecies) the distribution of haplotypes is not congruent with subspecies division (=geographic division). For example, no clear genetic differentiation within the *cytb* gene is found between the two subspecies *P. s. winkleri* from Luzon and *P. s. steerei*, which differ clearly in morphology and plumage (Gamauf and Preleuthner 1998). The same is true for the subspecies within clade 5: *P. p. torquatus* (Indo-Malayan mainland, Sumatra, Borneo) and *P. p. ptilorhyncus* (Java). Within clade 4 only *P. p. ruficollis* appears as a cluster, whereas the largest subspecies *P. p. philippensis* (Mayr 1939) and *P. p. orientalis*, which differ morphologically and in plumage characters more obviously, possess almost identical haplotypes (differing only by up to three substitutions). The sequences of *P. p. japonicus* (Pptiori14) as well as of the holotype of *P. p. gurneyi* (Pptiori15) (Stresemann 1940) from Lamaing/Mandalay, Myanmar, are found among *P. p. orientalis* specimens. The latter taxon name, although included in Brown and Amadon (1968), was never used since then. Thus, our data suggest that the usage of the name *P. p. gurneyi* (supported by Vaurie and Amadon 1962) is not meaningful and the (holotype) specimen is obviously a juvenile *orientalis* collected in its wintering quarter.

The distribution of haplotypes within clades 2–5 is in accordance with the assumption of quite recent radiations and colonization events on the various islands. Incomplete lineage sorting and/or frequent gene flow between populations of the different subspecies might account for the genetic homogeneity. On the other hand, the clear separation into two distinct groups within each of the species *P. ptilorhyncus* and *P. celebensis* is rather surprising and may be best explained on the basis of biogeographic considerations.

Biogeography

The five clades detected in the sequence analyses represent different levels of sequence divergence (see also Table 2). There are some reasons why we do not want to apply a molecular clock to date the splits between these lineages: (1) there are no dated fossils to calibrate this clock, (2) the 'universal' divergence rate (2% per My; as suggested by e.g. Klicka and Zink 1997) may be roughly correct, but probably not suitable for the more recent splits, and (3) the short section of the *cytb* gene used in our analysis may even not be representative for the complete gene with respect to evolutionary rate. Nevertheless, average sequence divergences can be used to reconstruct the succession of splits in connection with the oscillations of warm and cold phases during the Ice Ages.

The first level (5–6%) corresponds to the split between clade 1 (i.e. *P. apivorus*) and the rest and has most probably occurred already in the Pliocene (>1.7 My). Wherever the species representing the common ancestor of the extant *Pernis* taxa may have evolved (most probably in Southeast Asia), this first separation is also a west/east division, namely between the western *P. apivorus* and the Southeast Asian radiation. It could be hypothesized that an ancestral species expanded its range as a result of climatic amelioration into the western direction first and then in a ring-like manner around the large area of unsuitable habitat represented by the Himalayas forming a kind of 'ring-species' (Mayr 1963; Collinson 2001; Irwin et al. 2001). Alternatively one could assume an Asian/African vicariant distribution of the ancestral form (a similar example within Accipitridae:*Gypsbengalensis*/*indicus*/*tenuirostris* – *G. africanus*; Ferguson-Lees and Christie 2001). According to this hypothesis the African species *P. apivorus* would have shifted (due to climatic changes) its breeding range more and more up to the north. The connection between the west Palearctic and the Asian distribution ranges was interrupted by aridity in the Near and Middle East. Nevertheless, under this hypothesis, the question remains why *P. apivorus* does not breed in Africa.

The second level (3–5%) involves the separation of the three lineages representing clades 2, 3, and 4 + 5. This does not

necessarily mean that these lineages separated simultaneously, but rather that we cannot reconstruct the exact order of splits. In fact, the substitution rate within the *P. s. steerei–winkleri* clade (2) seems to be slightly lower compared to the others, which becomes apparent from the lower distances found in comparison to clade 1, but also to clades 3 or 4 and 5. During phases of low sea level (=cold periods) the ancestral form of the Southeast Asian radiation was probably distributed throughout Southeast Asia including the periodically dry fallen regions of the Sunda Shelf (Sumatra, Java, Borneo, Palawan), the isolated Philippines (Heaney 1985), and also Sulawesi (Whitten et al. 1987) across the Wallace line (Simpson 1977; van Oosterzee 1997). This continuous distribution area may have been split in the course of the following warm phase with high sea level resulting in the isolation of the populations on the Philippines (clade 2) and Sulawesi (clade 3) from the remaining populations in India, Indo-Malaysia, and the Greater Sundas (clades 4 + 5). Alternatively, the mainland may have been re-colonized during an interglacial from a refuge on the Sunda Shelf. The question why the Philippine and Sulawesi populations remained isolated in subsequent cold periods, can be explained by selective constraints caused by their morphological and ecological adaptation to tropical rain forest. Thus, they where no longer able to cross open water or larger areas devoid of forest (Gamauf et al. 1998a).

The third level (2–3%) represents the youngest split in our trees, namely between clades 4 and 5, which means the geographic separation between Southeast Siberia/India/Philippines (4) and Indo-Malaya/Greater Sundas (5). Whereas for the separation of island forms normally the rising sea level (change from cold to warm) is the most plausible scenario, in the case of the separation between lineages 4 and 5 two possibilities appear likely: (1) One could assume a continuous refuge throughout the Sunda Shelf and the adjacent continent in a cold period, which was split up in the following warm period when the continent was separated from the islands by rising sea levels. Then, the continental form could have spread north and finally reached its present wide distribution. (2) Another possibility is the assumption of two more remote refugial areas, one in the (dry fallen) Greater Sunda Shelf, and one, e.g. in the wooded lowlands of eastern China (which no longer exist because of human activities), or on the Indian subcontinent. In connection with population bottlenecks this scenario would also explain the rather low genetic variability within the two clades. The distances within clades are generally below 1% with the exception of those found among the sequences of the *P. p. ruficollis* cluster. A separation into a western (Indian) and an eastern (Chinese) population during the last cold period might account for that. The most recent expansion of the continental population led to the present huge distribution and the differentiation of the various subspecies. Similarly, differentiation of island forms of clades 2 (*winkleri* versus *steerei*) and 5 (*torquatus*, *ptilorynchus*, *palawanensis*) occurred very recently.

Migration behaviour and plumage characters as phylogenetic traits

Long distance migration was developed two times independently: in *P. apivorus*, which reaches even South Africa, and in *P. p. orientalis*, which migrates as far as to the Lesser Sundas. In both species migrating behaviour may have arisen as an answer to climatic changes during the Pleistocene. *Pernis p. orientalis* may have descended from an ancestral form living on the Indian subcontinent. *Pernis p. philippensis* is probably the most recent offshoot of migrating *P. p. orientalis*, overwintering at the Philippines. Thus, migration behaviour as a phylogenetically informative trait can result in misleading interpretations. The same is true for plumage characters, e.g. the presence or absence of a crest (*P. apivorus*, *P. celebensis*, *P. p. orientalis*, *P. p. philippensis*, *P. p. palawanensis* lack a crest). Also homogeneity of plumage colour and pattern, as found exclusively in the island taxa (*P. s. steerei*, *P. s. winkleri*, *P. celebensis*, *P. p. philippensis*, *P. p. ptilorhyncus*, *P. p. palawanensis*) may rather reflect ecological and behavioural adaptations (e.g. mimicry: Gamauf et al. 1998b; van Balen et al. 1999) than phylogenetic relationships. Island-rich Southeast Asia, a region that is a hot spot of diversification and speciation for many bird species (Stattersfield et al. 1998), also represents the centre of diversity for the Honey-buzzards. Together with climatic changes migration may have played a major part in the radiation of *Pernis*. For example, multiple colonization took place at the Philippines, where three taxa (two species) live sympatrically (*P. steerei*, *P. p. philippensis* as residents, and *P. p. orientalis* as wintering guest).

Acknowledgements

We thank R. G. Bijlsma (Wapse, The Netherlands) and H. Frey (University of Veterinary Medicine Vienna/EGS Haringsee, Austria) for providing blood of *P. apivorus*, and K. Saito (Hokuto Kushiro, Japan), T. Yamasaki (Tokyo, Japan), L. Poh and M. Chong (Kuala Lumpur, Malaysia) for moulted feathers of *P. p. orientalis* and *P. p. torquatus*, respectively. Without tissues of museum specimens this work would have been impossible, therefore, we are grateful to R. Prŷs-Jones, M. Adams, F. Steinheimer (The Natural History Museum/BMNH, Tring, UK), G. Barrowclough, P. Sweet (AMNH, NY, USA), G. Graves, J. Dean (USNM, Smithsonian Institution, Washington, DC, USA), E. Bauernfeind (NMW, Vienna, Austria), S. Frahnert (ZMB, Berlin, Germany), S. Eck (Zool. Mus. Dresden, Germany), R.v.d. Elzen (ZFMK Bonn, Germany), R. Dekker (Naturalis/RMNH-Leiden, The Netherlands), J. Fjeldså (Copenhagen, Denmark), A. Sumalde (Univ. Phil. at Los Baños, Philippines). We thank B. Däubl for excellent technical assistance and W. Mayer, W. Pinsker, and H. Winkler for helpful discussions and comments on the manuscript. Thanks also to J. Shergalin (Tallinn, EST) for doing the Russian translation. This work was supported by the Austrian Science Foundation (FWF Project No. 14096-BIO).

Zusammenfassung

Molekulare Phylogenie und Biogeografie der Wespenbussarde (Gattungen Pernis und Henicopernis)

Eine Teilsequenz des *Cytochrom b* Gens (382 bp) wurde von 35 Individuen (hauptsächlich Museumsmaterial) der Gattung *Pernis* amplifiziert und sequenziert. Inkludiert wurden alle validen Taxa (10) und zwei weitere (*P. p. gurneyi*, *P. p. japonicus*) mit zweifelhaftem taxonomischen Status. Weiters wurden die übrigen Vertreter der altweltlichen Perninae (*Henicopernis, Aviceda*) untersucht, um deren Verwandtschaft zum Genus *Pernis* festzustellen. Als Außengruppen-Taxa wurden Bartgeier *Gypaetus barbatus*, Schmutzgeier *Neophron percnopterus* und Mäusebussard *Buteo buteo* verwendet. In den aus den Sequenzen errechneten Bäumen stellt *Aviceda* die Schwestergruppe der Gattung *Pernis* dar. Die Gattungen, *Henicopernis* und die Altweltgeier *Gypaetus* und *Neophron* erscheinen mit der Gattung *Pernis* nur entfernt verwandt. Innerhalb der Gattung *Pernis* formen zwei der beschriebenen Arten (*P. apivorus*, *P. ptilorhyncus*) eine monophyletische Gruppe, die Verwandtschaftsverhältnisse der beiden anderen Clades, welche die drei Unterarten von *P. celebensis* repräsentieren, können jedoch auf der Basis dieser Sequenz nicht eindeutig geklärt werden. Obwohl diese Analyse auf relativ kurzen DNA-Abschnitten basiert, können die aus den Sequenzen abgeleiteten Stammbäume als erster Versuch angesehen

werden, die Phylogenie der Wespenbussard-Gattung *Pernis* und verwandter Genera mittels molekularer Methoden und unter Berücksichtigung evolutionsbiologischer, biogeographischer und systematischer Aspekte darzustellen.

References

van Balen, S.; Sözer, R.; Nijman, V.; Dennis, R.; Meijaard, E.; Jepson, P. R., 1999: Juvenile plumage of Javan Crested Honey Buzzard, with comments on mimicry in south-eastern Asian *Pernis* and *Spizaetus* species. Dutch Birding **21,** 192–198.

Boev, Z., 1996: Raptors and Owls (Aves: Falconiformes et Strigiformes) in the Archaeological Record. Historia Naturalis Bulgarica **6,** 83–92.

Brown, L. H., 1976: Birds of Prey. Feltham: Hamlyn Publishing Group Ltd.

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

Brown, L. H.; Urban, E. K.; Newman, K., 1982: The Birds of Africa, Vol. 1. London: Academic Press.

Coates, B. J., 1985: The Birds of Papua New Guinea, Vol. 1. Alderley: Dove Publications.

Collinson, M., 2001: Greenish Warbler, 'Two-barred Greenish Warbler', and the speciation process. British Birds **94,** 278–283.

DeBoer, L. E. M.; Sinoo, R. P., 1984: A karyological study of Accipitridae (Aves: Falconiformes), with karyotypic descriptions of 16 species new to cytology. Genetica **65,** 89–107.

Ferguson-Lees, J.; Christie, D.A., 2001: Raptors of the World. London: Ch. Helm and A. & C. Black.

Gamauf, A.; Preleuthner, M., 1998: A new taxon of the Barred Honeybuzzard *Pernis celebensis* from the Philippines. Bull. BOC **118,** 90–101.

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

Gamauf, A.; Preleuthner, M.; Winkler, H., 1998b: Aggressive mimicry in SE Asian birds of prey: Barred Honey buzzards *Pernis celebensis* vs. hawk-eagles *Spizaetus* spp. In: Fifth World Conference on Birds of Prey and Owls. Midrand, South Africa, p. 46.

Griffiths, C. S., 1994: Monophyly of the falconiformes based on syringeal morphology. The Auk **111,** 787–805.

Haring, E.; Kruckenhauser, L.; Gamauf, A.; Riesing, M. J.; Pinsker, W., 2001: The complete sequence of the mitochondrial genome of *Buteo buteo* (Aves, Accipitridae) indicates an early split in the phylogeny of raptors. Mol. Biol. Evol . **18,** 1892–1904.

Hartert, E., 1914: Die Vögel der paläarktischen Fauna. Berlin: Friedländer und Sohn.

Heany, L. R., 1985: Zoogeographic evidence for middle and late Pleistocene land bridges to the Philippine Islands. Mod. Quat. Res. SE Asia **9,** 127–143.

Helbig, A. J.; Seibold, I.; Martens, J.; Wink, M., 1995: Genetic differentiation and phylogenetic relationships of Bonelli's Warbler *Phylloscopus bonelli* and Green Warbler *P. nitidus*. J. Avian Biol. **26,** 139–153.

Holdaway, R. N., 1994: An exploratory phylogenetic 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. Berlin: WWGBP; Mountfield: Pica Press, pp. 601–649.

del Hoyo, J.; Elliott, D.; Sargatal, J. (eds), 1994: Handbook of the Birds of the World. Vol. 2. Barcelona: Lynx Edicions.

Irwin, D. E.; Bensch, S.; Price, T. D., 2001: Speciation in a ring. Nature **409,** 333–337.

Johansen, H., 1957: Die Vogelfauna Westsibiriens. J. Ornithol. **98,** 397–415.

Jollie, M., 1977: A contribution to morphology and phylogeny of the Falconiformes. Evolutionary Theory **1,** 285–298 (see also **2**, 115–300, **3**, 1–142).

Kislenko, G. S., 1974: Comparative ecology of the Oriental Honey buzzard and European Honey buzzard. In: Sixth All-Union Orn. Conf., Moscow: Moscow University Press, part 2, pp. 65–66 (in Russian).

Klicka, J.; Zink, R. M., 1997: The importance of recent Ice Ages in speciation: a failed paradigm. Science **277,** 1666–1669.

Linnaeus, C., 1758: Systema naturae. Ed. X, Holmie, p. 91.

Marchant, S.; Higgins, P. J., 1993: Handbook of Australian, New Zealand and Antarctic Birds. Melbourne: Oxford University Press.

Mayr, E., 1939: Ein neuer Wespenbussard von den Philippinen. Orn. Mber. **47,** 74–76.

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

Mosquitin, S. S., 1973: Materials on distribution and mode of life of some Siberian birds. Proc. Biol. Inst. Siberian. Acad. Sci. 16, 263–268.

Olsen, P., 1995: Australian Birds of Prey. Baltimore, MD: Johns Hopkins University Press.

van Oosterzee, P., 1997: Where Worlds Collide. Ithaca, NY: Cornell University Press.

Peters, J. L., 1931: Check-list of the Birds of the World, Vol. 1. Cambridge, MA: Harvard University Press.

Saitou, N.; Nei, M., 1987: The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4,** 406–425.

Seibold, I.; Helbig, A. J., 1995: Evolutionary history of New and Old World vultures inferred from nucleotid sequences of the mitochondrial cytochrome b gene. Philos. Trans. R. Soc. Lond. B **150,** 163–178.

Simpson, G. G., 1977: Too many lines: the limits of the oriental and Australian zoogeographic regions. Proc. Am. Philos. Soc. **121,** 102–120.

Stattersfield, A. J.; Crosby, M. J.; Long, A. J.; Wege, D. C., 1998: Endemic Bird Areas of the World: Priorities for Biodiversity Conservation. BirdLife Conservation Series No. 7. Cambridge, UK: BirdLife International.

Stepanyan, L. S., 1983: Superspecies and Sibling Species in Avifauna of the USSR. Moscow: Nauka Press, 296pp. (in Russian).

Stresemann, E., 1940: Zur Kenntnis der Wespenbussarde (*Pernis*). Arch. Naturgeschichte, N.F. 9**,** 137–193.

Stresemann, E.; Amadon, D., 1979: Order Falconiformes. In: Mayr, E.; Cottrell, G. W. (eds), Check-list of Birds of the World, Vol. 1, 2nd edn. Cambridge, MA.: Mus. Comp. Zoology.

Swann, H. K., 1926: A Monograph of the Birds of Prey (order Accipitres). Vol. 2. London: Wheldon and Wesley.

Swofford, D. L., 2002: PAUP* – Phylogenetic Analysis Using Parsimony (*and other methods). Ver. 4.0b6-10. Sunderland, MA: Sinauer.

Tyler, C., 1966: A study of egg shells of the falconiformes. J. Zool. **150,** 413–425.

Vaurie, C.; Amadon, D., 1962: Notes on the Honey Buzzards of eastern Asia. Am. Mus. Novit. **2111,** 1–11.

Weick, F., 1980: Greifvögel der Welt. Hamburg: Parey Verlag.

Wink, M., 1995. Phylogeny of Old and New World vultures (Aves: Accipitridae and Cathartidae) inferred from nucleotide sequences of the mitochondrial cytochrome b gene. Z. Naturforsch. **50c,** 868–882.

Wink, M.; Sauer-Gürth, H., 2000: Advances in the molecular systematics of African raptors. In: Meyburg, B.-U.; Chancellor, R. D. (eds), Raptors at Risk. Berlin: WWGBP; Surrey: Hancock House, pp. 135–147.

Whitten, A. J.; Mustafa, M.; Henderson, G. S., 1987: The Ecology of Sulawesi. Djakarta: Gadjah Mada University Press.

Authors' addresses: Anita Gamauf (for correspondence), E. Haring, Museum of Natural History, 1. Zoological Department, Burgring 7, A-1014 Vienna, Austria. E-mail: anita.gamauf@nhm-wien.ac.at, elisabeth.haring@nhm-wien.ac.at