Molecular Phylogeny of South American Screech Owls of the *Otus atricapillus* Complex (Aves: Strigidae) Inferred from Nucleotide Sequences of the Mitochondrial Cytochrome *b* Gene

Petra Heidrich[a], Claus König[b] and Michael Wink[a]

[a] Universität Heidelberg, Institut für Pharmazeutische Biologie, Im Neuenheimer Feld 364, D-69120 Heidelberg
[b] Staatliches Museum für Naturkunde, Rosenstein 1, D-70191 Stuttgart

Z. Naturforsch. **50c**, 294–302 (1995); received October 28/December 21, 1994

Otus atricapillus Complex, Bioacoustics, Speciation, Molecular Systematics, Cytochrome *b* Gene

The cytochrome *b* gene of 6 South American screech owls of the genus *Otus* (*O. choliba, O. atricapillus, O. usta, O. sanctaecatarinae, O. guatemalae,* and *O. hoyi*) and two Old World species (*Otus scops* and *Otus leucotis*) was amplified by polymerase chain reaction (PCR) and partially sequenced (300 nucleotides). *Otus atricapillus, O. guatemalae, O. hoyi* and *O. sanctaecatarinae* which are morphologically very similar, have been treated as belonging to a single species, *A. atricapillus* (Sibley and Monroe, 1990). Nucleotide sequences differ substantially between these taxa (6.3 to 8.8% nucleotide substitutions) indicating that they represent well established and distinct species which had been implicated already from ecological and bioacoustical analyses (König, 1991, 1994). The importance of vocal and ecological characters for the taxonomy of nocturnal birds is thus confirmed by our molecular analysis. Phylogenetic relationships were reconstructed between Old and New World owls using character state ("maximum parsimony"; PAUP 3.1.1) and distance matrix methods (neighbour-joining; MEGA).

Introduction

The genus *Otus* (family Strigidae, order Strigiformes) consists, according to Sibley and Monroe (1990) and Boyer and Hume (1991), of 53 and 41 species, respectively, occurring in the Old and New World. Whereas half of the taxa are recognized as distinct species according to morphological characters, the status of more than 20 others is still a matter of debate since morphology is often invariant in this group in contrast to the distinctive calls (König, 1991, 1994; Hekstra, 1982). This is especially true for South American screech-owls of the *Otus atricapillus* complex (Bond and Meyer de Schauensee, 1994; Fitzpatrick and O'Neill, 1986; Haffer, 1987; Hardy *et al.*, 1989; Hekstra, 1982; Marshall 1991; Sick, 1985; Weske and Terborgh, 1981; Wolters, 1975–1982). Sibley and Monroe (1990) classify *Otus atricapillus* and *O. watsonii* (these authors probably considered *O. usta* which up to now has been treated as a southern subspecies of *watsonii*) as distinct species and treat *O. atricapillus* (Temminck), *O. guatemalae* (Sharpe), *O. hoyi* König et Straneck, and *O. sanctaecatarinae* (Salvin) as belonging to a single species. However, König (1991, 1994) and König and Straneck (1989) have provided evidence that these taxa occupy different ecological niches and are distinguished by typical calls. Since in owls all vocalizations are inherited and not learned, these authors have stressed the importance of vocal differences in nocturnal birds to establish an efficient isolation mechanism as a prerequisite for speciation.

Using morphological and biological characters alone, the decision whether a taxon has the status of a species or subspecies will remain difficult, even if the voice obviously seems to be the most important factor in interspecific isolation mechanisms in owls. To evaluate the validity of the ecological and bioacoustical approach to define species within the *O. atricapillus* complex, methods of molecular systematics might help to decide these issues (Hillis and Moritz, 1990; Hoelzel, 1992; Avise, 1994). Most resolution can be obtained by comparing the nucleotide sequences of phylogenetically informative marker genes

Reprint requests to Prof. Dr. M. Wink.
Telefax: (06221) 564884.

0939–5075/95/0300–0294 $ 06.00

(Hoelzel, 1992; Kocher et al., 1989; Cooper *et al.*, 1992; Edwards *et al.*, 1991; Avise, 1994).

The mitochondrial cytochrome *b* gene has often been selected as a marker gene in recent years, since its sequence has been found informative for many phylogenetic and taxonomic problems of animals, especially of bird taxa whose speciation took place within the last 20 million years (Kocher *et al.*, 1989; Richman and Price, 1992; Helbig *et al.*, 1993, 1994; Seibold *et al.*, 1993, 1994a,b; Wink *et al.*, 1993a,b, 1994; Heidrich and Wink, 1994; Avise, 1994).

In this study partial cytochrome *b* nucleotide sequences of 6 South American and 2 Old World taxa of the genus *Otus* were determined and used to evaluate their degree of speciation in relation to acoustic differentiation and to reconstruct the phylogenetic relationship between them.

Materials and Methods

Material

The origin of owls of the *O. atricapilllus* complex is documented in Table III. *O. choliba* was collected by C. König in B. de Irigoyen, Misones (Argentina) and at Imaya Cocha, Napo (Ecuador) by the Zool. Museum Copenhagen. *O. leucotis* came from Rwanda (East Africa) (collector F. Henning/ B. Schottler) and *O. scops* from Crete (collector D. Ristow).

DNA-methods

Blood or tissues were stored in a modified EDTA-buffer (Arctander, 1988) at ambient temperatures in the field. DNA was extracted after digestion with proteinase K (Boehringer) according to Arctander (1988), Swatschek *et al.* (1993, 1994) and Heidrich *et al.* (1994).

Primer sequences used for PCR and direct sequencing were derived from Kocher *et al.* (1989) and are given in Seibold *et al.* (1994a), and Wink (1994). A 1026 bp portion of the cytochrome *b* gene was amplified using 1 µg of total DNA as target, 25 pmol each of primers A and F, 1.5 mM $MgCl_2$ and 2 units Taq-polymerase (Promega or AGS). After initial denaturation (4 min at 94 °C), 30 cycles of 45 sec at 94 °C, 60 sec at 45 °C and 90 sec at 72 °C were performed on a Biometra thermocycler. After 30 cycles the reaction temperature was maintained at 72 °C for 4 min and then lowered to 4 °C for further storage. PCR products were run on a 1% agarose gel, excised and extracted using the Qiaex gel purification kit (Diagen). After elution, the amplified DNA was precipitated with isopropanol and sodium acetate. The pellet was redissoled in 7.5 µl H_2O. Direct sequencing of the double-stranded DNA was carried out by the chain termination method at 37 °C using α-^{35}S-dATP as a radioactive marker and Sequenase 2.0 (USB) or T7-polymerase (Pharmacia) according to the distributor's specifications. Primer B was used as a sequencing primer. Products of the sequencing reactions were separated on a 6 % polyacrylamide/7 M urea gel by electrophoresis at 65 W. After drying, the gel was exposed to an X-ray film for 3–4 days.

Sequence analysis

Sequences were aligned with the cytochrome *b* gene of *Gallus gallus domesticus* (Desjardin and Morais, 1990). Phylogenetic trees were reconstructed using the maximum parsimony method (phylogeny program PAUP 3.1.1.; Swofford, 1993) and the neighbour-joining method (Saitou and Nei, 1987) (program package MEGA; Kumar *et al.*, 1993). In the neighbour-joining analyses genetic distances were calculated based on the Kimura 2-parameter or the Tamura-Nei model (Kumar *et al.*, 1993). With PAUP, heuristic algorithms were employed. Bootstrap analyses were performed to obtain confidence estimates for each furcation.

Results and Discussion

Base composition and mode of substitutions

Theoretically the abundance of each of the four DNA bases should be 0.25. Similar to the situation of mitochondrial genes in other animals (Irwin *et al.*, 1991; Kocher *et al.*, 1989; Edwards *et al.*, 1991; Kornegay *et al.*, 1993), guanine is significantly under and cytosine overrepresented (t-test, $p < 0.001$) probably reflecting a biased codon usage. This discrimination is even more expressed in the third codon postion where G has an abundance of 4.1%, C 49.7%, T 13.7% and A of 32.6%.

Within the dataset (Table I) we found 108 variable sites, of which 82 were parsimony informative.

Table I. Partial nucleotide sequences of the cytochrome *b* gene of 8 *Otus* species. Only variable characters are illustrated; numbers above sites refer to position 15289 (= 1) to 14990 (= 300) (H strand), as determined for the cyt *b* gene of *Gallus* (Desjardin and Morais, 1990).

				1	1111111111	1111111111	1111111111	1122222222	2222222222	2222222222	2222222222	22222222						
	1112233	3344444445	6677788890	0011112233	3344455566	6777788899	9900000011	1222233333	3444555556	6666777778	88889999							
	2581476925	7801346793	2814703951	7903692801	4706925801	3036912814	6703456925	8048923678	9258147890	3679012480	12470136							
Glaucidium passerinum	??????GTCG	GTGGTGCGTG	TAGGGAATGG	CGGGGTATGG	TTGATAGGTC	AGTGACTGGT	AGATGTGGCG	GTAGTAGGGT	GCTTGGGGCA	TGACATCGGT	TCCAGAGC							
Glaucidium perlatum	......???T	.G.TG...C.	.G..TGGC..	A..AACG...	...G.G...G	G...G?....	..G.....T.	.AG......C	.TCG..AATG	..G???????	????????							
O.choliba 1	GATAGA.GGT	AA.A.A..CA	...ATGGCC.	T.A..GGAAT	G.A.GGACGG	G..A.TCA.A	C?G..GTTT.	TAG....T..	.GGG.T.ATG	.AGTGCT...	.GTT.GTG							
O.choliba 2	......????	??...A..CA	...ATGGCC.	T.A..GGAAT	G.A.GGACGG	G..A.TCA.A	C?G..GTTT.	TAG....T..	.GGG.T.ATG	.AGTGCT...	.GTT.GTG							
O.atricapillus 1	...GAAAGTT	.G...ATACA	CGT..GGGT.	A....GGA.T	GCA.GG.TGT	GA.T.T....	.TGC...TT.	TAG..G.T..	AGGG.T.ATG	..GT..G.AC	..AT.GCG							
O.atricapillus 2	...GAAAGTT	.G...ATACA	CGT..GGGT.	A....GGA.T	GCA.GG.TGT	GA.T.T....	.TGC...TT.	TAG..G.T..	AGGG.T.ATG	..GT..T.A.	..AT.GCG							
O.atricapillus 3	...GAAAGTT	.G...ATACA	.GT..GGGT.	A....GGA.T	GCA.GG.TGT	GA.T.T....	.TGC...TT.	TAG..G.T..	AGGG.T.ATG	..GT..T.A.	..TT.GC?							
O.usta 1	GGTGAAAGTT	.G...ATACA	.GT....GT.	T....GGA.T	GCA.GG.TGT	GA.T.T....	.TGCA..TT.	TAG....T..	.GGG.C.ATG	..GT..T.A.	..AC.GCG							
O.usta 2	..TGAAAGTT	.G...ATACA	..T..G.GT.	T....GGA.T	GCA.GG.TGT	GA.T.T....	.TGCA..TT.	TAG....T..	.GGG.C.ATG	C.GT..T.A.	..AT.GCG							
O.usta 3	..TGAAAGTT	.G...ATACA	..T..G.GT.	T....GGA.T	GCA.GG.TGT	GA.T.T....	.TGCA..TT.	TAG....T..	.GGG.C.ATG	C.GT..T.A.	..AT.GCG							
O.sanctaecatarinae	.GTAAAAGTT	.G...A.AC.	.....GG.T.	TA...GGAAT	GCA.GG.TGT	GA.CGT....	.TG....TT.	TAG....TA.	AGGG.T?A?G	..??..G...	..TC?GC?							
O.guatemalae	......?GTT	.G.....ACA	.G...GGCTA	T....GGG.T	GC..AG.TGT	GA.TGT....	.TG....TT.	.A.....T.C	.GGG.T.ATG	..GT..T...	..GC.GCG							
O.hoyi1	......????	.G.....ACA	.G...GG.T.	.....GGAAT	GCA.GG.TGT	GA.T.....C	.TG..C.T..	TAGAC..T..	.TGGAC.ATG	..GT...???	????????							
O.hoyi2	GACGAA.GTT	.G.....AC.	.G...GG.T.	.....GGAAT	GCA.GG.TGT	GA.T.....C	.TG..C.T..	TAGAC..T..	.GGGAC.ATG	..GT.....C	..TT.GCG							
O.hoyi3	GACGAA.GTT	.G.....AC.	.G...GG.T.	.....GGAAT	GCA.GG.TGT	GA.T.....C	.TG..C.T..	TAGAC..T..	.GGGAC.ATG	..GT.....C	..TT.GCG							
O.leucotis	......????	??????..C.	....AG.CT.	G..A.G.G.T	GCA.CG..GT	..CTGTG...	..G.....TA	TAG...AT.C	.T.G.A.ATG	..GTGCT...	..ATTGTG							
O.scops 1	ATTAGGTATT	..A....ACA	.G...G.GT.	A...AGGGAT	G...AGT.GT	G..T.T.TA.	CTG..A..T.	.A....AT.C	.TGG.A.ATG	..GTG.TA..	G.AG.GTG							
O.scops 2	ATTAGGTATT	..A....ACA	.G...G.GT.	A...AGGGAT	G...AGT.GT	G..T.T.TA.	CTG..A..T.	.A....AT.C	.TGG.A.ATG	..GTG.TA..	G.AG.GTG							
O.scops 3	ATTAGGTATT	..A....ACA	.G...G.GT.	A...AGGGAT	G...AGT.CT	G..T.T.TA.	CTG..A..T.	.A....AT.C	.TGG.A.ATG	..GTG.TA..	G.AG.GTG							

. = base identical to that of the first line; ? = base could not be determined without ambiguity.

Table II. Pairwise genetic distances between sequences of the cytochrome *b* gene. Pairwise distances between taxa. Below diagonal: absolute distances; above diagonal: mean distances (adjusted for missing data).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*Glaucidium passerinum*	-	0.145	0.242	0.231	0.229	0.225	0.222	0.211	0.215	0.215	0.206	0.194	0.193	0.196	0.196	0.191	0.211	0.211	0.211
2	*Glaucidium perlatum*	34	-	0.177	0.173	0.167	0.167	0.162	0.171	0.175	0.175	0.157	0.128	0.169	0.167	0.167	0.126	0.145	0.145	0.145
3	*O.choliba* 1	66	41	-	0.004	0.149	0.142	0.138	0.144	0.137	0.137	0.134	0.144	0.148	0.141	0.141	0.146	0.161	0.161	0.164
4	*O.choliba* 2	60	39	1	-	0.138	0.131	0.125	0.135	0.127	0.127	0.124	0.135	0.137	0.135	0.135	0.146	0.150	0.150	0.154
5	*O.atricapillus* 1	63	39	43	36	-	0.007	0.014	0.038	0.038	0.038	0.070	0.081	0.084	0.079	0.079	0.148	0.148	0.148	0.152
6	*O.atricapillus* 2	62	39	41	34	2	-	0.007	0.031	0.031	0.031	0.070	0.073	0.084	0.083	0.083	0.141	0.141	0.141	0.145
7	*O.atricapillus* 3	60	38	39	32	4	2	-	0.032	0.032	0.032	0.063	0.071	0.080	0.077	0.077	0.143	0.144	0.144	0.147
8	*O.usta* 1	58	40	43	35	11	9	9	-	0.014	0.014	0.072	0.070	0.080	0.087	0.087	0.137	0.140	0.140	0.143
9	*O.usta* 2	59	41	40	33	11	9	9	4	-	0.000	0.073	0.077	0.084	0.085	0.085	0.129	0.140	0.140	0.143
10	*O.usta* 3	59	41	40	33	11	9	9	4	0	-	0.073	0.077	0.084	0.085	0.085	0.129	0.140	0.140	0.143
11	*O.sanctaecatarinae*	53	36	37	30	19	19	17	20	20	20	-	0.071	0.082	0.083	0.083	0.139	0.158	0.158	0.162
12	*O.guatemalae*	53	30	39	35	22	20	19	19	21	21	18	-	0.088	0.088	0.088	0.121	0.103	0.103	0.106
13	*O.hoyi* 1	46	39	35	32	20	20	19	19	20	20	19	21	-	0.008	0.008	0.153	0.130	0.130	0.134
14	*O.hoyi* 2	54	39	42	35	23	24	22	26	25	25	23	24	2	-	0.000	0.152	0.157	0.157	0.160
15	*O.hoyi* 3	54	39	42	35	23	24	22	26	25	25	23	24	2	0	-	0.152	0.157	0.157	0.160
16	*O.leucotis*	49	28	37	37	38	36	36	35	33	33	33	31	35	39	39	-	0.125	0.125	0.129
17	*O.scops* 1	58	34	48	39	43	41	41	42	41	41	44	28	31	47	47	32	-	0.000	0.003
18	*O.scops* 2	58	34	48	39	43	41	41	42	41	41	44	28	31	47	47	32	0	-	0.003
19	*O.scops* 3	58	34	49	40	44	42	42	43	42	42	45	29	32	48	48	33	1	1	-

About 83 nucleotide substitutions occur in the third position of a codon, which usually do not lead to amino acid substitutions. The resulting silent mutations are especially helpful for phylogenetic reconstructions since they are not adaptive as morphological characters. At the first and second position we observed 17 and 8 substitutions, respectively.

For most taxa (except *O. guatemalae* and *O. sanctaecatarinae*) at least 2–3 specimens were available. The degree of intraspecific variation which consisted of 1 to 4 base substitutions (= up to 1.4% difference) (Table II), was significantly smaller ($p < 0.001$, t-test) as compared to the differences encountered between species (substitution rate 6–16 %) (Table II). Genetic distances between *O. atricapillus* and *O. usta* which are treated as distinct species by most authors (Sibley and Monroe, 1990; Hume and Boyer, 1991) are 3.1–3.2% and are also significantly higher than intraspecific variations ($p < 0.001$, t-test). Since *O. guatemalae*, *O. hoyi* and *O. sanctaecatarinae* differ by 6.3 to 8.8% from *O. atricapillus* and between each other and since they are not closely related phylogenetically (Figs 1,2) it is very likely that these taxa represent distinct species, as indicated from ecological and acoustical analyses (Table III). The coefficient between transitions/transversions (transitions are nucleotide substitutions from A to G or C to T and vice versa whereas transversion are changes from pyrimidine to purines and vice versa, i.e. G to C or T and A to C or T) is relatively low. In recently diverged taxa ns/nv ratios are high since transitions are 20 times more common as transversions. Because of multiple substitutions this difference becomes smaller with time and taxa which are separated by more than 10–30 million years have nv/ns ratios approaching 1 (Kornegay *et al.*, 1993; Avise, 1994).

For comparisons between Old and New world *Otus* species a ns/nv coefficient of 1.5 ± 0.27 is obtained and 2.9 ± 0.74 for comparisons within

Table III. Origin of the samples and comparison of morphological and other biological characters of the *O. atricapillus* complex.

Parameter	*O. atricapillus*	*O. watsonii*	*O. usta*	*O. hoyi*	*O. guatemalae*	*O. sanctaecatarinae*
Origin of sample	Iguazu, Misiones (Argentina) No. 1–3	–	Rio Beni, La Paz (Bolivia) No. 1 Rio Napo, Loreto (Peru) No. 2 Pando (Bolivia) No. 3	Salta (Argentina) No. 1–3	Pasco (Peru) No. 1 San Martin (Peru) No. 2	Cerro tigre Misiones (Argentina) No. 1
Collector	C. König		LSUZM, Baton Rouge	C. König	LSUZM, Baton Rouge	C. König
Morphology						
Iris colour	brown/amber	amber yellow	brown	yellow	yellow	yellow/hazel
Wing length	~175 mm	~175 mm	~175 mm	~175 mm	~165 mm	~190 mm
Length	~230 mm	~230 mm	~230 mm	~230 mm	200 mm	~245 mm
Crown	blackish	rather dark	very dark	not dark	not dark	rather dark
Voice						
Male territorial Song (song A)	long trill up to 20 s	long sequence	long sequence	long trill	rather long trill	medium long trill ~8–10 s
Number of elements	14/s	7–8/s	2/s	11/s	14/s	14–15/s
Habitat	lowland rain-forest up to 600 m in tropical regions	lowland rain-forest north of the Amazon	Amazon rain-forest	montane and cloud forests "Southern Yungas"	mountain forest	Forests between 300 and 1000 m (mixed with *Araucaria*)
Distribution	SE-Brazil (north to Rio de Janeiro), NE-Argentina (northern Misiones), E-Paraguay	Surinam, E-Ecuador, Venezuela	Brazil, Peru, Bolivia	NW-Argentina, S-Bolivia	Mexico, south to N-Bolivia	SE-Brazil, Uruguay, NE-Argentina Sierra de Misiones

the South American *Otus* taxa. As expected, the highest ratio (e.g., 5.1 ± 2.62) can be obtained between species of the *O. atricapillus* complex indicating that this group diverged later from a common ancestor than the other South American *Otus* species. Sequence differences suggest that the taxa of the *O. atricapillus* complex, assuming a constant molecular clock for mitochondrial genes (Quinn *et al.*, 1991; Wilson *et al.*, 1987), were separated from a common ancestor probably several (approximately 3 to 4) million years ago (Table II). It is highly unlikely that the species of the *O. atricapillus* complex were still interbreeding a few centuries ago as suggested by Burton (1992).

Phylogenetic reconstructions

The distribution of 1000 randomly produced trees is significantly skewed to the right (g1 = −0.895) indicating that the data set contains a significant phylogenetic signal (Hillis and Huelsenbeck, 1992).

The character state method "maximum parsimony" implimented by PAUP 3.1.1. produced one

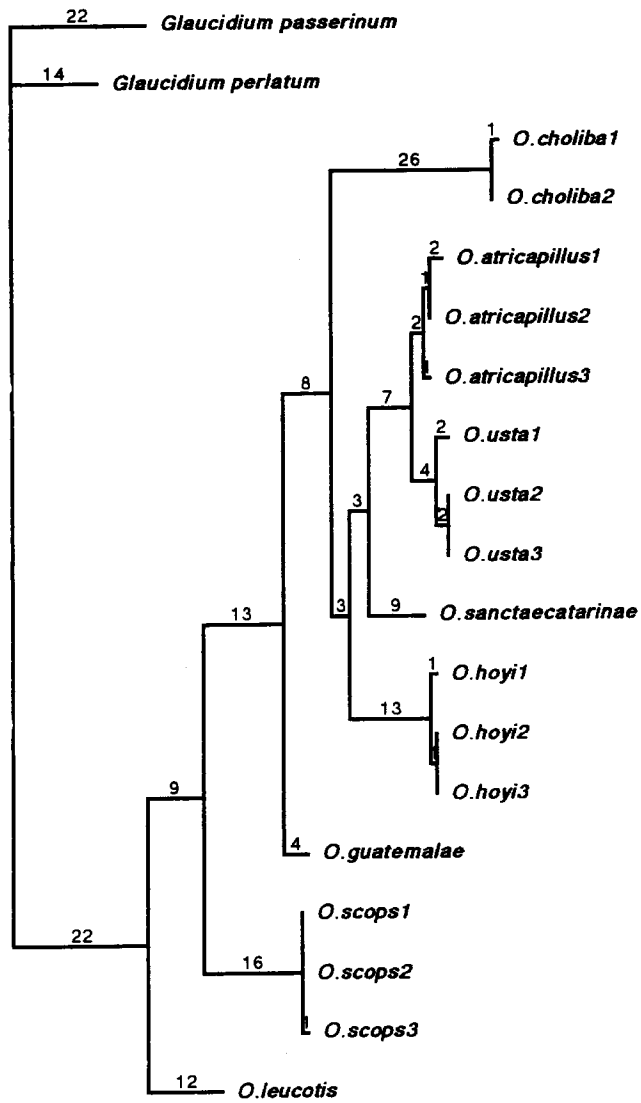


Fig. 1. Reconstruction of phylogenetic relationships within the genus *Otus* by maximum parsimony (using PAUP 3.1.1) with *Glaucidium perlatum* and *G. passerinum* as outgroups. In the phylogram numbers refer to nucleotide substitutions between taxa; branch length is proportional to substitution rates; CI= 0.729, RI= 0.803.

most parsimonious trees for the complete data set (Fig. 1). The shortest tree was 199 steps long (minimal length 145 steps, maximally 419 steps). Fig. 1 illustrates a phylogram of the *Otus* complex, in which figures correspond to the number of nucleotide substitutions between species. The distance matrix approach (MEGA 1.0) with the neighbour-joining method produced a phylogenetic tree (Fig.2) which is highly congruent with maximum parsimony tree, only the positions of *O. sanctaecatarinae* and *O. hoyi* are exchanged. Variation of the outgroup changed the topology of the phylogenetic trees only slightly, as can be seen from Fig. 3 in which *O. leucotis* instead of *Glaucidium* was selected as an outgroup and the South American *Otus* taxa as an ingroup.

These analyses show that the Old world taxa (*O. leucotis* and *O. scops*) are not included in the clade of screech-owls of the New World, reflecting that both groups must have diverged a long time ago from a common ancestor (see distances in Table II). Therefore, it must be discussed whether both groups should be treated as belonging to a single genus.

The analyses show that *O. atricapillus* and *O. usta* (apparently we had only samples of *O. usta* and none of true *O. watsonii*) appear as closely related sister species. According to the common topology of the phylogenetic trees (Figs 1–3) *O. sanctaecatarinae* and *O. hoyi* seem to belong to the *O. atricapillus* complex, but the respective bootstrap values (52 or 53%) are not significant. In both MP and NJ analyses *O. choliba* clusters outside the *O. atricapillus* complex. The position of *O. guatemalae* cannot be resolved with certainty (Figs 1–3) and the NJ and MP reconstructions suggest that this taxon does not belong to the *O. atricapillus* complex which would agree with the concept outlined in Burton (1992), that *O. guatemalae* might perhaps form a superspecies including *O. trichopsis, O. barbarus, O. marshalli, O. nudipes* and *O. clarkii.*

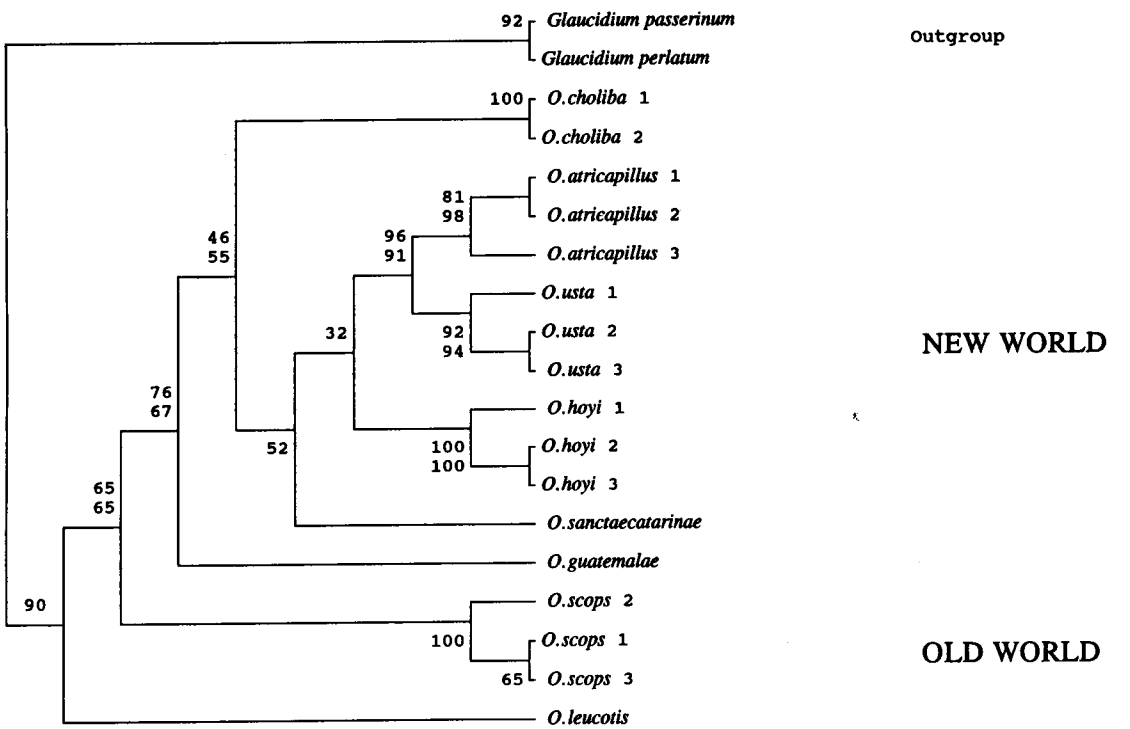
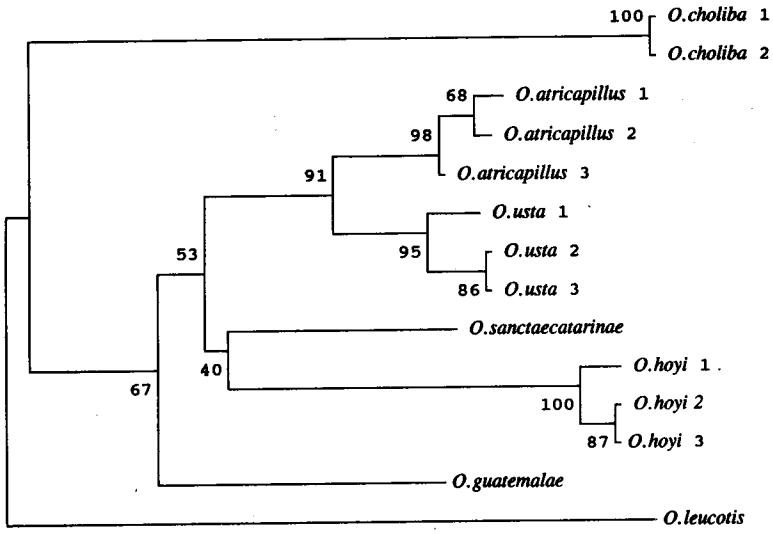


Fig. 2. Reconstruction of phylogenetic relationships within the genus *Otus* using the neighbor-joining method. The Tamura-Nei method was chosen as a distance algorithm to correct for differences in codon usage and ns/nv differences (Kumar *et al.*, 1993). Bootstrap values from 500 replicates (in %) give confidence estimates for each furcation; if two values are given, than the upper value refers to bootstrap data from MP and the lower to that of NJ analyses.



Scale: each - is approximately equal to the distance of 0.001606

Fig. 3. NJ analysis of the South American *Otus* complex chosing *O. leucotis* as an outgroup. Procedures as in Fig. 2.

Conclusions

This first analysis clearly indicates that the concept outlined in Sibley and Monroe (1990) to treat *O. atricapillus, O. sanctaecatarinae, O. hoyi* and *O. guatemalae* as a single species, *i.e.* as *O. atricapillus* cannot be supported by molecular evidence. On the contrary, the genetic data show that *O. atricapillus, O. watsonii* (apparently representing 2 taxa: *watsonii* and *usta), O. sanctaecatarinae, O. hoyi* and *O. guatemalae* represent independent and distinct species (Tables I–III; Figs 1–3).

It has been emphasized by several authors (König, 1991, 1994; König and Straneck, 1989) that ecological and bioacoustical characters are much more important for the classification of owls than morphological differences, since only the former characters can provide efficient isolation mechanisms in nocturnal animals to keep species separated. Because of the acoustical differences between species of the *O. atricapillus* complex (Table III), König (1991, 1994), König and Straneck (1989) had recommended to treat its members, *i.e., O. atricapillus, O. watsonii* (and *O. usta*), *O. sanctaecatarinae, O. hoyi* and *O. guatemalae* as distinct species. This conclusion can be supported by our molecular analysis confirming the importance of ecological and bioacoustical characters for taxonomy.

Acknowledgements

We thank Ute Kahl for technical assistance and D. Ristow, F. Henning, B. Schottler, J. Fjeldså (Zoologisk Museum, Kobenhavn), and the Museum of Zoology, Lquisiana State University (LSUZM), Baton Rouge for providing blood or tissue samples.

Arctander P. (1988), Comparative studies of avian DNA by restriction fragment length polymorphism analysis. J. Orn. **129,** 205–216.

Avise J. C. (1994), Molecular Markers, Natural History and Evolution. Chapmann & Hall, London.

Bond J. and Meyer de Schauensee R. (1941), Descriptions of new birds from Bolivia. Part IV. – Notul. Nat. **93,** 2.

Burton J. A. (1992), Owls of the World, Their Evolution, Structure and Ecology. Peter Lowe, London.

Cooper A., Mourer-Chauvire C., Chambers G. K., von Haeseler A., Wilson A. C. and Pääbo S. (1992), Independent origins of New Zealand moas and kiwis. Proc. Natl. Acad. Sci. **89,** 8741–8744.

Desjardins P. and Morais R. (1990), Sequence and gene organisation of chicken mitochondrial genome. J. Mol. Biol. **212,** 599–634.

Edwards S. V., Arctander P. and Wilson A. C. (1991), Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. Proc. Royal Soc. London, Series B, **243,** 99–107.

Fitzpatrick J. W. and O'Neill J. P. (1986), *Otus petersoni*, a new screech-owl from the eastern Andes, with systematic notes on *Otus colombianus* ands *O. ingens*. Wilson Bull. **98** (1), 1–14.

Haffer J. (1987), Über Superspezies bei Vögeln. Annln. naturhist. Mus. Wien (B) **88/89.** 147–166.

Hardy J. W., Coffey B. B. and Reynard G. B. (1989), Voices of New World Owls. Ara Records, nr. 16. – Gainesville, Fla. (Audio-Cassette).

Heidrich P., König C. and Wink W., Bioakustik und molekulare Systematik amerikanischer Sperlingskäuze (Aves: *Glaucidium* spp.). Stuttgt. Beitr. Naturkde. (in press).

Heidrich P. and Wink M. (1994), Tawny owl (*Strix aluco*) and Hume's tawny Owl (*Strix butleri*) are distinct species: Evidence from nucleotide sequences of the cytochrome *b* gene. Z. Naturforsch. **49c,** 230–234.

Helbig A. J., Salomon M., Wink M. and Bried J. (1993), Absence of mitochondrial gene flow between European and Iberian "chiffchaffs" (Aves: *Phylloscopus collybita collybita, P. (c.) brehmii*). The taxonomic consequences. Results drawn from PCR and DNA sequencing. Compt. Rend. l'Acad. Sciences, Paris, **316,** Série III, 205–210.

Helbig S., Seibold I., Martens J. and Wink M., Genetic differentiation and phylogenetic relationships of Bonelli's warbler (*Phylloscopus bonelli*) and green warbler (*P. nitidus*) (submitted).

Hekstra G. P. (1982), Description of twenty-four new subspecies of American *Otus* (Aves, Strigidae). Bull. zool. Mus. Amsterdam **9,** 49–63.

Hillis D. M. and Moritz C. (1990), Molecular Systematics. Sinauer Press.

Hillis D. M. and Huelsenbeck J. P. (1992), Signal, noise and reliability in molecular phylogenetic analyses. J. Hered. **83,** 189–195.

Hoelzel A. R. (1992), Molecular Genetic Analysis of Populations. IRL Press, Oxford.

Irwin D. M., Kocher T. D. and Wilson A. A. (1991), Evolution of the cytochrome *b* gene in mammals. J. Mol. Evol. **32,** 128–144.

Kocher T. D., Thomas W. K., Meyer A., Edwards S. V., Pääbo S., Villablanca F. X. and Wilson A. C. (1989), Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. **86,** 6196–6200.

König C. (1991), Taxonomische und ökologische Untersuchungen an Kreischeulen (*Otus* spp.) des südlichen Südamerika. J. Orn. **132,** 209–214.

König C. and Straneck R. (1989), Eine neue Eule (Aves: Strigidae) aus Nordargentinien. Stuttgt. Beitr. Naturkde. Ser. A., **428,** 1–20.

König C. (1991), Zur Taxonomie und Ökologie der Sperlingskäuze (*Glaucidium* spp.) des Andenraumes. Ökol. Vögel (Ecol. Birds) **13,** 15–76.

König C. (1994), Lautäußerungen als interspezifische Isolationsmechanismen bei Eulen der Gattung *Otus* aus dem südlichen Südamerika. Stuttgt. Beitr. Naturkde. Ser. A., **511,** 1–35.

Kornegay J. R., Kocher T. H., Williams L. A. and Wilson A. C. (1993), Pathways of lysozyme evolution inferred from the sequences of cytochrome *b* in birds. J. Mol. Evol. **37,** 367–379.

Kumar S., Tamura K. and Nei M. (1993), MEGA – Molecular Evolutionary Genetics Analysis. Version 1.0. Pennsylvania State University.

Marshall J. T. (1991), The variable screech owl (*Otus atricapillus*) and its relatives. Wilson Bull. **103** (2), 314–315.

Quinn T. W., Shields G. F. and Wilson A. C. (1991), Affinities of the Hawaiian goose based on two types of mitochondrial DNA data. Auk **108,** 585–593.

Richman A. D. and Price T. (1992), Evolution of ecological differences in the Old World leaf warblers. Nature **355,** 817–821.

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

Sambrook J., Fritsch E. F. and Maniatis T. (1989), Molecular Cloning. A Laboratory Manual. 2nd ed. CSHL, Cold Spring Harbour, New York.

Seibold I., Helbig A. J. and Wink M. (1993), Molecular systematics of falcons (family Falconidae). Naturwissenschaften **80,** 87–90.

Seibold I., Fentzloff C. and Wink M., Rekonstruktion der Phylogenie der Seeadler (Gattung *Haliaeetus*) anhand der Nukleotidsequenzen des mitochondriellen Cytochrom *b* Gens (submitted).

Seibold I., Helbig A., Meyburg B., Negro J. and Wink M., Genetic differentiation and molecular phylogeny of European Aquila eagles according to cytochrome *b* nucleotide sequences (in preparation).

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

Sick H. (1985), Ornitologia Brasileira. Uma introduçao. – Vol. **I.** Brasília.

Stewart C.-B. (1993), The powers and pitfalls of parsimony. Nature **361,** 603–607.

Swatschek I., Ristow D., Scharlau W., Wink C. and Wink M. (1993), Populationsgenetik und Vaterschaftsanalyse beim Eleonorenfalken (*Falco eleonorae*). J. Orn. **134,** 137–143.

Swatschek I., Ristow D. and Wink M. (1994), Mate fidelity and parentage in Cory's shearwater (*Calonectris diomedea*). – Field studies and DNA-fingerprinting. Mol. Ecol. **3,** 259–262.

Swofford D. L. (1993), PAUP: Phylogenetic Analysis Using Parsimony. Version 3.1.1. Illinois.

Weske J. S. and Terborgh J. W. (1981), *Otus marshalli,* a new species of screech-owl from Perú. Auk **98** (1), 1–7.

Wilson A. C., Ochman H. and Prager E. M. (1987), Molecular time scale for evolution. Trends Genet. **3,** 241–247.

Wink M. (1994), PCR in der molekularen Evolutionsforschung, in: PCR im medizinischen und biologischen Labor (M. Wink and H. Wehrle, eds.). GIT-Verlag, Darmstadt, pp. 166–184.

Wink M., Heidrich P., Kahl U., Witt H. H. and Ristow D. (1993a), Inter- and intraspecific variation of the nucleotide sequence for cytochrome *b* in Cory's shearwater (*Calonectris diomedea*), Manx shearwater (*Puffinus puffinus*) and Fulmar (*Fulmarus glacialis*). Z. Naturforsch. **48c,** 504–508.

Wink M., Heidrich P. and Ristow D. (1993b), Genetic evidence for speciation of the Manx shearwater (*Puffinus puffinus*) and the Mediterranean shearwater (*P. yelkouan*). Vogelwelt **114,** 226–232.

Wink M., Kahl U. and Heidrich P. (1994), Lassen sich Silber-, Weißkopf- und Heringsmöwe (*Larus argentatus, L. cachinnans* und *L. fuscus*) molekulargenetisch unterscheiden? J. Orn. **135,** 73–80.

Wolters H. E. (1975–1982), Die Vogelarten der Erde. Hamburg, Berlin.