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EVOLUTIONARY RELATIONSHIPS AMONG THE POTOOS (NYCTIBIIDAE) BASED ON ISOZYMES

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ABSTRACT.—Isozyme electrophoresis was used to assess genetic variation in potoos (Nyctibiidae), a distinctive Neotropical family of caprimulgiform nightbirds. Interspecific levels of genetic differentiation among potoos are extremely high (range of Nei's D = 0.191–1.172) and are comparable to intergeneric levels of differentiation in other bird families. In addition, levels of genetic differentiation between populations of both *Nyctibius grandis* and *N. griseus* from east and west of the Andes are comparable to the high genetic distances found in cross-Andes comparisons in other isozyme studies of Neotropical birds. These data suggest that extant potoo lineages are quite old, and that substantial genetic diversity exists in potoos that is not conveyed in the current taxonomy, in which most potoo species lack named or described intraspecific variation, and all species share a single genus.

Phylogenetic analysis of isozyme data supports the monophyly of Nyctibiidae through comparisons with outgroups from five other caprimulgiform families. Our results also support the monophyly of a clade composed of *Nyctibius maculosus*, *N. leucopterus*, and *N. griseus*, and confirm *maculosus* and *leucopterus* as sister taxa. The relationships of other potoos remain essentially unresolved, although there is weak support for the placement of *N. bracteatus* as the basal taxon. Relationships among caprimulgiform families are also essentially unresolved by these data, although there is some support for a clade composed of Caprimulgidae, Aegothelidae, and Eurostopodidae. The very high genetic distances from *Steatornis* to all other caprimulgiforms indicate that it represents the earliest branching lineage in the order.

RESUMEN.—Electroforesis de proteínas fue usado para estudiar la variación genética de los nictibios (Nyctibiidae), una familia Neotropical de aves nocturnas. Los niveles de diferencia entre las especies de nictibios son altos (Nei's D = 0.191 hasta 1.172), tan alto como los niveles que se encuentran entre los géneros en otras familias de aves. Los niveles de diferencia entre las dos especies con representantes en los dos lados de los Andes (el occidente y el oriente), *Nyctibius grandis* y *N. griseus*, están de acuerdo con esos observados en otros estudios de aves de quienes los representantes ocurren en los ambos lados de las montañas. Estos resultados sugieren que los lineajes evolucionarios de nictibios son antiguos y que bastante diversidad genetica existe que no se puede reconocer con la taxonomia corriente.

Un análisis filogenético de los datos indica que los nictibios pertenecen a un grupo monofilético y diferente de otros grupos de aves nocturnas. Los resultados tambien apoyan la existencia de un grupo monofilético compuesto de *N. maculosus*, *N. leucopterus* y *N. griseus*, en que las especies *maculosus* y *leucopterus* tienen una relación mas cercana que con *griseus*. Las relaciones evolucionarias de los otros nictibios no fueron resolvadas, sin embargo los datos sugieren que *N. bracteatus* es la especie mas antigua. Tampoco resolvamos las relaciones entre las familias caprimulgiformas, aunque los datos sugieren que Caprimulgidae, Aegothelidae y Eurostopodidae son consanguineos. La grande distancia genética que existe entre *Steatornis* y otras familias en el estudio, indica que *Steatornis* es la familia mas antigua de las aves nocturnas.

The potoos (Nyctibiidae) are an exclusively Neotropical family of nocturnal birds characterized by their distinctive mimicry of vertical tree stubs, upon which they often perch. Mimicry is achieved by pointing their bill upward, closing their eyes, and laying their tail flat along the branch (Sick 1993). Their cryptic behavior, nocturnal habits, and tropical distribution have made them one of the most poorly known groups of birds. However, recent fieldwork in South America

has yielded new information on vocalizations and life history of several little-known taxa that clarifies species boundaries and provides new material for anatomical and molecular studies (Remsen and Traylor 1983; Schulenberg et al. 1984; Parker et al. 1985; Cohn-Haft 1993).

All potoos are currently united within the genus *Nyctibius* (Monroe and Sibley 1993). Most treatments of the family recognize five to seven potoo species (Chapman 1926; Peters 1940; Schulenberg et al. 1984; Sibley and Monroe 1990), depending on whether the Middle American form, *jamaicensis* (Northern Potoo), is given specific status or lumped within *griseus* (Common Potoo), and whether *maculosus* (Andean Potoo) is lumped within *leucopterus* (White-winged Potoo) (Schulenberg et al. 1984). Confusion concerning the specific status of *maculosus* began when Peters (1940) reduced it to a subspecies of *leucopterus*. This was presumably based on Chapman's (1926) conclusion that *maculosus* was an Andean representative of *leucopterus*, although Chapman did not explicitly state that he considered them conspecific. More recent analyses of morphology and voice strongly support the species status of *leucopterus* and *maculosus* (Schulenberg et al. 1984; Cohn-Haft 1993).

As with most Neotropical avian taxa, there have been few attempts to elucidate the evolutionary relationships within this family. The Northern Potoo has been hypothesized to be the sister taxon to *grandis* (Great Potoo) based on their vocalizations, rather than to the phenotypically similar Common Potoo (Davis 1978). Schulenberg et al. (1984) proposed that *maculosus* is a highland relative of *griseus*, not *leucopterus*, based on similarities in size and plumage. Mariaux and Braun (1996) recently performed a molecular phylogenetic survey of Nyctibiidae using DNA sequence data from the mitochondrial cytochrome *b* (cyt *b*) gene. They found evidence for a *maculosus-leucopterus* clade, confirming Chapman's (1926) early view. However, they were not able to fully resolve relationships among potoos, partly due to unexpectedly high levels of divergence among potoo cyt *b* sequences. To confirm and extend those observations, we examined nuclear genetic markers generated from electrophoresis of isozymes to perform a phylogenetic analysis of Nyctibiidae.

METHODS

We use the taxonomy of Sibley and Monroe (1993) as the most recent comprehensive treatment of Caprimulgiformes. All available frozen tissue samples of *Nyctibius* were obtained for this study ($n = 14$). Specimens examined in this study included one to three individuals of each currently recognized potoo species (Table 1), aside from *N.* [*griseus*] *jamaicensis*, of which no samples were available. One individual from each of five other caprimulgiform families was used as outgroups (Table 1). Because the SOD locus could not be resolved for *Podargus strigoides*, an individual of *Podargus papuensis* was scored for this locus.

Protein electrophoresis was performed on Titan III cellulose acetate plates (Helena Laboratories Inc.) according to methods described by Richardson et al. (1986). Tissue homogenates were prepared by grinding approximately 50 mg of heart, liver, and pectoral muscle in 500 µl of distilled water. The mixture was spun in a Brinkman 5415C Eppendorf centrifuge at 14,000 rpm for 2 min. The resulting supernatant was divided into 20 µl aliquots and frozen (−80°C) for subsequent electrophoretic analyses. Running conditions for all loci appear in Table 2. Electromorphs were coded alphabetically in order of relative mobility from the origin with the most anodally migrating allele as "a."

BIOSYS-1 (Swofford and Selander 1981) was used to compute Cavalli-Sforza and Edwards (1967) and Nei (1978) genetic distances (*D*), and to perform a UPGMA cluster analysis using the Cavalli-Sforza and Edwards (1967) chord distance. Phylogenetic analyses were performed using FREQPARS (available electronically via anonymous ftp from onyx.si.edu; see Swofford and Berlocher 1987) and PAUP (Swofford, 1993; the edition used was a prerelease version of PAUP* 4.0).

RESULTS

Genetic Variation

Interspecific variation.—Levels and patterns of variation at 23 presumed genetic loci from 20 enzyme systems were resolved for all ingroup and outgroup taxa (Table 3). One locus represents an unknown dehydrogenase (UDH) that appeared as a lightly staining but well resolved locus on SORDH. Because three additional loci from three enzyme systems (GPT, ME-1, NP; Table 3) could not be fully resolved for all outgroups, they were not included in the phylogenetic analyses. Twenty-two of the 26 loci (85%) were variable among the potoos; the monomorphic loci were AK, GOT, GPI, and MDH-1. There were no monomorphic loci when outgroups were

TABLE 1

SPECIMENS EXAMINED IN THIS STUDY. SPECIMEN NUMBERS REFER TO THE TISSUE CATALOG AND NOT THE VOUCHER SPECIMEN. IN ADDITION TO SAMPLES AVAILABLE AT THE UNITED STATES NATIONAL MUSEUM OF NATURAL HISTORY (USNM), TISSUE SAMPLES WERE PROVIDED BY THE FROZEN TISSUE COLLECTIONS OF THE FOLLOWING INSTITUTIONS (IN DECREASING ORDER OF AMOUNT BORROWED): LOUISIANA STATE UNIVERSITY MUSEUM OF ZOOLOGY (BATON ROUGE; LSUMZ), MUSEUM OF VICTORIA (AUSTRALIA; MV), ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA (PHILADELPHIA; ANSP)

Taxon/specimen number	Collector	Locality
NYCTIBIIDAE		
Nyctibius aethereus (NAET)		
LSUMZ B10877	A. S. Meyer	PERU: depto. Ucayali: SE. slope Cerro Tahuayo.
LSUMZ B11236	D. C. Schmitt	PERU: depto. Ucayali: SE. slope Cerro Tahuayo.
Nyctibius bracteatus (NBRA)		
LSUMZ B4509	S. W. Cardiff	PERU: depto. Loreto; Lower Río Napo region, E. bank Río Yanayacu, ca. 90 km N Iquitos.
LSUMZ B20270	M. Cohn-Haft	BRAZIL: Amazonas; Munic. Manaus, km 34 ZF-3, FAZ. Esteio, ca. 80 km N Manaus.
LSUMZ B20318	K. V. Rosenberg	BRAZIL: Amazonas; Munic. Manaus, km 41 ZF-3, Faz. Esteio, ca. 80 km N Manaus.
Nyctibius grandis (NGRA)		
USNM B3223	R. T. Brumfield	PANAMA: prov. Bocas del Toro; 6 km E Changuinola on road from Changuinola to Almirante.
LSUMZ B8954	C. G. Schmitt	BOLIVIA: depto. Pando; Nicolás Suarez, 12 km by road S Cobija, 8 km W on road to Mucden.
LSUMZ B15415	J. M. Bates	BOLIVIA: depto. Santa Cruz, Velasco, Pre-Parque Nacional: "Noel Kempff Mercado," 30 km E Aserradero Moira.
Nyctibius griseus (NGRI)		
USNM B3252	M. J. Braun	PANAMA: prov. Panamá; Chiva Chiva Rd.
ANSP B3238	F. Sornoza M.	ECUADOR: prov. Sucumbios; Imuya Cocha.
Nyctibius leucopterus (NLEU)		
LSUMZ B20267	M. Cohn-Haft	BRAZIL: Amazonas: Munic. Manaus; km 34 ZF-3, Faz. Esteio, ca. 80 km N Manaus.
LSUMZ B20315	M. Cohn-Haft	BRAZIL: Amazonas: Munic. Manaus; km 34 ZF-3, Faz. Esteio, ca. 80 km N Manaus.
LSUMZ B20319	M. Cohn-Haft	BRAZIL: Amazonas: Munic. Manaus; km 34 ZF-3, Faz. Esteio, ca. 80 km N Manaus.
Nyctibius maculosus (NMAC)		
LSUMZ B271	M. J. Braun	PERU: depto. Cajamarca; Lucuma on Sapalache-Carmen Trail.
LSUMZ B1825	T. S. Schulenberg	PERU: depto. Pasco; Santa Cruz, about 9 km SSE Oxapampa.
CAPRIMULGIDAE		
Chordeiles minor (CHOR)		
LSUMZ B5279	L. Hale	USA: Louisiana: Cameron Par.; Holly Beach, ¼ mi N Holly Beach Hwy.
EUROSTOPODIDAE		
Eurostopodus mystacalis (EURO)		
MV JWC 129	J. Wombey	AUSTRALIA: Australian Capital Territories; Canberra; 35°17′S, 149°08′E.
AEGOTHELIDAE		
Aegotheles cristatus (AEGO)		
MV C450	J. Wombey	AUSTRALIA: Queensland; Kroombit Tops; 24°26′S, 150°43′E.
PODARGIDAE		
Podargus papuensis		
MV C876	J. Wombey	AUSTRALIA: Queensland; Silver Plains; 13°59′S, 143°33′E.
Podargus strigoides (PODA)		
LSUMZ B8654	A. P. Capparella	AUDUBON ZOO, New Orleans, Louisiana.
STEATORNITHIDAE		
Steatornis caripensis (STEA)		
LSUMZ B7474	D. E. Willard	VENEZUELA: terr. Amazonas; Cerro de la Neblina Camp VII, 1,800 m.

TABLE 2

ENZYMES EXAMINED, BUFFERS USED, AND RUNNING TIME FOR EACH ENZYME

Enzyme (E.C. no.)	Abbreviation	Number of loci	Running buffer[a]	Running time (hr)[b]
Aconitate hydratase (4.2.1.3) (aconitase)	ACON	1 (anodal)[d]	C	1
Adenosine deaminase (3.5.4.4)	ADA	1	B	1
Adenylate kinase[c] (2.7.4.3)	AK	1	C	1
Alanine aminotranserase (2.6.1.2) (glutamate-pyruvate transaminase)	GPT	1	B	1
Aspartate aminotransferase[c] (2.6.1.1) (glutamate-oxaloacetate transaminase)	GOT	1 (anodal)[d]	B	1
Creatine kinase[c] (2.7.3.2)	CK	2	D	1
Esterase (α-napthyl acetate) (3.1.1.1)	EST	1	C	1
Fumarate hydratase[c] (4.2.1.2) (fumarase)	FUM	1	A	2
Glucose-phosphate isomerase[c] (5.3.1.9)	GPI	1	B	1.5
Glutathione reductase (1.6.4.2)	GSR	1	E	1
α-Glycerophosphate dehydrogenase (1.1.1.8) (glycerol-3-phosphate dehydrogenase)	αGPD	1	B	1.5
Guanine deaminase (3.5.4.3)	GDA	1	B	0.75
Isocitrate dehydrogenase[c] (1.1.1.42)	IDH	2	A	2
Lactate dehydrogenase (1.1.1.27)	LDH	2	A	2
Malate dehydrogenase[c] (1.1.1.37)	MDH	2	C	1
Malic enzyme (1.1.1.40) (NADP-malate dehydrogenase)	ME	2	B	1
Mannose phosphate isomerase[c] (5.3.1.8)	MPI	1	B	1
Phosphoglucomutase[c] (2.7.5.1)	PGM	2	B	1
6-Phosphogluconate dehydrogenase (1.1.1.44)	6PGD	1	B	1.5
Purine nucleoside phosphorylase (2.4.2.1)	NP	1	B	0.75
Peptidases (3.4.11)				
Leucine-alanine	LA	1	A	0.75
Leucine-glycine-glycine	LGG	1	A	1
Phenylalanine-proline	Phe-Pro	2	A	.75
Valine-leucine	VL	1	A	1
Pyruvate kinase[c] (2.7.1.40)	PK	1	A	1.5
Sorbitol dehydrogenase (1.1.1.14) (L-iditol dehydrogenase)	SORDH	1	C	1
Superoxide dismutase (1.15.1.1)	SOD	1 (anodal)[d]	A, B	1
Unknown dehydrogenase[c] (1.1.1.?)	UDH	1	C	1

[a] A = 0.01 M Citrate-phosphate (10 mM di-sodium hydrogen orthophosphate, 2.5 mM citric acid), pH 6.4, B = 0.02 M Phosphate (11.6 mM di-sodium hydrogen orthophosphate, 8.4 mM sodium di-hydrogen orthophosphate), pH 7.0, C = 0.05 M Tris-maleate (50 mM Tris, 20 mM maleic acid), pH 7.8, D = 0.015 M Tris-EDTA-borate-$MgCl_2$ (15 mM Tris, 5 mM di-sodium EDTA, 10 mM magnesium chloride, 5.5 mM boric acid), pH 7.8, E = 0.13 M Tris-EDTA-borate (130 mM Tris, 2.2 mM di-sodium EDTA, 6 mM sodium hydroxide, 71.3 mM boric acid), pH 8.9. Recipes found in Richardson et al. (1986).

[b] At 200 V or 7 mA.

[c] Conservative loci suggested for birds by Les Christidis (pers. comm.) with the exception of UDH, which was considered conservative based on the small number of alleles.

[d] Indicates direction of migration of scored locus. Most vertebrates have more than one locus for these enzyme systems.

included. Eleven additional loci (ACON-1&2, EST, LA-1&2, LDH-2, LGG, ME-2, PGM-2, PHEPRO-1, and SORDH) showed variation among the potoos, but either could not be fully resolved or exhibited uninterpretable variation for some species. When these additional loci are considered, 92% of the loci are variable within the potoos.

Genetic distances (Table 4) among potoo species are extremely high (average Nei's $D = 0.655$; range = 0.191–1.172). Likewise, genetic distances between potoo species and the outgroups are high, dramatically illustrated by the absence of shared alleles between *N. bracteatus* (Rufous Potoo) and *Aegotheles cristatus* (Tables 3 and 4). In fact, the smallest average genetic distance between the potoos and an outgroup is 2.031 with *Eurostopodus mystacalis*.

TABLE 3

MATRIX OF ALLELE FREQUENCIES USED FOR PHYLOGENETIC ANALYSIS. SPECIES ACRONYMS ARE AS IN TABLE 1. LOCI FOLLOWED BY ASTERISKS WERE CONSIDERED CONSERVATIVE

		Species										
Locus	Allele	NMAC	NLEU	NGRI	NAET	NGRA	NBRA	CHOR	EURO	AEGO	STEA	PODA
ADA	a	—	—	—	—	—	—	—	—	—	1.0	—
	b	—	—	—	—	—	—	—	1.0	—	—	1.0
	c	—	—	—	—	—	—	—	—	1.0	—	—
	d	1.0	0.833	1.0	1.0	1.0	—	0.5	—	—	—	—
	e	—	0.167	—	—	—	—	—	—	—	—	—
	f	—	—	—	—	—	1.0	0.5	—	—	—	—
AK*	a	—	—	—	—	—	—	—	—	—	—	1.0
	b	—	—	—	—	—	—	1.0	—	1.0	—	—
	c	1.0	1.0	1.0	1.0	1.0	1.0	—	1.0	—	1.0	—
CK-1	a	—	—	—	—	—	—	—	1.0	—	—	—
	b	1.0	—	1.0	—	—	1.0	—	—	—	—	—
	c	—	—	—	—	—	—	—	—	—	1.0	—
	d	—	1.0	—	—	—	—	—	—	—	—	—
	e	—	—	—	—	—	—	1.0	—	—	—	—
	f	—	—	—	—	—	—	—	—	1.0	—	—
	g	—	—	—	1.0	—	—	—	—	—	—	—
	h	—	—	—	—	—	—	—	—	—	—	1.0
	i	—	—	—	—	1.0	—	—	—	—	—	—
CK-2*	a	—	—	—	—	—	—	—	—	—	—	1.0
	b	—	—	—	—	—	—	—	—	1.0	—	—
	c	—	—	—	—	—	—	1.0	1.0	—	1.0	—
	d	—	—	—	—	—	1.0	—	—	—	—	—
	e	—	—	1.0	1.0	1.0	—	—	—	—	—	—
	f	1.0	1.0	—	—	—	—	—	—	—	—	—
FUM*	a	—	—	—	—	0.667	—	—	—	—	—	—
	b	—	—	—	—	0.333	—	—	—	—	—	—
	c	—	—	—	—	—	—	—	—	—	—	1.0
	d	—	—	—	—	—	—	—	—	1.0	—	—
	e	—	—	—	—	—	—	—	1.0	—	—	—
	f	—	—	—	—	—	—	1.0	—	—	—	—
	g	—	—	—	—	—	—	—	—	—	1.0	—
	h	1.0	1.0	1.0	—	—	1.0	—	—	—	—	—
	i	—	—	—	1.0	—	—	—	—	—	—	—
GDA	a	—	—	—	—	1.0	—	—	—	—	—	—
	b	—	—	1.0	—	—	—	—	—	—	—	—
	c	—	—	—	—	—	—	—	—	—	—	1.0
	d	1.0	—	—	—	—	—	—	—	—	—	—
	e	—	1.0	—	—	—	—	—	—	—	—	—
	f	—	—	—	—	—	—	1.0	—	—	—	—
	g	—	—	—	1.0	—	—	—	—	—	—	—
	h	—	—	—	—	—	1.0	—	—	—	—	—
	i	—	—	—	—	—	—	—	1.0	—	—	—
	j	—	—	—	—	—	—	—	—	—	1.0	—
	k	—	—	—	—	—	—	—	—	1.0	—	—
GOT*	a	—	—	—	—	—	—	—	—	—	—	1.0
	b	—	—	—	—	—	—	1.0	1.0	1.0	1.0	—
	c	1.0	1.0	1.0	1.0	1.0	1.0	—	—	—	—	—
αGPD	a	—	—	—	—	—	—	—	1.0	—	—	—
	b	—	—	—	—	—	—	—	—	1.0	—	—
	c	—	—	—	—	—	0.333	—	—	—	—	—
	d	1.0	0.667	1.0	—	1.0	0.667	—	—	—	—	—
	e	—	—	—	—	—	—	—	—	—	1.0	—
	f	—	0.333	—	1.0	—	—	—	—	—	—	—
	g	—	—	—	—	—	—	—	—	—	—	1.0
	h	—	—	—	—	—	—	1.0	—	—	—	—

TABLE 3
CONTINUED

Locus	Allele	Species										
		NMAC	NLEU	NGRI	NAET	NGRA	NBRA	CHOR	EURO	AEGO	STEA	PODA
GPI*	a	—	—	—	—	—	—	—	—	0.5	—	—
	b	—	—	—	—	—	—	1.0	—	—	—	—
	c	—	—	—	—	—	—	—	1.0	0.5	—	—
	d	—	—	—	—	—	—	—	—	—	1.0	1.0
	e	1.0	1.0	1.0	1.0	1.0	1.0	—	—	—	—	—
GSR	a	—	—	1.0	1.0	1.0	—	—	—	—	—	—
	b	—	1.0	—	—	—	—	—	—	—	—	—
	c	—	—	—	—	—	—	—	—	—	1.0	—
	d	1.0	—	—	—	—	—	—	—	—	—	—
	e	—	—	—	—	—	—	1.0	—	—	—	—
	f	—	—	—	—	—	—	—	1.0	—	—	—
	g	—	—	—	—	—	1.0	—	—	—	—	—
	h	—	—	—	—	—	—	—	—	1.0	—	—
	i	—	—	—	—	—	—	—	—	—	—	1.0
IDH-1	a	—	—	0.25	—	0.667	—	—	—	—	—	—
	b	—	—	—	—	—	0.167	—	—	—	—	—
	c	1.0	1.0	0.75	1.0	0.333	0.833	—	—	—	—	—
	d	—	—	—	—	—	—	1.0	—	—	1.0	—
	e	—	—	—	—	—	—	—	—	0.5	—	—
	f	—	—	—	—	—	—	—	1.0	—	—	—
	g	—	—	—	—	—	—	—	—	—	—	1.0
	h	—	—	—	—	—	—	—	—	0.5	—	—
IDH-2*	a	—	—	—	—	—	—	—	—	0.5	1.0	—
	b	—	—	—	—	—	—	—	0.5	—	—	—
	c	1.0	1.0	1.0	—	—	—	—	—	—	—	—
	d	—	—	—	—	—	—	1.0	—	0.5	—	0.5
	e	—	—	—	—	—	—	—	0.5	—	—	—
	f	—	—	—	1.0	1.0	1.0	—	—	—	—	0.5
LDH-1	a	—	—	—	—	—	—	—	—	1.0	—	—
	b	1.0	1.0	1.0	—	—	—	—	—	—	1.0	—
	c	—	—	—	1.0	—	—	—	1.0	—	—	—
	d	—	—	—	—	1.0	—	—	—	—	—	—
	e	—	—	—	—	—	1.0	1.0	—	—	—	—
	f	—	—	—	—	—	—	—	1.0	—	—	—
	g	—	—	—	—	—	—	—	—	—	—	1.0
MDH-1*	a	—	—	—	—	—	—	—	—	1.0	1.0	1.0
	b	1.0	1.0	1.0	1.0	1.0	1.0	—	—	—	—	—
	c	—	—	—	—	—	—	—	1.0	—	—	—
	d	—	—	—	—	—	—	1.0	—	—	—	—
MDH-2*	a	—	—	—	—	—	—	—	—	—	—	1.0
	b	1.0	1.0	1.0	1.0	0.833	—	1.0	1.0	1.0	1.0	—
	c	—	—	—	—	0.167	1.0	—	—	—	—	—
MPI*	a	—	—	—	—	—	1.0	—	—	—	—	—
	b	—	—	—	—	—	—	—	—	1.0	—	—
	c	—	—	—	0.25	0.667	—	—	—	—	—	—
	d	—	—	0.25	0.75	—	—	—	—	—	—	—
	e	—	—	—	—	0.333	—	—	—	—	—	—
	f	1.0	1.0	0.75	—	—	—	—	—	—	—	—
	g	—	—	—	—	—	—	—	—	—	0.5	—
	h	—	—	—	—	—	—	1.0	1.0	—	—	—
	i	—	—	—	—	—	—	—	—	—	0.5	—
	j	—	—	—	—	—	—	—	—	—	—	1.0
6PGD	a	—	—	—	0.25	—	—	—	—	—	—	—
	b	—	—	—	—	—	—	—	1.0	—	—	—
	c	1.0	—	—	—	—	—	—	—	—	—	—
	d	—	—	—	—	—	—	—	—	—	—	1.0

TABLE 3
CONTINUED

Locus	Allele	Species										
		NMAC	NLEU	NGRI	NAET	NGRA	NBRA	CHOR	EURO	AEGO	STEA	PODA
	c	—	0.833	1.0	0.75	1.0	—	—	—	—	—	—
	f	—	—	—	—	—	—	1.0	—	1.0	—	—
	g	—	—	—	—	—	1.0	—	—	—	—	—
	h	—	0.167	—	—	—	—	—	—	—	—	—
	i	—	—	—	—	—	—	—	—	—	1.0	—
PGM-1*	a	—	—	—	—	—	1.0	—	—	—	—	1.0
	b	—	—	—	—	—	—	1.0	1.0	1.0	—	—
	c	1.0	1.0	1.0	1.0	1.0	—	—	—	—	—	—
	d	—	—	—	—	—	—	—	—	—	1.0	—
PHE-PRO-2	a	—	—	—	—	—	—	1.0	1.0	—	—	—
	b	—	—	—	—	1.0	—	—	—	—	—	—
	c	—	—	—	—	—	—	—	—	—	—	1.0
	d	—	—	—	—	—	—	—	—	1.0	—	—
	e	—	—	—	—	—	1.0	—	—	—	—	—
	f	1.0	1.0	1.0	1.0	—	—	—	—	—	1.0	—
PK*	a	—	—	—	—	—	—	—	1.0	—	—	—
	b	—	—	—	—	—	—	—	—	—	—	1.0
	c	1.0	1.0	1.0	—	—	—	1.0	—	—	—	—
	d	—	—	—	—	—	1.0	—	—	—	—	—
	e	—	—	—	—	1.0	—	—	—	1.0	1.0	—
	f	—	—	—	1.0	—	—	—	—	—	—	—
SOD	a	—	—	—	—	—	—	—	—	0.5	1.0	—
	b	—	—	—	1.0	—	—	—	—	—	—	—
	c	—	—	—	—	—	—	1.0	—	—	—	—
	d	—	—	—	—	—	—	—	1.0	0.5	—	—
	e	1.0	1.0	1.0	—	1.0	—	—	—	—	—	—
	f	—	—	—	—	—	1.0	—	—	—	—	—
	g	—	—	—	—	—	—	—	—	—	—	1.0
UDH*	a	—	—	—	—	—	—	—	—	—	—	1.0
	b	—	—	—	—	—	—	—	—	1.0	—	—
	c	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—	—	—
	d	—	—	—	—	—	—	—	—	—	1.0	—
VL	a	—	—	—	—	—	—	—	—	1.0	—	—
	b	—	—	—	—	—	—	1.0	—	—	—	—
	c	—	—	—	—	—	—	—	—	—	1.0	—
	d	—	—	—	—	—	—	—	—	—	—	1.0
	e	—	—	0.25	—	—	—	—	—	—	—	—
	f	—	—	—	1.0	—	—	—	1.0	—	—	—
	g	1.0	1.0	0.75	—	1.0	1.0	—	—	—	—	—

The small sample sizes may have resulted in some inflation of genetic distances because lower frequency alleles shared between taxa were unlikely to be detected. To assess the contribution of small sample size to genetic distance, we calculated pairwise genetic distances between individuals within a species. In this analysis, the average genetic distance among individuals within species in our data set (see Appendix) was 0.04 over all possible comparisons (n = 12). If comparisons among populations of *griseus* and *grandis* separated by the Andes are eliminated from the analysis (see *Intraspecific Variation* section below), the average genetic distance is 0.02. These results illustrate that the extremely high genetic distances found among potoo species are not simply an artifact of small sample size.

Examination of the genetic distance matrix reveals two striking patterns (Table 4). First, *griseus* consistently has among the lowest genetic distances to all other potoo taxa, a pattern also revealed in the matrix of raw distances generated from cyt *b* sequences (Mariaux and Braun 1996). Secondly, *bracteatus* consistently has the highest genetic distance to all other potoo taxa. These patterns may reflect lineage-specific reduction or acceleration in evolutionary rate. An alternative explanation for the high distances to *bracteatus* is that it represents the basal potoo

TABLE 4

GENETIC DISTANCE MATRIX BASED ON 23 LOCI RESOLVED FOR ALL TAXA. UPPER MATRIX, NEI (1978) UNBIASED GENETIC DISTANCE; LOWER MATRIX, CAVALLI-SFORZA AND EDWARDS (1967) CHORD DISTANCE. NEI'S GENETIC DISTANCE BETWEEN NBRA AND AEGO WAS UNOBTAINABLE BECAUSE THEY SHARED NO ALLELES. THE CAVALLI-SFORZA AND EDWARDS GENETIC DISTANCE BETWEEN NBRA AND AEGO IS NOT 1.0 BECAUSE OF CORRECTION FACTORS. FOR ACRONYMS REFER TO TABLE 1

Taxon	NMAC	NLEU	NGRI	NAET	NGRA	NBRA	CHOR	EURO	AEGO	PODA	STEA
NMAC	******	0.191	0.198	0.811	0.679	0.865	1.872	2.026	3.090	2.026	3.125
NLEU	0.388	******	0.208	0.708	0.622	0.976	1.869	1.999	3.063	1.999	3.098
NGRI	0.394	0.410	******	0.539	0.419	0.881	1.838	1.992	3.056	1.992	3.091
NAET	0.677	0.643	0.589	******	0.578	1.172	2.186	1.716	3.068	2.004	3.795
NGRA	0.637	0.619	0.541	0.601	******	0.979	2.233	2.039	2.440	2.039	3.774
NBRA	0.684	0.713	0.690	0.753	0.709	******	2.189	2.412	∞∞∞∞∞	3.105	2.700
CHOR	0.825	0.826	0.825	0.846	0.847	0.846	******	1.168	1.374	1.727	3.807
EURO	0.840	0.840	0.840	0.818	0.841	0.860	0.751	******	1.693	1.727	3.114
AEGO	0.881	0.881	0.881	0.881	0.862	0.900	0.781	0.809	******	1.470	2.856
PODA	0.840	0.840	0.840	0.840	0.841	0.881	0.818	0.818	0.787	******	2.420
STEA	0.881	0.881	0.881	0.886	0.886	0.866	0.886	0.881	0.870	0.860	******

lineage, and simply has not shared a recent common ancestor with the other extant potoo taxa. Regardless, these patterns are indicative of rate variation, and the distance-based methods of phylogenetic inference used herein that assume rate constancy (i.e., UPGMA) should be interpreted with caution.

Intraspecific variation.—Nine additional loci (ACON-1, EST, LA-1, LDH-2, LGG, ME-2, PGM-2, PHEPRO-1, and SORDH) were scored for the analysis of intraspecific differentiation within *griseus*, *grandis*, and *bracteatus* for a total of 34 or 35 loci (EST was not resolved for *griseus*). The genetic distance (Nei 1978) between samples of *griseus* on opposite sides of the Andes was 0.131 based on 34 loci (0.034 for the 23 loci used in phylogenetic analyses; Tables 3 and 4). *Nyctibius grandis* had a similar across-Andes genetic distance of 0.188 based on 35 loci, but the distance based on 23 loci (Nei's $D = 0.101$) was considerably higher than that of *griseus*. Levels of genetic differentiation between populations on opposite sides of the Andes are consistently higher than levels of genetic differentiation between populations on the same side of the Andes (0.188 versus 0.010 for *grandis*). These values are quite high for intraspecific comparisons in birds, but are consistent with levels of genetic differentiation across the Andes that have been found in a taxonomically diverse array of avian species (Brumfield and Capparella 1996). It is noteworthy that the magnitude of genetic divergence between cross-Andean populations of *grandis* approaches that separating *maculosus*, *leucopterus*, and *griseus* (Table 4).

The two populations of *bracteatus*, between which the Río Negro represents the largest potential barrier to dispersal, had a genetic distance of 0.035 based on 35 loci. Although this value is relatively low, it suggests that some genetic differentiation exists among populations of *bracteatus* because the genetic distance between two individuals from the same population was 0.010. Analysis of more specimens will be necessary to determine if significant genetic structure exists in *bracteatus*.

Phylogenetic Analysis

Phylogenetic analyses were performed on the allele-frequency data shown in Table 3. Only phylogenetically informative loci consistently scoreable in all taxa were included. We chose the FREQPARS method (Swofford and Berlocher 1987) as our primary method of analysis. FREQPARS implements a parsimony method for polymorphic character data that assigns (for any given tree) a set of ancestral allele-frequency arrays that minimize the total amount of frequency change implied by the reconstruction, with change measured in terms of Manhattan distance between nodes (the "MANAD" criterion). Although our sample sizes are small, methods of analysis that incorporate frequency information are, in fact, less sensitive to sampling error than coarser "presence-absence" coding strategies (Swofford and Berlocher 1987).

The FREQPARS program has limited searching capabilities and is best used by evaluating user-defined trees that cover the range of trees likely to be optimal. Because over 34 million unrooted trees are possible for the 11 taxa included in our study, evaluation of all possible trees was impractical. However, only 99,225 unrooted trees are consistent with monophyly of the six potoo taxa. We evaluated all of these trees using FREQPARS; this strategy guarantees finding the optimal tree(s) assuming only that the potoos are monophyletic (see Potoo monophyly section below). The input treefile for this analysis was constructed by creating a dummy data matrix containing a single uninformative character and performing an exhaustive search using the "topological constraints" feature of PAUP with the "collapse zero-length branches" option deselected. The resulting trees were then exported in FREQPARS format.

Potoo relationships.—A single most-parsimonious tree resulted (219.668 "steps" or units of allele-frequency change) from the FREQPARS analysis, placing *aethereus* as the basal potoo taxon (Fig. 1A). On this tree, a *grandis-bracteatus* clade is sister to a clade composed of *maculosus*, *leucopterus*, and *griseus*, with *maculosus* and *leucopterus* appearing as sister taxa within the latter clade. The cost of rejecting either the *maculosus-leucopterus-griseus* or the *maculosus-leucopterus* clade is 222.268–219.668 = 2.500 steps, equivalent to 1.25 allelic substitutions. The five next-most-parsimonious trees were less than one-half step longer than the most parsimonious (each 220.002 steps) and should probably be treated as equally parsimonious given the small sample sizes. All six trees agree on the ([*maculosus*, *leucopterus*], *griseus*) relationship. However, basal to this clade, all permutations of potoo relationships occur, with the exception that *grandis* never appears as the basal taxon.

The extremely high levels of genetic divergence found among the potoos raise the possibility that the true phylogenetic signal might be obscured by high rates of substitution at loci evolving too rapidly to provide reliable information. Consequently, we performed a second FREQPARS

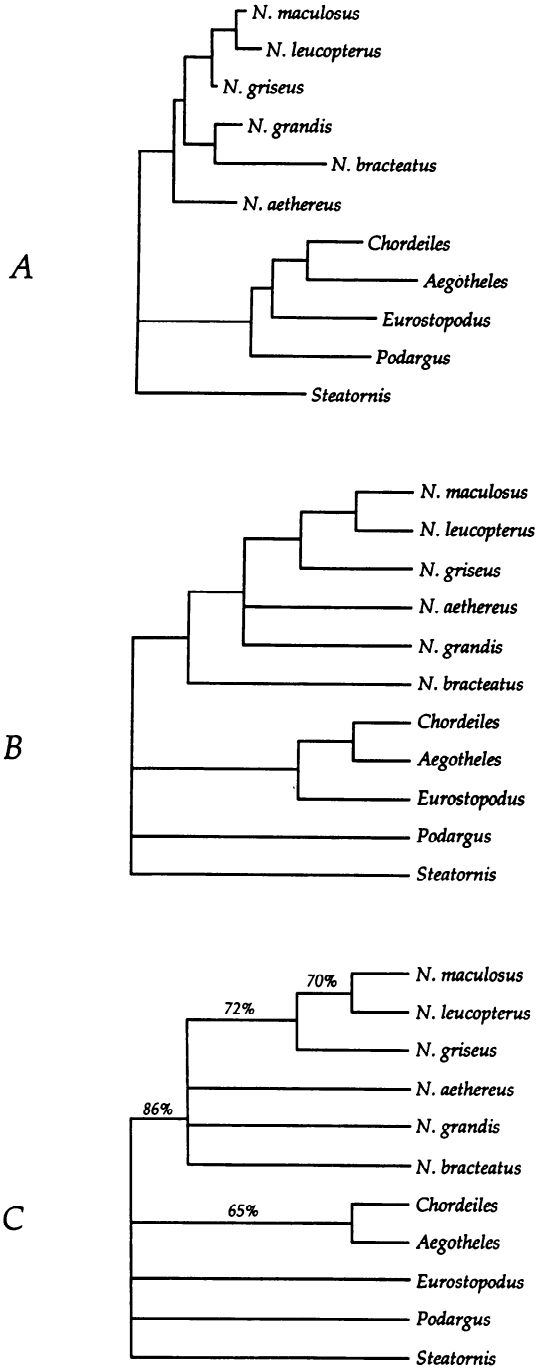

FIG. 1. Cladograms of caprimulgiform relationships based on parsimony (FREQPARS) analysis of allele-frequency data from isozyme loci (Table 3). The potoos were constrained to be monophyletic, and the trees are drawn rooted to *Steatornis* based on its large genetic distance to all other taxa (Table 4). A) Most-parsimonious tree (219.668 steps) of caprimulgiform relationships based on 23 isozyme loci (Table 3). Branch lengths reflect amount of allele-frequency change (measured as Manhattan distance) between each pair of nodes. B) Strict consensus of nine most-parsimonious trees (97.020 steps) based on data from 12 conservative loci (Table 3). Branch lengths do not reflect genetic distance. C) 50% Majority-rule consensus tree resulting from bootstrap analysis under MANOB criterion. Values shown on branches reflect the percentage of 1,000 bootstrap replicates in which the corresponding clade was found.

analysis in which only 12 loci considered to be conservatively evolving in birds (Table 3) were included. Nine equally parsimonious trees (97.020 steps) were found; the strict consensus (also the majority rule consensus) of these nine trees is shown in Figure 1B. The trees from the conserved-loci-only analysis preserved most aspects of the all-loci analysis, except that *bracteatus* consistently appears as the basal potoo taxon, a relationship supported by an apparent PGM-1^a synapomorphy in the remaining five potoo taxa.

Taken jointly, we interpret the FREQPARS analyses as supporting a terminal clade composed of *maculosus*, *leucopterus*, and *griseus* based on the synapomorphic alleles MPI^f, IDH-2^c, LDH-1^b, and PK^c. The presence of some of these alleles in other taxa is most parsimoniously interpreted as homoplasy, perhaps due to coincident migration of nonidentical alleles. The monophyly of *maculosus* plus *leucopterus* is supported by the shared allele CK-2^f. These analyses failed to resolve definitively whether *aethereus* or *bracteatus* is the basal potoo taxon, but suggest that *grandis* is not. Although distance analyses (see below) provide weak support for a *grandis-aethereus* relationship, no allelic synapomorphies for such a grouping are evident in the data matrix, and FREQPARS analysis rejects this relationship. The only unambiguous synapomorphy for the *grandis* plus *bracteatus* clade is the allele MDH-2^c, which occurs at low frequency in *grandis*; evidence for this clade should therefore be regarded as tentative in light of the small sample sizes.

Potoo monophyly.—The analyses described above do not directly address the question of whether the six potoo species constitute a monophyletic group, as potoo monophyly was assumed. To evaluate the evidence for potoo monophyly, we used a technique (Berlocher and Swofford in press; available in PAUP* 4.0) that obtains an exact solution to the "MANOB" criterion of Swofford and Berlocher (1987), which is a good approximation to the MANAD criterion. MANOB requires that allele-frequency arrays assigned to each internal node of the tree (hypothetical ancestral taxa) be chosen from the set of allele-frequency arrays observed in the terminal taxa. In this analysis, each unique allele-frequency array is treated as a character-state, and "stepmatrices" are created in which the cost of transformation between any pair of states is the Manhattan distance between the allele-frequency arrays represented by the two states. The generalized parsimony (Sankoff and Rousseau 1975; Swofford and Maddison 1992) algorithms available in PAUP can then be used for tree searches, overcoming the limitations of FREQPARS.

An unconstrained search using MANOB found the same most-parsimonious tree and tree-length as the exact FREQPARS analysis, demonstrating that in this case MANOB's approximation to MANAD is perfect. By performing a constrained search in PAUP, we found that the shortest tree incompatible with potoo monophyly required 223.668 steps for the full set of loci. Thus, the cost of rejecting potoo monophyly is 223.668–219.668 = 4.0 steps, equivalent to two complete allelic substitutions. The comparable analysis using the conserved-locus set yields a cost of rejecting potoo monophyly of 100.668–97.020 = 3.648 steps. In view of these results and the uniformly greater genetic distances between potoos and non-potoos than within potoos, we believe that potoo monophyly is reasonably well-supported by the allozyme data.

Support for potoo monophyly, as well as for relationships within potoos, was also evaluated with the bootstrap procedure (Felsenstein 1985), using the PAUP* MANOB approximation. The bootstrap proportions shown in Figure 1C reflect the extent to which these groupings might be supported by an independent sample of loci, although in many cases they provide conservative estimates of the probability that each group represents a true phylogenetic clade (Hillis and Bull 1993). The bootstrap results indicate reasonably strong support for potoo monophyly, with somewhat weaker support for the (*maculosus*, *leucopterus*) and (*griseus*, (*maculosus*, *leucopterus*)) clades.

Caprimulgiform relationships.—*Steatornis* was used to root the FREQPARS analysis of all taxa. Although *Steatornis* is not an unequivocal candidate for the basal caprimulgiform, the high genetic distances between it and all other taxa (Table 4) make it the best *ad hoc* outgroup. In addition, a weighted parsimony analysis of cyt *b* sequence data, including *Gallus gallus* as an outgroup, placed *Steatornis* as the basal caprimulgiform (Mariaux and Braun 1996). In the FREQPARS analysis of all loci (Fig. 1A), the most parsimonious tree joins *Chordeiles* and *Aegotheles* as sister taxa based on the shared allele AK^a. This is inconsistent with the UPGMA and minimum evolution analyses (see below), in which *Chordeiles* and *Eurostopodus* are sister taxa. There are no unambiguous synapomorphies that support *Chordeiles* and *Eurostopodus* as sister taxa, and constraining *Chordeiles* and *Eurostopodus* to be monophyletic requires an additional 2.67 steps relative to the most parsimonious FREQPARS topology (222.668 steps). With respect to the non-potoo taxa, the analysis of the conserved-loci-only data set is consistent with the all-loci data

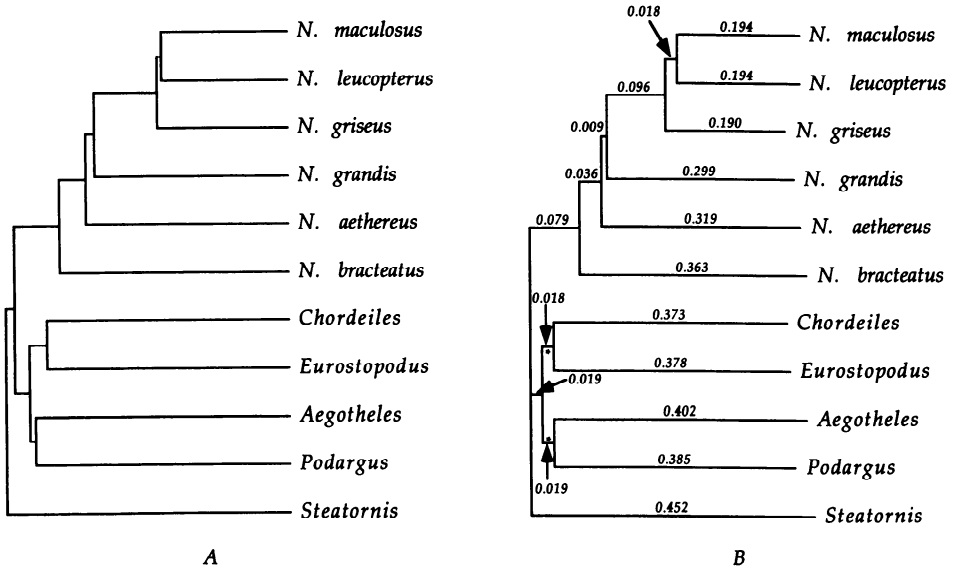

FIG. 2. Distance analyses of caprimulgiform relationships based on chord distances of Cavalli-Sforza and Edwards (1967) derived from allele frequencies at 23 isozyme loci. A) UPGMA phenogram. B) Optimal minimum evolution tree (Kidd and Sgaramella-Zonta 1971, Rzhetsky and Nei 1992) found by branch-and-bound search under the constraint of potoo monophyly.

set. However, the grouping of *Podargus* as a sister taxon to the *Chordeiles-Aegotheles-Eurostopodus* clade is not unequivocally supported, and is therefore absent from the consensus of the nine equally parsimonious trees (Fig. 1B). The *Chordeiles-Aegotheles* clade is the only one for which bootstrap support exceeds 50% (Fig. 1C).

Distance analyses.—The data of Table 3 were also analyzed using several distance methods for comparison. The results of a UPGMA cluster analysis are shown in Figure 2A. Figure 2B shows the optimal tree under the "minimum evolution" criterion of Rzhetsky and Nei (1992) (originally described as "LS-length" by Kidd and Sgaramella-Zonta 1971). This tree was found by a branch-and-bound search under the constraint of potoo monophyly and was also found in every one of 100 unconstrained heuristic searches using the random-addition-sequence option of PAUP*. The score for the minimum-evolution tree is slightly better (3.837 vs. 3.842) than the tree found by the neighbor-joining method (Saitou and Nei 1987) (not shown), and one additional tree had a score better than the neighbor-joining tree. All of these contain a *Chordeiles-Eurostopodus* clade absent from the FREQPARS trees. Except for this discrepancy (addressed above), all trees from the distance analyses are generally consistent with the tree from the conserved-loci FREQPARS analysis, differing only in rearrangements around the short branches indicated by asterisks in Figure 2B. In particular, *bracteatus* is consistently the sister taxon to the remaining potoos, and the (*griseus*, (*maculosus*, *leucopterus*)) clade is consistently present.

DISCUSSION

Intraspecific geographic variation.—Although our sample sizes are small, the genetic distances found between populations of *griseus* and *grandis* from opposite sides of the Andes are clearly large, indicating that significant geographic variation does exist in these taxa, and supporting the notion that the Andes have played an important role in the diversification of Neotropical birds restricted to the humid lowlands (Chapman 1917; Haffer 1967; Brumfield and Capparella 1996). It is unclear from the literature whether the populations of *griseus* we sampled from opposite sides of the Andes are currently united within the same subspecies (Chapman 1926; Peters 1940; Meyer de Schauensee 1964). All populations of *grandis* that we sampled are recognized as the same subspecies (Land and Schultz 1963). The isozyme data suggest that a careful examination of morphological characters and vocalizations may reveal significant differences between *cis-* and *trans*-Andean populations of both *griseus* and *grandis*.

Relationships among potoos.—The large mouth and eyes, gray-brown vermiculated plumage,

and distinctive body form give most potoos a common general appearance that likely explains why they have traditionally been classified in a single genus. *Nyctibius bracteatus*, however, is strikingly different, with unvermiculated rufous plumage and pronounced white spots on the wing coverts and flanks that make it appear like *Steatornis*. However, the isozyme data demonstrate that not only are the potoos monophyletic, but they have probably not recently shared a common ancestor with another caprimulgiform.

The fossil record provides little help in resolving relationships and divergence dates within Nyctibiidae, although a fossil of *griseus* from the Pleistocene (20,000 years ago) of coastal Brazil confirms that diversification events within *Nyctibius* are at least as old as the late-Pleistocene (Brodkorb 1971). In addition, the high degree of overlap in current potoo distributions obfuscates any insight into evolutionary relationships based on biogeography.

The isozyme data strongly support the monophyly of a terminal clade composed of *maculosus*, *leucopterus*, and *griseus*, based on synapomorphic alleles at MPI, IDH-2, LDH-1, and PK. Genetic distances among these three species are remarkably similar, indicating a relatively rapid radiation among them. Interestingly, cyt *b* sequence divergences among these three taxa were also similar, although the taxonomic relationships of *griseus* remained unresolved by parsimony analyses of those data (Mariaux and Braun 1996). The similarity in genetic distances among these three species highlights the controversy that has surrounded their relationships (Chapman 1926; Peters 1940; Schulenberg et al. 1984; Cohn-Haft 1993; Mariaux and Braun 1996).

All analyses (FREQPARS, UPGMA, minimum evolution, neighbor-joining) performed on the isozyme data indicate that *maculosus* and *leucopterus* are sister taxa (Figs. 1–2). However, close examination of the data reveals that the monophyly of *maculosus* and *leucopterus* rests largely on a single synapomorphy at the CK-2 locus, assuming monophyly of the *maculosus-leucopterus-griseus* clade. Polymorphisms present in *griseus* and not in *maculosus* and *leucopterus* (IDH-1, MPI, and VL) also contribute to the clustering of *maculosus* and *leucopterus* in the analyses, yet the small available sample size renders allele-frequency estimates imprecise. Increased sample sizes of both individuals and loci should help in verifying relationships within this clade. Although the possibility of rate deceleration in *griseus* further complicates interpretation of the genetic distance data, such a deceleration may actually be responsible for the difficulty in placing *griseus*, because it would tend to make *griseus* appear more closely related to *maculosus* and *leucopterus* than it really is. On the whole, the isozymes provide weak support for the sister taxon relationship of *maculosus* and *leucopterus*. This same grouping is strongly supported by cyt *b* sequence data, however (Mariaux and Braun 1996). Taken together, we believe the genetic data provide a reasonably firm resolution of this trichotomy in favor of a *maculosus-leucopterus* clade, as originally proposed by Chapman (1926).

Of the 23 loci in which two or more potoo species shared an allele, *bracteatus* possessed a unique allele at 12 of those loci. This level of divergence suggests that either *bracteatus* represents the basal branch of the potoo lineage or it has undergone an accelerated rate of evolution relative to the other potoos (e.g., Fig. 1A). The presence of a synapomorphic allele for all other potoos at PGM-1 (see also ADA and MDH-2) favors the former interpretation. Although the FREQPARS analysis of all loci was unable to clearly resolve whether *bracteatus* or *aethereus* represents the basal potoo taxon, the FREQPARS analysis of conservative loci and all distance analyses placed *bracteatus* in that position. We conclude that the isozymes provide some evidence for the placement of *bracteatus* as the basal potoo, but consider this conclusion tentative based on these data alone. Unfortunately, the cyt *b* data did not elucidate the placement of *bracteatus* (Mariaux and Braun 1996).

The relationships of *aethereus* and *grandis* also remain problematic. It seems unlikely, however, that *grandis* is the basal potoo. The FREQPARS analysis of all loci suggested that *aethereus* may represent the basal lineage, but this relationship was unsupported in the analysis of conservative loci. Of the 23 loci in which two or more potoo species share an allele, *aethereus* and *grandis* possess unique alleles at seven and five of those loci, respectively. The high number of unique alleles in *bracteatus*, *aethereus*, and *grandis* suggest an ancient divergence among these potoos. Unfortunately, the high number of autapomorphies obfuscates attempts to resolve their relationships. Relationships of these potoos will most easily be resolved through examination of more conservative genetic markers.

Relationships among caprimulgiforms.—The fossil record indicates an early divergence date among caprimulgiforms. Fossils of Nyctibiidae (*Euronyctibius kurochkini*), Aegothelidae, and Podargidae all appear in the fossil record of France by the upper Eocene (Mourer-Chauviré 1982, 1987). These fossils demonstrate that not only was the diversification of caprimulgiforms ancient, but also that current distributions are relicts of once more extensive ranges. The uniformly high

isozyme genetic distances among caprimulgiform families are consistent with their apparent age. Sibley and Ahlquist (1990:412, 840) also found high genetic divergences among caprimulgiform families using DNA-DNA hybridization data.

The unvermiculated rufous plumage and aberrant behavior of *Steatornis*, a frugivorous echo-locating troglodyte, make it the most distinctive caprimulgiform. Phylogenetic analysis of all caprimulgiform families based on cyt *b* sequences (including *Gallus gallus* as an outgroup) placed *Steatornis* as the basal caprimulgiform taxon (Mariaux and Braun 1996). The occurrence of a fossil oilbird in the early Eocene (ca. 50 Mya) of Wyoming (Olson 1987) confirms that Steatornithidae has had a long and complex history. Sibley and Ahlquist (1990:412, 840) presented an alternative phylogenetic view based on UPGMA analysis, which placed Nyctibiidae and Steatornithidae as sister taxa in a terminal clade. However, another analysis presented by these authors (1990:819) linked *Steatornis* with *Podargus* in a terminal taxon, and a reanalysis of these data (Harshman 1994) produced a "star" phylogeny of caprimulgiform families, indicating a lack of resolution in the data.

The large isozyme genetic distances to *Steatornis* (Table 4) make it a likely candidate for the earliest branching lineage, as indicated by the UPGMA phenogram and minimum evolution tree (Fig. 2). However, because we did not include any non-caprimulgiform outgroup taxa, we could not determine cladistically whether *Steatornis* is the basal caprimulgiform. In addition, there are no unambiguous isozyme synapomorphies that unite all other caprimulgiform families. In sum, we believe that the available evidence indicates that *Steatornis* is the earliest branching lineage of the caprimulgiforms, although this position cannot be considered incontrovertible.

Our analyses of the isozyme data result in various groupings of the other caprimulgiform families (Figs. 1–2). The high genetic distances involved probably make resolution of their relationships difficult. One interesting linkage that does arise in the FREQPARS analyses is that of *Chordeiles* and *Aegotheles* with *Eurostopodus*. This putative clade is supported by an isozyme synapomorphy at PGM-2. It also received some support from parsimony analysis of the cyt *b* data (Mariaux and Braun 1996), although it did not appear in the phylogenies of Sibley and Ahlquist (1990) or Cracraft (1981). Further data are required to confirm or refute this hypothesis of relationship.

Potoo taxonomy.—The levels of isozyme genetic divergence found among the currently recognized potoo species may be the highest ever found within a single genus of birds. Randi et al. (1991) examined levels of allozyme divergence among genera within Strigiformes (Strigidae and Tytonidae), an order hypothesized to represent the sister taxon to Caprimulgiformes (Sibley et al. 1988; Bleiweiss et al. 1994). Although they did not analyze intrageneric differentiation, they found levels of genetic differentiation among genera within Strigidae (average Nei's D = 0.88) comparable to those found among species within Nyctibiidae (average Nei's D = 0.65). An isozyme analysis of genetic differentiation among the procellariiform families (Barrowclough et al. 1981) found an average interfamilial genetic distance (average Nei's D = 0.68; range = 0.336 to 1.214) less than that found among potoo species.

The amount of genetic variation that exists among potoo species is not adequately conveyed in the current nomenclature, in which all potoos share a single genus. Simply elevating all of the current species to monotypic genera, however, will not greatly improve the utility of the taxonomy, and would, in fact, remove any phylogenetic information. If additional support is found for the *maculosus-leucopterus-griseus* clade, an alternative treatment would be to place these three taxa in one genus, and elevate *aethereus*, *grandis*, and *bracteatus* as monotypic genera. This classification would retain phylogenetic information while also recognizing the high genetic differentiation among the taxa. Another possible arrangement would be to treat *bracteatus* as a monotypic genus, leaving the other species in *Nyctibius*. Again, this treatment can only be recommended if additional support for *bracteatus* as the basal taxon can be mustered. We prefer the conservative approach of retaining the traditional taxonomy until additional data allow more certain resolution of potoo phylogeny.

The paucity of specimens of most potoos in museum collections has prevented detailed analyses of geographic variation and speciation. A conspicuous case in point is that of *leucopterus* and *maculosus*, two dramatically differentiated species that were conflated well into the 1980's due to lack of comparative material (Schulenberg et al. 1984). Conservative morphology, sometimes coupled with intrapopulational plumage variability, has made discerning species and subspecies limits difficult even in well-collected taxa like *griseus* and *grandis*. For example, although the Northern Potoo, *jamaicensis*, has often been treated as a subspecies of *griseus*, it is still uncertain whether the two are even close relatives (Davis 1978). Further analyses of genetics, vocalizations, and morphology will clarify variation within several of the currently recognized

species. Such studies may identify other geographically delineated taxa differentiated at or near the species level. Populations of both *griseus* and *grandis* on opposite sides of the Andes seem to be prime candidates, as they appear to be well-differentiated genetically, based on the limited samples available. In particular, the amount of genetic divergence between populations of *grandis* from opposite sides of the Andes is comparable to that found among *maculosus*, *leucopterus*, and *griseus*.

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This paper really began on a mountainside in northern Peru on June 30, 1980, when M.J.B. and Theodore A. Parker III recorded the first *Nyctibius maculosus* for the country. Parker and Braun immediately realized that the bird must be specifically distinct from *N. leucopterus*, contrary to the literature of the time. For sharing the thrill of discovery on that day, and much knowledge, insight, and inspiration before and since, M.J.B. and R.T.B. acknowledge a deep debt to Ted Parker. We thank the many skilled field workers named in Table 1 for collecting samples, and Les Christidis, Van Remsen, Diana Reynolds, Mark Robbins, Fred Sheldon, and Bob Zink for providing them to us. Mario Cohn-Haft, Van Remsen, and Tom Schulenberg provided helpful reviews of the manuscript. Fernanda Zermoglio helped with the Spanish translation of the Abstract.

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APPENDIX

GENOTYPE FOR EACH INDIVIDUAL AT ALL LOCI. REFER TO TABLE 1 FOR TAXON ACRONYMS. ALLELE DESIGNATIONS IN UPPER CASE ARE REFERABLE ONLY TO VARIATION WITHIN A SINGLE SPECIES.

Individual	ACON	ADA	AK	CK-1	CK-2	EST	FUM	GDA	GOT	αGPD	GPI	GPT	GSR	IDH-1	IDH-2
1825 (NMAC)	S	d	c	b	f	—	h	d	c	d	e	c	d	c	c
271 (NMAC)	F	d	c	b	f	—	h	d	c	d	e	c	d	c	c
20315 (NLEU)	—	d	c	d	f	—	h	e	c	df	e	h	b	c	c
20319 (NLEU)	—	de	c	d	f	—	h	e	c	df	e	h	b	c	c
20267 (NLEU)	—	d	c	d	f	—	h	e	c	d	e	h	b	c	c
3238 (NGRI)	F	d	c	b	e	—	h	b	c	d	e	cg	a	ac	c
3252 (NGRI)	S	d	c	b	e	—	h	b	c	d	e	g	a	c	c
10877 (NAET)	—	d	c	—	e	—	i	g	c	f	e	d	a	c	f
11236 (NAET)	—	d	c	g	e	—	i	g	c	f	e	d	a	c	f
15415 (NGRA)	S	d	c	i	e	S	a	a	c	d	e	i	a	ac	f
8954 (NGRA)	S	d	c	i	e	S	a	a	c	d	e	i	a	ac	f
3223 (NGRA)	F	d	c	i	e	F	b	a	c	d	e	i	a	a	f
20270 (NBRA)	F	f	c	b	d	S	h	h	c	cd	e	b	g	c	f
20318 (NBRA)	F	f	c	b	d	F	h	h	c	cd	e	b	g	bc	f
4509 (NBRA)	S	f	c	b	d	F	h	h	c	d	e	b	g	c	f
5279 (CHOR)	—	df	b	e	c	—	f	f	b	h	b	f	e	d	d
129 (EURO)	—	b	c	a	c	—	e	i	b	a	c	a	f	f	be
450 (AEGO)	—	c	b	f	b	—	d	k	b	b	ac	e	h	eh	ad
7474 (STEA)	—	b	a	h	a	—	c	j	a	g	d	—	i	g	df
8654 (PODA)	—	a	c	c	c	—	g	c	b	e	d	—	c	d	a

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APPENDIX

EXTENDED

LA	LDH-1	LDH-2	LGG	MDH-1	MDH-2	ME-1	ME-2	MPI	NP	6PGD	PGM-1	PGM-2	PHE-PRO-1	PHE-PRO-2	PK	SOD	SORDH	VL	UDH
—	b	—	—	b	b	c	—	f	ad	c	c	—	—	f	c	e	—	g	c
—	b	—	—	b	b	c	—	f	eh	c	c	—	—	f	c	e	—	g	c
—	b	—	—	b	b	c	—	f	g	e	c	—	—	f	c	e	—	g	c
—	b	—	—	b	b	c	—	f	g	e	c	—	—	f	c	e	—	g	c
—	b	—	—	b	b	c	—	f	g	eh	c	—	—	f	c	e	—	g	c
FS	b	b	F	b	b	c	F	f	hi	e	c	F	F	f	c	e	F	g	c
M	b	b	F	b	b	c	S	df	h	e	c	F	F	f	c	e	F	eg	c
—	c	—	—	b	b	c	—	d	f	ae	c	—	—	f	f	b	—	f	c
—	c	—	—	b	b	c	—	cd	f	e	c	—	—	f	f	b	—	f	c
S	d	a	F	b	b	—	S	c	f	e	c	F	S	b	e	e	F	g	c
F	d	a	F	b	bc	a	F	c	f	e	c	F	S	b	e	e	F	g	c
S	d	a	F	b	b	a	F	e	fh	e	c	F	F	b	e	e	F	g	c
BD	e	b	F	b	c	ab	F	a	c	g	a	F	F	e	d	f	F	g	c
DE	e	b	F	b	c	b	F	a	bc	g	a	F	F	e	d	f	F	g	c
AC	e	b	F	b	c	b	F	a	c	g	a	F	F	e	d	f	F	g	c
—	e	—	—	d	b	—	—	h	—	f	b	—	—	a	c	c	—	b	c
—	g	—	—	c	b	—	—	h	—	b	b	—	—	a	a	d	—	f	c
—	a	—	—	a	b	—	—	b	—	f	b	—	—	d	e	ad	—	a	b
—	b	—	—	a	a	—	—	j	—	d	a	—	—	c	b	g	—	d	a
—	h	—	—	a	b	—	—	gi	—	i	d	—	—	f	e	a	—	c	d