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MORPHOLOGY, GENETICS AND THE VALUE OF VOUCHER SPECIMENS: AN EXAMPLE WITH *CATHARTES* VULTURES

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ABSTRACT.—Similarity of two of the cathartid vultures, the Greater and Lesser Yellow-headed vultures (*Cathartes melambrotus* and *C. burrovianus*) has caused field-identification problems. The primary means of distinguishing those vultures are the different flight profiles and general habitat preferences. As part of a larger study of cathartid phylogeny, we sequenced cytochrome *b* for six specimens of the two species. Sequences segregate into two groups, with two of the four Lesser Yellow-headed Vulture specimens clustering with the Greater Yellow-headed specimens. This incongruence led us to reexamine the two apparently misidentified specimens. The first bird, a specimen from the Sedgwick County Zoo, Kansas, had been acquired in 1960 and identified as a yellow-headed vulture. The name on the label of this specimen was not changed after *melambrotus* was established as a separate species in 1964. The second specimen, from Amapá, Brazil, had been identified based on observations of habitat and flight behavior. Because this voucher specimen was available for study, we were able to reexamine the specimen and corroborate the molecular identification as a Greater Yellow-headed Vulture. Without these voucher specimens, we would have misinterpreted the results from the molecular data. This is a reaffirmation of the importance of voucher specimens for accurate scientific work.

KEY WORDS: *yellow-headed vultures*; Cathartes burrovianus; Cathartes melambrotus; *voucher specimens*; *cytochrome b*; *genetics*.

Morfología, genética y el valor de los especimenes de gaveta: Un ejemplo con los buitres del genero *Cathartes*

RESUMEN.—La similaridad entre dos buitres del genero *Cathartes: Cathartes melambrotus* y *C. burrovianus* ha causado problemas de identificación en campo. La diferencia principal para distinguir estas especies son el perfil de vuelo y sus preferencias de hábitat. Como parte de un estudio de filogenia de los *Cathartidae*, hicimos una secuencia del citograma b para seis especimenes de dos especies. Las secuencias fueron separadas en dos grupos, con dos de los cuatro especimenes de *Cathartes burrovianus* agrupados con los especimenes de *Cathartes melambrotus*. Está incongruencia permitió re-examinar a dos especimenes incorrectamente identificados. El primero, un espécimen del zoológico del Condado de Sedgwick, Kansas, fue adquirido en 1960 y fue identificado como *Cathartes burrovianus*. El nombre en el rotulo de este espécimen no fue cambiado después de que *melambrotus* fue establecido como especie aparte en 1964. El segundo espécimen, procedente de Amapá, Brasil, había sido identificado con base en observaciones de hábitat y comportamiento de vuelo. Debido a que este espécimen estaba disponible para estudio, pudimos re-examinar el ave y corroborar la identificación molecular como *Catarthes melambrotus*. Sin estos especimenes de gaveta, hubiéramos mal interpretado los resultados de los datos moleculares. Esta es un reafirmación de la importancia de los especimenes de gaveta para el trabajo científico.

[Traducción de César Márquez]

Morphologically similar avian species can be difficult to distinguish in the field. Typically, species identical in appearance are identified by where they are located (habitat), by their song or calls (e.g., *Empidonax* flycatchers), and possibly other characters such as behavior (Zimmer et al. 2001). Here, we present genetic data and provide an example of how voucher specimens were vital in the interpretation of results and in the identification

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Table 1. Wetmore's (1964) measurements (mm) of Yellow-headed Vultures.

	LESSER YELLOW-HEADED VULTURE				GREATER YELLOW-HEADED VULTURE	
	C. B. BURROVIANUS		*C. B. URUBITINGA*		*C. MELAMBROTUS*	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Tail length	195–225	193–230	205–238	204–236	252–275	272–285
Central rectrix width	42–49	43–49	43–50	43–51	59–70	60–67

of species of New World Vultures (*Cathartes*). This genus currently comprises three species, the Turkey Vulture (*Cathartes aura*) and the Greater and Lesser Yellow-headed vultures (*C. melambrotus* and *C. burrovianus,* respectively).

The two yellow-headed vultures are so similar that they were only recognized as separate species when Wetmore (1964) revised the genus. Wetmore designated the Greater Yellow-headed Vulture as a separate species based on differences in overall plumage color and in size. The distinctive measurements are length of the tail and the width of the central rectrices (Wetmore 1964; Table 1). The Lesser Yellow-headed Vulture was subdivided into two subspecies: *C. burrovianus burrovianus,* the smaller, northern form occurring from Mexico to Venezuela and the slightly larger *C. burrovianus urubitinga,* which occurs from Venezuela and Colombia south to northern Argentina and Uruguay.

The similarity of the Greater and Lesser Yellow-headed vultures has caused problems in identifying birds in the field (Blake 1977, de Schauensee and Phelps 1978); the slight differences in size and head color are not useful in most field circumstances. The primary means of distinguishing these two species are different flight profiles and general habitat preferences. Hilty and Brown (1986) also emphasize differences in general coloration (the Greater Yellow-headed Vulture is darker overall with less lighter coloration on the flight feathers) and in the color of primary quills (white in the lesser yellow-headed, dark in the greater yellow-headed) that can be seen from above in a flying bird. Because these plumage features are difficult to use in many field situations, the two species are likely to be most frequently identified by location and method of hunting. The Lesser Yellow-headed Vulture is considered to be a bird of marshes, flat grass, and open wetland habitats (de Schauensee 1970, Houston 1994, Hilty and Brown 1986), rarely found in forests (del Hoyo et al. 1994). In contrast, the Greater Yellow-headed Vulture occurs in mature lowland forests and along forest edges (de Schauensee and Phelps 1978, Houston 1994, Sick 1993), rarely wandering over grassland (Hilty and Brown 1986). It hunts by flying over the forest canopy, using a sense of smell to locate carrion (del Hoyo et al. 1994, Houston 1994). Possibly because of its habitat preference, it is considered to soar at greater heights, with a steadier flight pattern, and with wings flatter than the lesser yellow-headed (Hilty and Brown 1986, Sick 1993). The potential for some overlap to occur at forest edges with flooded grassland only increases the difficulty of evaluating some sight records (see Ridgely and Greenfield 2000).

As part of a larger study of cathartid phylogeny, tissue specimens of Greater and Lesser Yellow-headed vultures were obtained from museums and from a zoo (Table 2), and a mitochondrial gene, cytochrome *b,* was amplified and sequenced for six individuals.

In this paper, we report on sequence divergence between the two species. In addition, we discuss the misidentification of two specimens, the usefulness of various methods of identifying these species, and, reiterate the importance of voucher specimens for accurate faunistic and taxonomic studies.

METHODS

Taxon Sampling. Four specimens originally identified as *C. burrovianus,* and two specimens identified as *C. melambrotus* were analyzed (Table 2).

DNA Extraction, Amplification, and Sequencing. DNA was extracted from frozen tissue samples using DNAzol (Molecular Research), according to manufacturers' instructions and then subjected to PCR reactions. Primers (Table 3) were used to amplify and sequence overlapping regions of both strands of the mitochondrial cytochrome *b* gene.

PCR reactions were run in a PTC-200 Peltier Thermal Cycler machine. Double-stranded DNA was generated in 30 μl solutions run at 40 cycles: 20 sec at 94°C, 15 sec at 55°C, and 1 min at 72°C. The double-stranded DNA template was purified using Geneclean II (Bio 101 Inc., Vista,

Table 2. Voucher numbers and locations of the birds used in this study.

SPECIES	VOUCHER NO.	LOCATION
Cathartes melambrotus	LSUMNS[a] B9005	Pando Department, Bolivia
	LSUMNS B7175	Loreto Department, Peru
Cathartes burrovianus	KUNHM[b] 89344	Yucatan, Mexico
	KUNHM 1872	Yucatan, Mexico
	MPEG[c] CH-268	Amapá, Brazil
	SCZ[d] 4550	Sedgwick County Zoo

[a] Louisiana State University Museum of Natural Sciences.
[b] University of Kansas Natural History Museum.
[c] Museu Paraense Emilio Goeldi.
[d] Sedgwick County Zoo.

CA) and resuspended with 18 μl of ultra pure water. Two μl were used as template for cycle sequencing using a Prism™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit. Protocol for the 6 μl reactions was: preheating for 1 min at 95°C, then 35 cycles at 95°C for 15 sec, 50°C for 15 sec, and 60°C for 4 min. The product was precipitated using 74 μl of an ethanol/$MgCl_2$ mixture, cleaned with 95% ethanol and resuspended in 2.2 μl of a 6 to 1 solution of formamide-EDTA. Two μl of the sequenced product were loaded into a 6% acrylamide gel and analyzed in an ABI Model 377 DNA sequencer.

RESULTS

The cytochrome *b* sequences of these species have been deposited in Genbank (Accession numbers AF494339–AF494342). Sequences of the six specimens segregate into two groups (Table 4), with two of the four Lesser Yellow-headed Vultures (SCZ 4550 and MPEG CH-268) clustering with the Greater Yellow-headed specimens. Within each of these two groups, sequences diverge by 0.09% (one character), between the two groups divergence is 2.7% (31 characters). This level of divergence is similar to the number of sites that differ in comparisons of each of these species to the Turkey Vulture (*C. aura*).

Table 3. The sequences of primers used to amplify overlapping regions of both strands of cytochrome *b*.

NAME	LOCATION[a]	SEQUENCE (5′ to 3′)
L14851[b]	14851	CCTACTTAGGATCATTCGCCCT
H15149[c]	15298	GCCCCTCAGAATGATATTTGTCCTCA
L15162[c]	15311	CTACCATGAGGACAAATATC
H15780	15780	TAGGAATAGGATTAGTACGGAGGCAG
L15636	15636	CTAACAACCCTAGCCCTATTCTCACC
H16057	16057	CTCTGGTAACAAGACCAATG

[a] Based on chicken sequence (Desjardins and Morais 1990).
[b] Groth (1998).
[c] Helm-Bychoswki and Cracraft (1993).

The incongruence of these results led us to reexamine the two apparently misidentified Lesser Yellow-headed Vulture specimens. A tail measurement (MPEG CH-268, length 267 mm) or a measurement of the central retrix (SCZ 4550, width 62 mm) was taken. These measurements clearly fall within the range of the Greater Yellow-headed Vulture (Table 1).

DISCUSSION

The reason for the apparent misidentification of the Sedgwick County Zoo specimen (SCZ 4550) is straightforward. It had been acquired from the Cincinnati Zoo, which bought the bird in 1960, four years before Wetmore's (1964) taxonomic revision. It was identified correctly at the time as a yellow-headed vulture (*C. burrovianus*) and that identification was not changed or updated when the species within that genus were revised.

The second specimen (MPEG CH-268) illustrates the potential pitfalls associated with such similar species. In his study of the birds of the state of Amapá, Novaes (1974) cited one 1902 specimen of Lesser Yellow-headed Vulture from Cunani (02°48′N, 51°06′W, Paynter and Traylor 1991), but could not locate specimens of the Greater Yellow-headed Vulture. Despite the lack of specimens, he felt certain that Greater Yellow-headed Vultures occurred in the state at least in the forested western half.

The Amapá individual (MPEG CH-268) used in this study was collected specifically for Griffiths' on-going molecular studies, because at the time the Lesser Yellow-headed Vulture was not represented in any tissue collections. Large open-coun-

Table 4. Nucleotide sites that differ in sequences of cytochrome *b* of *Cathartes melambrotus* and *C. burrovianus* and the bird from Amapá, Brazil. The Amapá specimen is identical to *C. melambrotus* except at a single site (1085).

	NUCLEOTIDE SITE																
SPECIMEN	72	117	165	195	228	249	273	285	327	396	501	534	594	603	628	648	696
C. melambrotus	T	C	C	G	C	G	C	T	G	T	A	G	T	A	C	C	G
Amapá specimen	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C burrovianus	C	T	T	A	T	A	T	C	A	C	G	A	C	G	T	T	A
	699	747	751	753	801	819	843	858	1038	1050	1062	1085	1107	1113			
C melambrotus	T	C	C	T	C	A	C	C	T	T	A	C	C	A			
Amapá specimen	.	.	.	.	.	.	.	.	.	.	.	T	.	.			
C burrovianus	C	T	T	A	T	G	T	T	C	C	G	T	.*	G			

* *C. burrovianus* KUNHM 89344 has a T in position 1107.

try taxa are not often part of modern collections, and vultures may be among the most under-represented groups in modern avian collections. At the time of collection, this particular individual was perched 4 m above the ground on the edge of a gallery forest next to a seasonally-flooded grassland at Lago Cujubím (1°39′N, 50°55′W). During the previous several days, yellow-headed vultures had been seen soaring low over the adjacent open grasslands. Based on the habitat and the flight behavior and light primary shafts of a number of individuals, all had been identified as Lesser Yellow-headed Vultures. There are some fingers of primary forest in the region, but the nearest continuous forest lies some 10 km to the west. Thus, it was assumed that this bird was a Lesser Yellow-headed Vulture. The genetic data clearly refute this and reexamination of the specimen supports the reidentification as a Greater Yellow-headed Vulture. When the problem in the sequences became apparent, we reexamined the voucher specimens. Morphological measurements confirmed the information from the sequence comparisons, that this was, indeed, a Greater Yellow-headed Vulture. This specimen (MPEG CH-268) now represents the first documented record of a Greater Yellow-headed Vulture for Amapá and clearly suggests that this species ventures some distance into the wet grasslands of this region.

Debating the value of specimen-based research, of collecting, and, implicitly, of natural history collections, is becoming increasingly contentious within the scientific community. Editorials and editorial policy in leading ornithological journals appear to question various aspects of collecting (British Ornithologists' Club 2001, British Ornithologists' Union 1995). Papers are published based on sequence data, with little or no information about the specimens from which the sequences were derived (Ruedas et al. 2000).

That non-specimen based research has the potential to be sloppy science, with no opportunity to reexamine or verify data, has been noted (Ruedas et al. 2000). Comprehensive statements have been published about the importance of voucher specimens for accurate scientific work, and the importance of collections for science, in general (e.g., Winker et al. 1991, Remsen 1995, Winker 1996, Peterson et al. 1998, Ruedas et al. 2000). In this report, we are not attempting to add to this general review. Rather, we are verifying the necessity of voucher specimens for molecular work. Without the ability to reexamine the specimens, we might have misinterpreted the results from the molecular data used in this study.

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