Painting of the unique specimen of *C. estudilloi* prepared by Albert E. Gilbert from a photograph taken by Dr J. Estudillo Lopez and kindly made available by Dr S. Strahl.

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DNA EVIDENCE CONCERNING THE IDENTITIES OF *CRAX VIRIDIROSTRIS* SCLATER, 1875, AND *C. ESTUDILLOI* ALLEN, 1977

Leo Joseph[1], Beth Slikas[2], Karen Rankin-Baransky[1], Boldgiv Bazartseren[1], Deryn Alpers[1] & Albert Earl Gilbert[3]

[1]Department of Ornithology, Academy of Natural Sciences of Philadelphia, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103-1195 USA. *Email*: joseph@acnatsci.org

[2]Department of Zoological Research, National Zoological Park, Smithsonian Institution, Washington, DC 20008-2598 USA.

[3]PO Box 107, Bridgewater, CT 06752 USA.

Abstract. We examined 474 base pairs of mitochondrial DNA (mtDNA) sequences to determine whether the nominal curassow species *Crax viridirostris* Sclater, 1875, and *Crax estudilloi* Allen, 1977, both known from single specimens, should be considered representatives of a species to be known as *C. viridirostris* Sclater, 1875, or whether both are descended from hybridization events involving species of the Great Curassow *C. rubra* complex. The mtDNA sequences of *estudilloi* were identical to those of the Blue-billed Curassow *C. alberti*, and those of *viridirostris* were identical to those of the Yellow-knobbed Curassow *C. daubentoni*. We argue that the simplest interpretation of these findings is that at unknown times in the ancestry of *estudilloi* and *viridirostris*, hybridization events occurred involving maternal parents of *alberti* and *daubentoni*, respectively. This interpretation is easily reconciled with *viridirostris* having been a captive bird in a European zoo but is not so easily reconciled with *estudilloi* having been obtained as a chick in Bolivia. We discuss this difficulty and other possible explanations of the data, all of which we consider to be less preferable than the hybrid origin interpretation. *Accepted 1 March 1999*.

Key words: Cracid taxonomy, hybridization, mitochondrial DNA, Crax estudilloi, Crax viridirostris.

INTRODUCTION

Among the unsolved riddles of Neotropical ornithology are the identities of two curassows (Craciformes: Cracidae: *Crax*, *sensu* Sibley & Ahlquist 1990), each known from single specimens and sharing the otherwise unique characteristic in the genus *Crax* of a greenish cere (Plate, Fig. 1). Here we report on an attempt using molecular methods to clarify the identities of the specimens on which these two names were based. We begin with an

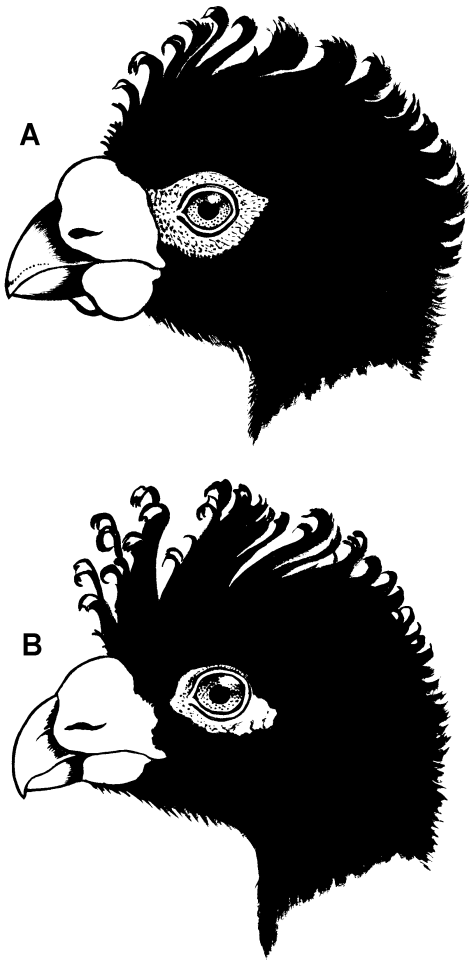

FIG. 1. Comparison of the heads of the unique specimens of *C. viridirostris* (A) and *C. estudilloi* (B) to show differences in bill, cere and bare ocular skin. Note the two independent (i.e., not connected, bulbous wattles on either side of mandible of *viridirostris* lacking in *estudilloi*. Also note different shape of bills. The bicolored bill of both is also notable – see text. Prepared by Albert E. Gilbert based on examination of the holotype of *C. viridirostris* and on a photograph of *C. estudilloi* taken by Dr J. Estudillo Lopez and kindly made available by Dr S. Strahl.

account of the histories of the two names and we cite pertinent literature in some detail because much of it was published in journals that are not readily accessible. For the same reason, we have included several illustrations of the birds involved.

Crax viridirostris Sclater, 1875. In 1875, P.L. Sclater felt "somewhat unwillingly constrained to place on our register a Curassow which I met with in the Gardens of the Zoological Society of Amsterdam, during a recent visit, and which, having been most kindly lent to me by Mr Westerman, is now in the [London Zoological] Society's gardens." After the bird died it was prepared as a study skin at the Natural History Museum, London. Sclater (1875) described this bird as a new species, *Crax viridirostris*. (We have not yet attempted to see whether there is any surviving documentation that might reveal more of the origins of this bird.) Based on its plumage of glossy black with white crissum and white-tipped rectrices, it is a male. Sclater (1875) further noted that *viridirostris* "is perhaps most closely allied to *Crax alberti* [the Blue-billed Curassow which is endemic to northern Colombia and is the only blue-cered *Crax* species], having a large caruncle on the lower mandible as in that species. But this and the swollen cere are of a pale green colour instead of blue." Later, Sclater (1879) wrote that *alberti* "is unquestionably considered its nearest ally, and it is possible that the change of cere from blue into greenish-yellow may be an individual variation."

Taibel (1950) published a 1939 photograph of a captive bird fitting the description of *viridirostris* but known to be a male hybrid between blue-cered *alberti* and yellow-cered Great Curassow *C. rubra*. He concluded that *viridirostris* must have been based on a hybrid, a conclusion easily reconciled with the original specimen of *viridirostris* having been a captive bird in a European zoo. Vaurie (1967, 1968) and Delacour & Amadon (1973) placed *viridirostris* in the synonymy of *alberti*.

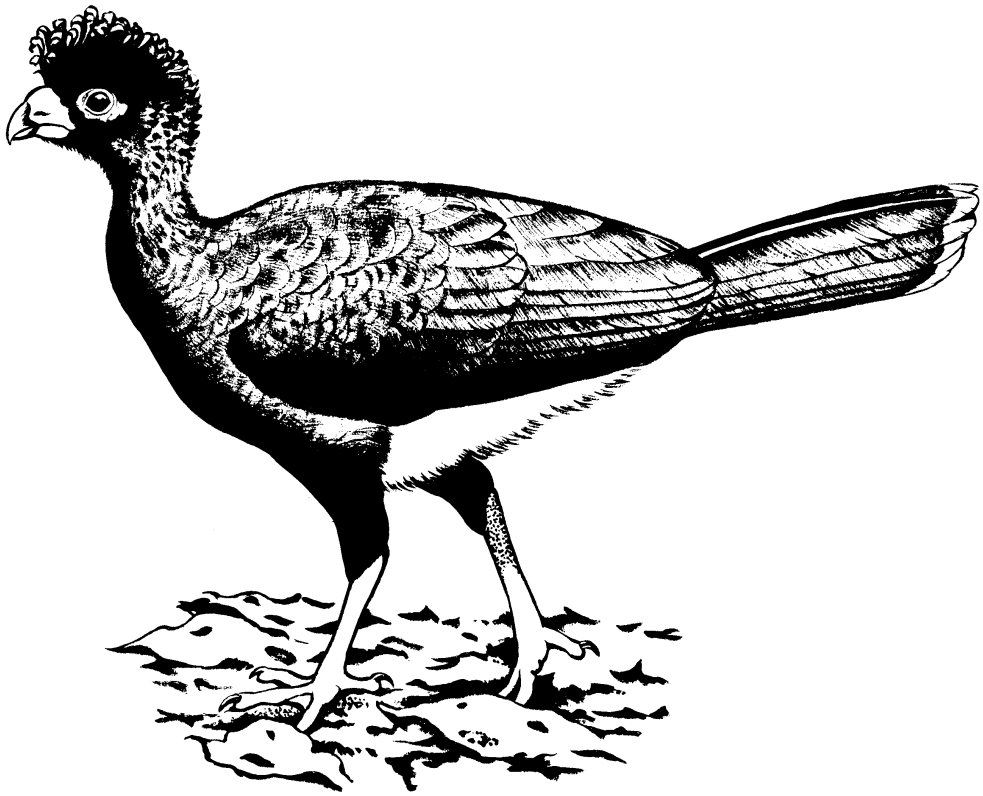

FIG. 2. Sketch of the unique individual of *C. estudilloi*, prepared by Albert E. Gilbert, based on a photograph of the live bird taken by Dr J. Estudillo Lopez and kindly made available by Dr S. Strahl. Note the white on the rectrices and plumage similar to *viridirostris* with main differences being on the head and bill (see Fig. 1).

Lastly in connection with introducing *viridirostris*, Dr Daniel Brooks forwarded the following remarks (pers. comm.) made after he examined the unique specimen of *viridirostris*: "The entire top of the head comprised short curls extending far back, as I've noted for *C. alector* compared to other *Crax*. Also the head (and neck) appear slightly larger, and the head is shaped more like *alector* than other species of *Crax*. The measurements (cm) are: wing chord 39, tail 41, tarsus 11. There is a bare eye patch (not characteristic of *alberti* or *blumenbachii*) and as with other male *Crax*, the body is completely black with a white abdomen . . . the bill is bicoloured - mostly blackish with a horn coloured tip . . . The casque ornamentation has a very small bulbous structure on top, and two small independent (not connected in centre) bulbous structures below. Overall, the casque ornamentation is quite shrivelled, reduced and a dark blackish colour, with only a hint of dark green, mostly on one side. This is probably to do with aging of the specimen (prepared in 1875) or less likely, the specimen was not yet sexually mature (i.e., 3 years)."

In further correspondence, Dr Brooks speculated that *viridirostris* may have been derived through hybridization between *alector* and *globulosa*.

Crax estudilloi Allen, 1977. The name *Crax estudilloi* first appeared in an editorial note preceding an article by Dr Jesus Estudillo Lopez about a putative new species of curassow (Lopez 1977; see Plate and Fig. 2). The name was apparently offered as a suggestion for formal dedication. The spirit in which the suggestion was made was one of recognizing Dr Estudillo's work on and knowledge of the Cracidae. Unfortunately and unintentionally, however, nomenclatural priority was established. As Vuilleumier & Mayr (1987) later remarked: "the name is valid and is available in the literature, although the format of the description fails to follow the rules of the International Code of Zoological Nomenclature and the journal in which the description was published is very obscure. Such practice must be discouraged very emphatically."

Dr Estudillo described his discovery of *estudilloi* thus (E. Lopez 1977): "I found the bird three years ago in Bolivia on the eastern slopes of the Andes near the village of Ixima [*sic* = Ixiamas, 222 metres asl, see Paynter 1992] in an indian hut at the shore of the Tequije River that flows into the Rio Benii [*sic* = Beni]. Unfortunately the indian who had the bird didn't speak Spanish so much to my regret I was not able to get any information about where he found the bird. When I got the Curassow it was a young chick, probably not older than 5–6 weeks."

The bird, whose provenance from the above remarks cannot be certainly attributed, was then raised to adulthood in aviaries in Mexico. One of us, A. E. Gilbert, examined colour photographs of the live bird and prepared a painting from them. He noted that as an adult, *estudilloi* closely resembled *viridirostris*, but there were several notable differences: *estudilloi* had a light aquamarine green knob and cere (not yellow-green); it lacked wattles, and by its glossy black plumage with white crissum and white-tipped rectrices, it too was presumably a male. Its bill was bi-coloured, being ivory-buff at its tip and for most of its length but with a narrow, vertical stripe of black bordering the cere. The large periophthalmic ring was dull grey with prominent patches of bright aquamarine. It was said to have a whistling call, but to our knowledge no sound recordings were made. Dr François Vuilleumier of the American Museum of Natural History reported examining the bird in 1983 when it was seven years of age and noted that it looked much like another adult *Crax* in the aviaries. That bird, he was told, was a hybrid, though its putative parental species were unknown (Vuilleumier & Mayr 1987). After the *estudilloi* individual died, its carcass was donated to Louisiana State University's Museum of Natural Science through the efforts of Drs Estudillo Lopez and Stuart D. Strahl (Remsen & Traylor 1989), but it was so decomposed when it was received that it was prepared as a skeleton with some feathers and breast muscle also preserved (Cardiff & Remsen 1994). Remsen & Traylor (1989), Vuilleumier & Mayr (1987) and Vuilleumier *et al.* (1992) further discussed the introduction of *C. estudilloi* Allen, 1977, into the literature and considered it a "*species inquierenda*". We also note, however, that Dr Estudillo invited many ornithologists to view this bird at his aviary and that he made another trip to Bolivia, hoping to obtain more birds, especially a female, but was unsuccessful.

Remsen & Traylor (1989) and del Hoyo (1994) remarked that the abnormal greenish cere colour of *estudilloi* has been or might be attributed to various factors such as inadequate or inappropriate nutrition and hybridization, and noted the need for further study of the bird's identity.

In view of the above, we formulated two hypotheses concerning the identities of *estudilloi* and *viridirostris*: either they are a single species to be known as *C. viridirostris* Sclater, 1875, or they are hybrids between one or more combinations of species from the *C.*

TABLE 1. List of specimens studied and their provenance when known.

Taxon	Sample number	Provenance	Sequenced for	
			cytochrome *b*	t-lys/ATPase 8
C. estudilloi	LSUMZ B29828	Bolivia (see text)	+	+
C. viridirostris	NHM 1889.6.1.302	London Zoo	+	+
C. alberti	6184	Captive bird	+	+
C. alberti	1006	Wild-caught, locality unknown; now captive in Houston Zoological Gardens	+	–
C. daubentoni	6364	Captive bird	–	+
C. daubentoni	6305	Captive bird	+	+
C. rubra	LSUMZ B14200	Captive bird	+	+
C. rubra	LSUMZ B10362	Captive bird	+	+
C. fasciolata	LSUMZ B18929	Captive bird	+	+
C. fasciolata	LSUMZ B16810	Captive bird	+	+
C. globulosa	LSUMZ B10360	Captive bird	+	+
C. globulosa	LSUMZ B5359	Captive bird	+	–
C. alector	ANSP 8225	Iwokrama Reserve, Guyana	+	–

rubra complex to which they clearly belong based on the generally black body plumage and recurved crests. (In the Discussion we consider the further alternative that both *estudilloi* and *viridirostris* could be separate, valid species.) If they are hybrids, the putative Bolivian provenance of *estudilloi* would suggest that the two *Crax* species occurring in that country, yellow-cered *fasciolata* and red-cered *globulosa*, would be the most likely candidate parental species of at least that bird. If anything, however, it seems unlikely that these two species should produce hybrid progeny with a greenish cere. Perhaps a more likely combination is one that involves blue- and yellow-cered species as parents. However, the only blue-cered species of the *C. rubra* complex, *C. alberti*, is endemic to northern Colombia and is not known to occur naturally anywhere near Bolivia. Because the type specimen of *estudilloi* was reportedly obtained from Bolivian indians, natural hybridization involving *alberti* seems unlikely. *C. viridirostris*, on the other hand, was a captive bird in a zoo and could more feasibly have been a hybrid between *alberti* and any of the following yellow-cered taxa, viz., *C. rubra*, the Yellow-knobbed Curassow *C. daubentoni*, or the nominate subspecies of the Black Curassow, *C. a. alector*. The shape of the cere or its red colour would seem to render unlikely the possibility of any of the following as parental taxa: the western subspecies of Black Curassow *C. alector erythrognatha*, the Wattled Curassow *C. globulosa*, and the Red-billed Curassow *C. blumenbachii*.

We have used maternally inherited mitochondrial DNA (mtDNA) techniques to test these hypotheses through fresh examination of the original specimens on which the names

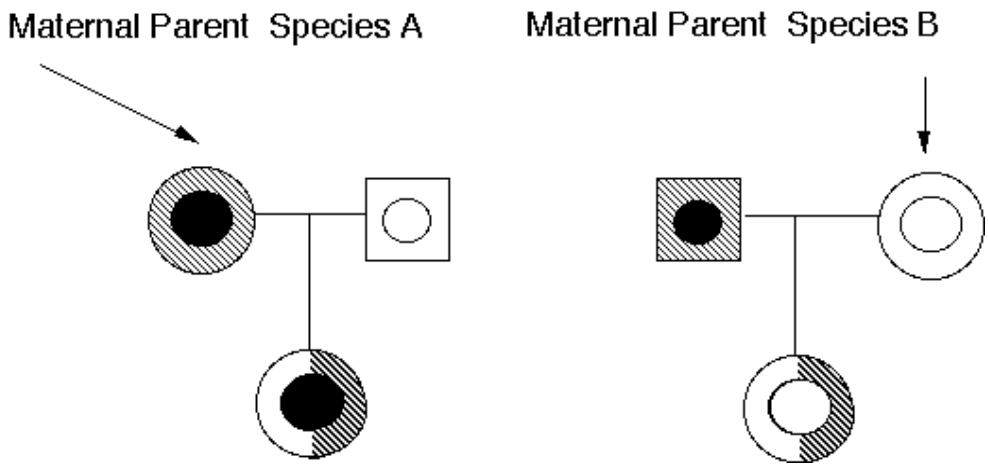

FIG. 3. Diagram showing the rationale of the approach used here to determine the status of *C. estudilloi* and *C. viridirostris*. Males are represented by squares and females by circles. MtDNA of species A is represented by the dark inner circles and that of species B by the clear inner circles. Nuclear DNA is indicated in the outer squares and circles which are hatched for species A and clear for species B. Both offspring in this example are hybrid females between species A and B. They have equal contributions of nDNA from each parent as shown by the combinations of shading and non-shading in the outer circles of the hybrids. In this example they have different mtDNA because their maternal parents were different species and this is shown by the different patterns of the inner circles.

were based. If *estudilloi* and *viridirostris* are conspecific, then we predicted that (1) their mtDNA sequences would be at least as divergent from those of other *Crax* species as the latter are from each other, (2) the level of divergence between *viridirostris* and *estudilloi* would be within the range of intraspecific divergences in *Crax*, and (3) in a phylogenetic analysis, their mtDNA sequences would align with each other and apart from other *Crax* species. By the alternative hybrid origin hypothesis, the mtDNA sequences should closely match at least one other *Crax* species depending on whether the maternal parents were the same or different species. This expectation hinges on the fact that mtDNA of the maternal species involved in a hybridization event will appear unchanged not just in the immediate F1 progeny but in every generation of the matriline descending from the female in the original hybridization until it is changed by mutation (see Fig. 3). Also under a hybrid origin, the mtDNAs of *estudilloi* and *viridirostris* should align in a phylogenetic analysis with their putative maternal parent(s). More specifically, if the maternal parents of *estudilloi* and *viridirostris* were the same species, then the mtDNA sequence divergence between *estudilloi* and *viridirostris* should lie within the range of intraspecific divergences in that putative parental species. If their maternal parents were different species, then the mtDNA divergence between *estudilloi* and *viridirostris* should be within the range of divergence between the two putative maternal parent species. Finally, in a phylogenetic analysis the two individuals should cluster with the species identified as the putative maternal parent(s).

MATERIALS AND METHODS

Samples. Table 1 lists the samples of all members of the *C. rubra* complex that we have been able to study and their provenance. All samples except that from *alector* were from birds raised in captivity. Details follow for the key samples of *estudilloi* and *viridirostris*. For *estudilloi*, a piece of decomposed muscle tissue stored in alcohol and which had itself been subsampled from a parent sample (LSU B29828) in DMSO, was obtained from the sole specimen of *C. estudilloi* Allen, 1977. For *viridirostris*, a piece of bone and scute taken by R. Fleischer from the unique specimen of *C. viridirostris* Sclater, 1875, held in the Natural History Museum, England (NHM 1889.6.1.302) was used. All other samples were of whole blood (*alberti*) or liver and heart in DMSO buffer or frozen.

DNA extraction and PCR amplification. Following recommended protocols for comparisons between "ancient" and recent DNA samples (Austin *et al.* 1997), all extractions and sequencing of *estudilloi* and *viridirostris* were done in a dedicated ancient DNA laboratory before work commenced on the other species in a separate location. Accordingly, mtDNAs from *estudilloi* and *viridirostris* were first extracted and sequenced by B. Slikas in Washington at the Smithsonian National Zoological Park (SNZP), where the laboratory is in a building free of "modern" DNA extracts and PCR products. All other species were analysed in L. Joseph's laboratory at the Academy of Natural Sciences of Philadelphia (ANSP). ANSP work did include an attempt to replicate the *estudilloi* and *viridirostris* sequences, but only after the initial SNZP sequences had been obtained. Also at ANSP, after all initial sequences had been obtained, fresh extractions were prepared and sequenced in order to assess the repeatability of the sequences.

Extractions at SNZP of the *estudilloi* and *viridirostris* samples followed standard protocols for ancient DNA samples: all tubes and reagents were UV-treated for > 20 minutes prior to use, pipettors were disassembled and cleaned with a 10% bleach solution after each extraction set, and only filter tips were used on pipettors. Each extraction set (5 samples) included an extraction control (i.e., a tube containing only extraction buffer, carried through the same steps as the actual samples).

At SNZP, small (1mm x 3mm) pieces of tissue from the samples of *estudilloi* and *viridirostris* were chopped finely with a sterile scalpel blade and incubated overnight at 55°C in 750 ml of extraction buffer (10 mg/ml DTT, 1 mg/ml proteinase K, 1% SDS, 10 mM Tris, 2 mM EDTA, 10 mM NaCl) on a rotating platform. After incubation, the samples were purified with two phenol extractions, followed by a single chloroform extraction. The samples were concentrated with Centricon-30 columns (Amicon, Inc.), and the volume of each was adjusted to 120 ml with sterile, UV-treated water. Each extract was aliquotted into four tubes, and the tubes were heated at 65°C for 10 minutes to destroy any DNAase. The extracts were stored at -20°C in the ancient DNA laboratory. PCR reactions were set up in the ancient DNA laboratory, but PCR and post-PCR steps were performed in the main genetics laboratory, housed in a different building. At ANSP, genomic DNA was extracted for all samples except ANSP 8225 with the QIAGEN blood and tissue extraction kits following the manufacturer's instructions. MtDNA was purified from ANSP 8225, which was a frozen tissue sample, with the Wizard MiniPrep kit (Beckman *et al.* 1993).

At SNZP, PCR amplifications were done in 50 µl reactions with 3 µl of template and final concentrations (mM) of $MgCl_2$, dNTPs and primers of 1.5, 0.2 and 0.6, respectively, and 0.2 µl of Amplitaq Gold *Taq* polymerase;

45 cycles were run with an annealing temperature of 48°C. PCR amplifications at ANSP were done in 25 µl volumes with equal volumes of template and reagent mix in which final concentrations (mM) of $MgCl_2$, dNTPs and primers were 0.95, 0.19 and 0.2, respectively, with 0.06 µl of GIBCO *Taq* polymerase and no bovine serum albumin; 30 cycles were run with annealing mostly at 48°C and in one case at 50°C.

Two segments of the mitochondrial genome were amplified and sequenced. A fragment of 375 base pairs (bp) of the cytochrome *b* gene was amplified using primers L14990 (5'- AAA AGC TTC CAT CCA ACA TCT CAG CAT GAT GAA A -3') and H15305 (5'- AAA CTG CAG CCC CTC AGA ATG ATA TTT -3'), where the numbers give the position of the 3' end of the primer in the chicken *Gallus gallus* mitochondrial genome (Desjardins & Morais 1990). For *C. estudilloi*, a second fragment of 358 bp, including a portion of the genes for CO II and lysine transfer RNA (hereafter t-lys), the entire ATPase 8 gene and a portion of that for ATPase 6, was amplified using primers L8929 (5'- GGA CAA TGC TCA GAA ATC TGC GG -3' - cited as CO2GQL by Greenberg *et al.* 1998) and H9240 (5'- GTC RAA GAA RCT TAG GTT CAT -3' - cited as A6MNH by Greenberg *et al.* 1998) and both of which were developed by G. Seutin and E. Bermingham. For *C. viridirostris*, a smaller fragment (235 bp) was amplified using primers L9051 (5'- CAC CAG CAC TAG CCT TTT AAG -3') and H9241 (5'- TGG TCG AAG AAG CTT AGG TTC A -3'). At ANSP, only the first two of these primer pairs were used.

Sequencing and analyses. Amplified products at SNZP were sequenced using cycle sequencing with dye-labelled ddNTPs and visualized with an ABI 373 automated sequencer. Those sequences were edited and aligned using Sequencer 3.0 (1995; Gene Codes Corporation, Ann Arbor, MI). ANSP amplification products were purified using the QIAGEN PCR product purification kit following the manufacturer's instructions. They were then sequenced with a Thermo-Sequenase fluorescent labelled primer cycle sequencing kit (Amersham RPN 2438) and visualized with a Licor Long ReadIR 4200 bi-directional automated sequencer. All *Crax* sequences were aligned at ANSP with CLSTAL-V (Higgins *et al.* 1992). PAUP 3.1.1 (Swofford 1993) was used to calculate sequence divergences and to derive phylogenetic trees using parsimony with steepest descent, random addition of taxa, mid-point rooting and a range of weighting schemes (transversions weighted 5:1, 10:1 over transitions, no weighting). Since results with and without weighting were similar, we present results with no weighting. As different combinations of individuals and genes were sequenced, phylogenetic analysis was first done on the data from the two genes separately and then on a pooled data set only for those individuals sequenced for both genes.

RESULTS

A total of 474 bp of sequence were aligned across all taxa examined except *alector* for ATPase 8, for which good sequence was not obtained (Appendix). All sequences have been lodged in Genbank, accession numbers AF106485-AF106507. The aligned sequences comprised 253 bp from the cytochrome *b* gene and 221 bp from t-lys/ATPase subunit 8 genes. Two or more individuals were sequenced in the following combinations of species and genes: *alberti, rubra*, *fasciolata* and *globulosa* for cytochrome *b* and *daubentoni*, *rubra* and *fasciolata* for t-lys/ATPase 8 (Appendix). Sequences of *estudilloi* and *viridirostris* obtained at the SNZP laboratory were from the heavy strand primers H15331 and H9260. Some t-lys/ATPase 8 sequence from *estudilloi* was

later obtained independently at the ANSP laboratory with the light strand primer L8929. Though different instruments and protocols were used in the two laboratories for DNA extraction, PCR and sequencing, the reverse complement sequence of the ANSP L8929 *estudilloi* sequence matched the SNZP H9260 *estudilloi* sequence precisely.

In the cytochrome *b* sequences, three, one and twenty-one polymorphic sites were at first, second and third codon positions, respectively. The mean proportions of A, C, G and T nucelotides were 0.25, 0.36, 0.15 and 0.24, respectively, the relatively low proportion of G being characteristic of avian mtDNA. These sequences were aligned with published cytochrome *b* sequences from a Plain Chachalaca *Ortalis vetula* (Genbank accession number L08384) and translated completely into amino acids; amino acid replacements were at only three sites relative to the *estudilloi* reference sequence. In the ATPase 8 sequences excluding the t-lys sequences at the 5' end of the t-lys/ATPase 8 region, there were four, three and thirteen polymorphic sites at first, second and third positions, respectively; the mean proportions of A, C, G and T were 0.32, 0.31, 0.10 and 0.23, respectively. The ATPase 8 sequences (excluding t-lys) also translated completely and the resulting amino acid sequences were aligned with homologous *Rhea* amino acid sequences (Genbank accession number Y16884) (Appendix). Amino acid replacements were at six sites relative to the *estudilloi* reference sequence.

The sequences of *estudilloi* were identical with those of *alberti*, and sequences of *viridirostris* were identical with those of *daubentoni*. Sequence identity was also observed as follows: for cytochrome *b* - between the pairs of *alberti* and between both *fasciolata* samples and one *rubra* sample, B10362; for t-lys/ATPase 8 - between the pair of *daubentoni* individuals and between *rubra* B10362 and *globulosa* B10360. Depending on whether calculated from the sequences separately or in combination, uncorrected intraspecific sequence divergences ranged from zero to 4.7% with mean values of 1.3% or 2.1%. Again depending on whether calculated from the cytochrome *b* and t-lys/ATPase 8 sequences separately or in combination, interspecific divergences not involving *estudilloi* and *viridirostris* ranged from zero to 6.9% with mean values varying from 3.8% to 4.0%. Sequence divergences between *estudilloi* and *viridirostris* ranged from 4.7% (cytochrome *b*) to 7.0% (t-lys/ATPase 8).

In phylogenetic analyses, the cytochrome *b* data yielded four most parsimonious trees of 30 steps with a consistency index of 0.83. In the strict and 50% consensus trees, *daubentoni* aligned with *viridirostris* and *estudilloi* with *alberti*. The sequences from *rubra*, *globulosa* and *fasciolata* were paraphyletic. One *rubra* sequence, B14200, was the sister of the *estudilloi/alberti* sequences with which it formed a separate clade. The other *rubra* sequence, B10362, aligned with *fasciolata*. Similarly, one *globulosa* sequence, B10360, aligned with *alector* and the other, B5359, lay on a separate branch. Analysis of the t-lys/ATPase 8 data yielded a single tree of 27 steps with a consistency index of 0.93. The tree had two clades, one with *estudilloi/alberti* and the *viridirostris/daubentoni* sequences and another in which the two *fasciolata* sequences were the sister to the two *rubra* and single *globulosa* sequences. The two *rubra* sequences were again paraphyletic. Analysis of the pooled data produced a single shortest tree of 55 steps with a consistency index of 0.89 and with a topology broadly similar to that of the cytochrome *b* tree. The *rubra* sequences were again paraphyletic, with *rubra* sample B14200 the sister of *estudilloi/alberti*. Because of the extent of paraphyly, the sequences were checked by repeating afresh all extraction and sequencing protocols for t-lys/ATPase 8 from the original tissue samples

and the sequences so obtained agreed with the initial sequences.

DISCUSSION

Our central aim was to use mtDNA sequences to discriminate between the two hypotheses that *estudilloi* and *viridirostris* are a single valid species or that they are descended from hybridization events involving one or more combinations of *Crax* spp. The following key findings emerged from our study. The sequences we obtained of *estudilloi* and *alberti* are identical and those of *viridirostris* and *daubentoni* are identical. Sequence identity was also seen intraspecifically in *daubentoni* for t-lys/ATPase 8 and in *alberti* and *fasciolata* for cytochrome *b* and interspecifically between one *rubra* individual and the *fasciolata* individuals for cytochrome *b*. The divergence between *estudilloi* and *viridirostris*, aside from being equal to that between *alberti* and *daubentoni*, is well within the range of other interspecific values and is twice the mean intraspecific divergence, though at the high end of the intraspecific range. In phylogenetic analyses *estudilloi* aligned with *alberti*, *viridirostris* with *daubentoni*, and sequences from *rubra*, *fasciolata* and *globulosa* were not monophyletic.

In terms of our main goals and expectations, the simplest interpretation of our results is that both *estudilloi* and *viridirostris* are descended from hybridization events at an undetermined time in the past involving maternal parents of *alberti* and *daubentoni*, respectively. This interpretation explains the sequence identity between *estudilloi* and *alberti* and that between *viridirostris* and *daubentoni*. It is also consistent with the sequence divergence between *estudilloi* and *viridirostris* being close to the mean of interspecific comparisons and well above that for intraspecific values. It is easily reconciled with the known captive origin of *viridirostris*, but not so easily with the putative Bolivian provenance of *estudilloi*. This is because the parent of *estudilloi* inferred from the DNA data, *alberti*, is a relatively narrowly distributed endemic from northern Colombia not known to occur anywhere near Bolivia. To reconcile this interpretation of the sequence data with the putative Bolivian origin of *estudilloi*, one has to postulate unlikely events such as inadvertent mixing of samples at the source of the *estudilloi* material or an ancient hybridization event that involved *alberti* in the ancestry of the *estudilloi* individual. In this regard, native peoples across the Amazon region have from time immemorial kept curassows as pets. The possibility of a hybridization event occurring under such circumstances cannot be ruled out. We also note that although opportunities for natural hybridization to occur among the members of the *C. rubra* complex are few, Blake (1955) remarked that *alberti* and *rubra* have been collected a few miles apart in the valley of the upper Rio Sinu in Colombia. Thus, hybridization between them in that area is possible. As *rubra* is a yellow-cered species, one might predict that green-cered individuals resembling *estudilloi* could be found in that region if habitat remains. Note that the bicoloured bill pattern of *estudilloi* described in the Introduction is identical to that seen in male *rubra*, for which it is diagnostic. We note that members of the *rubra* complex are essentially allopatric so that opportunities for natural hybridization among any pairs of species will be few.

By the alternative view that *estudilloi* and *viridirostris* are conspecific, the sequences we obtained from them would clearly be paraphyletic. Given the paraphyly we have observed in the other species (and which we discuss below), this need not necessarily be unreasonable. However, it would be disconcerting in view of the existence of only two specimens collected more than 100 years apart, one of which was a captive bird in a European zoo. The lack of field observations

of such a species adds to the concern. The observations that sequence divergences between *estudilloi* and *viridirostris* were twice the mean of intraspecific values and close to the mean of interspecific comparisons in *Crax* would be difficult to explain under this interpretation, although we note again that the divergence between them is at the high end of observed intraspecific values.

Two other interpretations might also be considered. First, *estudilloi* and *viridirostris* may both be valid species, but it would then be difficult to explain the mtDNA sequence identity between *estudilloi* and *alberti* and that between *viridirostris* and *daubentoni*. Also by this view, multiple origins or losses of the greenish cere colour would almost certainly have to be postulated, such as the colour having independently evolved in *estudilloi* and *viridirostris* or having been ancestral and lost in other taxa. These sequences seem unlikely. Also, one might consider that *estudilloi* and *viridirostris* are simply colour phases of *alberti* and *daubentoni*, respectively. Several *Crax* species do have distinctive colour phases of plumage, and some of these were originally described as different species. Thus, *Crax annulata* Todd, 1915, was based on a rare black and white female plumage of *C. alberti*, and *Crax incommoda* Sclater, 1872, was similarly based on a rare variant female plumage of *C. daubentoni*. This interpretation is easily reconciled with the sequence identity between *estudilloi* and *alberti* and that between *viridirostris* and *daubentoni*, but an explanation would be needed for why the most distinctive feature of these birds, their greenish ceres, arose independently in aberrant individuals of *alberti* and *daubentoni*. Also by this view, the putative Bolivian provenance of the *estudilloi* individual would imply that *alberti* has a most remarkable and unusual distribution pattern among Neotropical birds. In short, we consider these two explanations unlikely.

Although one might argue that our data cannot absolutely reject the hypothesis that *estudilloi* and *viridirostris* are representatives of a species to be known as *C. viridirostris* Sclater, 1875, we conclude from our data that hybrid origins of *estudilloi* and *viridirostris* must be seriously considered as the preferable hypothesis. This is despite the difficulty of reconciling hybrid origins with the putative Bolivian origin of *estudilloi*. We consider the likelihood of *estudilloi* and *viridirostris* both being distinct, valid species to be vanishingly small. Our conclusion should be tested with study of nuclear DNA markers.

Finally, we comment briefly on the paraphyly observed among our samples of *rubra, globulosa* and *fasciolata*. This needs to be interpreted in the light of the captive origins of the birds used in this study, the availability of pedigree data for only some of them, and the propensity of *Crax* species to hybridize in captivity (Delacour & Amadon 1973, del Hoyo 1994). We reiterate that until it is changed by mutation, the mtDNA of the maternal species involved in a hybridization event will appear unchanged in every generation of the matriline descending from the female in the original hybridization. In the case of *Crax* spp, the external phenotype of male progeny from females on that matriline could come to resemble the paternal parent in the original cross if the males are derived through repeated backcrossing to individuals of the paternal species. For an example of this phenomenon see Joseph & Moritz (1993). Therefore, unpedigreed birds used in this study that had the external phenotype of one species could easily carry the mtDNA of another species. A corollary of this argument is that the mtDNA of *viridirostris* that we observed as being identical to that of *daubentoni* may in fact have been of another species if hybridization events had occurred in the ancestry of the captive *daubentoni* individuals we used. The same might be said of our observation that the mtDNA of *estudilloi* is

identical with that of *alberti* but in that case one of the *alberti* individuals was a wild-caught bird. Nonetheless, we consider these possibilities unlikely if only because two individuals of both *daubentoni* and *alberti* were involved. In conclusion, until a phylogenetic analysis of *Crax* spp is done with wild-caught individuals, it would be premature to attach much significance to the paraphyly we have observed among these species. Rather, we suggest that it should flag awareness in later work of the possible presence of paraphyly in these species.

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APPENDIX. Alignments of light strand cytochrome *b* and t-lys/ATPase 8 sequences examined in this study from *Crax* spp. Sequences are given in the 5′ to 3′ direction from left to right, top to bottom. Sample numbers (see Table 1 for details) of the indicated individuals are as follow: *estudilloi* B29828; *alberti*1 – 6184; *alberti*2 – 10006; *viridirostris* – NHM 1889.6.1.302; *daubentoni*1 – 6304; *daubentoni*2 – 6305; *rubra*1 – B14200; *rubra*2 – B10362; *fasciolata*1 – B18929; *fasciolata*2 – B16810; *globulosa*1 – B10360; *globulosa*2 – B5359; *alector* – ANSP 8225.

1. Alignment of cytochrome *b* sequences.

```
estudilloi    ACCCAAATCCTCACTGGCCTCCTACTGGCCATACACTACACTGCAGACACTACCCTCGCCTTCTCCTCCGTAGCTCATAC
alberti1      ................................................................................
alberti2      ................................................................................
viridirostris ........................................C...............................C..C..
daubentoni2   ........................................C...............................C..C..
rubra1        ........................A..............C...................................C..
rubra2        ........................................C.............................G........
fasciolata1   ........................................C.............................G........
fasciolata2   ........................................C.............................G........
globulosa1    ............................T...........C.............................G........
globulosa2    ........................................C.....................T..................
alector       ............................T...........C.............................G........


estudilloi    ATGCCGGAACGTCCAGTATGGCTGACTAATCCGCAACCTACACGCAAACGGTGCCTCATTCTTCTTCATCTGCATCTACC
alberti1      ................................................................................
alberti2      ................................................................................
viridirostris ......A.........................................T..C...........................
daubentoni2   ......A.........................................T..C...........................
rubra1        ..................C.....................................C.......................
rubra2        ...T..A........A.....................................C.......................
fasciolata1   ...T..A........A.....................................C.......................
fasciolata2   ...T..A........A.....................................C.......................
globulosa1    ...T..A........A.....T...............................C..T....................
globulosa2    ......A...........C.....................................C..T....................
alector       ......A........A.....T...............................C..T....................
```

APPENDIX. Continuation.

Alignment of cytochrome *b* sequences. Continuation.

```
estudilloi     TGCACATTGGCCGCGGCCTCTACTACGGCTCATACCTTTACAAGGAAACCTGAAACACAGGAGTTATCCTCCTACTAACGCTTATAGCAACTG
alberti1       ................................................................................
alberti2       ................................................................................
viridirostris .C..............T.........................A.............G..................GT.............
daubentoni2   .C..............T.........................A.............G..................GT.............
rubra1        .C.....................................T..A.................A..............GT.............
rubra2        .C..............T.........................A.............G..................GTA............
fasciolata1   .C..............T.........................A.............G..................GTA............
fasciolata2   .C..............T.........................A.............G..................GTA............
globulosa1    .C.....C.........T.........................A..................................GTA............
globulosa2    .C..............T.........................A..................................GT.............
alector       .C.....C.........T.........................A..................................GTA............
```

2. Alignment of t-lys/ATPase 8 sequences. The first 70 bp are from the t-lysine gene and the start codon of the ATPase8 gene is underlined.

```
estudilloi     ATTAAGAAGCTATGAATCAGCACTAGCCTTTTAAGCTAGAGAAAGAGGCCCACCCATCCTCCTTAATGGCATGCCTCAGCTAAA
alberti1       ...............................................................................
viridirostris ??????????????????????????????????..G.............................A..............
daubentoni1   ...................................G.............................A..............
daubentoni2   ...................................G.............................A..............
rubra1        ..................G...?..........G.................?.....................A.?...
rubra2        ........?.........?.G...?........?..G.......?.........C.....................A.....
fasciolata1   ...................................G.................C...................C..A.....
fasciolata2   ........................G.........G.................C.................?C..A.....
globulosa1    ..................G...?..........G.................C.....................A.....
```

APPENDIX. Continuation.

Alignment of t-lys/ATPase 8 sequences. Continuation.

```
estudilloi    CCCAAATCCATGATTTATCATCTTCCTATTGACATGATTAACCCTCTCCCTGCTTATTCAACCAAAATTGTTATCATTTACCCC
alberti1      ................................................................................
viridirostris ............G..C.C.................................A.....C...........A.............
daubentoni1   ............G..C.C.................................A.....C...........A.............
daubentoni2   ............G..C.C.................................A.....C...........A.............
rubra1        ...G...........C.C............A.................T.................C..............
rubra2        ............G....C.............................................................
fasciolata1   ............G....C.....................................................C.............
fasciolata2   ............G....C.....................................................C.............
globulosa1    ............G....C.............................................................


estudilloi    AACAAACCCTCCATCAAACAAAGCCCTGGTAACTAAAACCACCCCATGAACCT
alberti1      ...........................................................
viridirostris ........TC..................AC.............T.........
daubentoni1   ........TC..................AC.............T.........
daubentoni2   ........TC..................AC.............T.........
rubra1        .........C.................A.C.......................
rubra2        .........C.................A.C.........?.....G.......
fasciolata1   .........C.................A.C......................?
fasciolata2   .........C.................A.C................???????
globulosa1    .........C.................A.C.........?.....G.......
```