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Source: The Auk, 129(2) : 197-204

Published By: American Ornithological Society

URL: https://doi.org/10.1525/auk.2012.11259

The Auk

An International Journal of Ornithology

Vol. 129 No. 2 April 2012

The Auk 129(2):197–204, 2012

Printed in USA.

RAPID COMMUNICATIONS

PHYLOGENETIC RELATIONSHIPS OF THE EXTINCT CAROLINA PARAKEET (*CONUROPSIS CAROLINENSIS*) INFERRED FROM DNA SEQUENCE DATA

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Abstract.—We obtained the first DNA sequences from the extinct Carolina Parakeet (*Conuropsis carolinensis*) and used these data to infer the phylogenetic relationships of this iconic North American parrot. We compared our sequences of the mitochondrial COI and ND2 genes obtained from multiple Carolina Parakeet museum specimens to homologous sequences from individuals representing 43 species in 28 genera of Neotropical parrots (Tribe Arini), and four species from more distantly related Old World species of the Order Psittaciformes. Bayesian and maximum likelihood analyses place *C. carolinensis* on a long branch within a well-supported clade of parakeets that also includes *Aratinga solstitialis*, *A. auricapillus*, and *Nandayus nenday*. These species of *Aratinga* (but not *N. nenday*) closely resemble *C. carolinensis* in the presence of yellow and orange head plumage and blue feathers in the wings. Our data do not support a close relationship with the Monk Parakeet (*Myiopsitta monachus*), with which the Carolina Parakeet shares fully feathered ceres, a putative adaptation for cold tolerance that appears to have evolved independently in both species. Given the high level of sequence divergence from all sampled species, we recommend continued recognition of the monotypic genus *Conuropsis*. Taxonomic revision of the highly polyphyletic genus *Aratinga* is needed. *Received 23 November 2011, accepted 13 February 2012.*

Key words: Arini, Carolina Parakeet, *Conuropsis carolinensis*, extinct species, historic DNA, phylogenetics, systematics.

Relaciones Filogenéticas del Perico Extinto *Conuropis carolinensis* Inferidas con Datos de Secuencias de ADN

Resumen.—Obtuvimos las primeras secuencias de ADN de la especie extinta *Conuropis carolinensis* y usamos estos datos para inferir las relaciones filogenéticas de este simbólico perico norteamericano. Comparamos nuestras secuencias de los genes mitocondriales COI y ND2 obtenidos de varios especímenes de museo de *C. carolinensis* con secuencias homólogas de individuos representativos de 43 especies de 28 géneros de loros neotropicales (tribu Arini) y de cuatro especies del orden Psittaciformes más lejanamente relacionadas habitantes del viejo mundo. Los análisis bayesianos y de máxima verosimilitud ubicaron a *C. carolinensis* en una rama larga dentro de un clado de pericos bien sustentado, que incluye a *Aratinga solstitialis, A. auricapillus* y *Nandayus nenday*. Estas especies de *Aratinga* (pero no *N. nenday*) se parecen bastante a *C. carolinensis* en la presencia de plumaje amarillo y naranja en la cabeza y plumas azules en las alas. Nuestros datos no sustentan una relación cercana con *Myiopsitta monachus*, que comparte con *C. carolinensis* la cera completamente emplumada. Esta presunta adaptación relacionada con la tolerancia al frío parece haber evolucionado independientemente en cada especie. Dado el alto nivel de divergencia genética de *C. carolinensis* con relación a todas las especies muestreadas, recomendamos continuar con el reconocimiento del género monotípico *Conuropsis*. Se necesita una revisión taxonómica del género *Aratinga*, que es áltamente polifilético.

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The Auk, Vol. 129, Number 2, pages 197–204. ISSN 0004-8038, electronic ISSN 1938-4254.

DOI: 10.1525/auk.2012.11259

Prior to its decline and extinction, the Carolina Parakeet (*Conuropsis carolinensis*) was distributed patchily throughout the eastern half of North America as far north as the southern shores of Lake Erie and Lake Ontario, making it by far the most northerly distributed parrot (Psittaciformes) in the Americas.[1,2] Once common and abundant, the species was in decline by the 1830s and was rare by the end of the 19th century, by which time its distribution was largely limited to the swamps of Florida.[3] The last reliable sightings were in the late 1920s, but unconfirmed reports suggest that it persisted in Florida, Georgia, and South Carolina into the late 1930s.[3] Although the exact timing and cause of its demise are unknown, it is generally acknowledged that Carolina Parakeets were shot for sport, food, and feathers and to guard against crop depredations. Destruction of bottomland forests likely also played a role in the species' extinction.[3] Snyder hypothesized that a preference for eating weedy cockleburs may have brought it into contact with diseases carried by settlers and their livestock.[4] The Carolina Parakeet now stands with the Passenger Pigeon (*Ectopistes migratorius*) as an iconic example of the ability of humans to extirpate even widespread and abundant continental bird species.[5,6]

The extinction of the Carolina Parakeet occurred before any systematic study of its ecology and habits was undertaken, and what little is known of its life history and historical distribution has been pieced together from the fragmentary accounts of 19th-century naturalists. Daniel McKinley synthesized reports of its regional occurrence[7,8,9] and summarized the available details of its nesting biology[10] and seasonal movements.[11] Noel F. R. Snyder combined this work with interviews of the few remaining persons with first-hand knowledge of wild Carolina Parakeets and his own experience with parrot biology to provide the most complete picture to date of the natural history of this species.[3,4] On the heels of Snyder's work, additional insights concerning the natural history and extinction of the Carolina Parakeet will likely come from only two sources: reasoned inference from studies of related extant species, and additional study of museum specimens.

Inference of evolutionary patterns requires an informed hypothesis of the evolutionary history of a clade. The genus *Conuropsis* was erected as a monotypic genus by Linnaeus.[12] Most workers have suggested that *Conuropsis* was most closely related to the genus *Aratinga*; this inference is based on shared morphological features, including a long, pointed tail and wings, feathered cheeks and lores, and comparatively broad and heavy bills.[1,4] Snyder noted the shared presence of fully feathered ceres in *Conuropsis* and the Monk Parakeet (*Myiopsitta monachus*) of temperate South America, but expressed considerable uncertainty as to the true affinities of *Conuropsis*.[4] Here, we use mitochondrial DNA sequences obtained from museum specimens of *Conuropsis* and representatives from a broad sampling of Neotropical parrots to reconstruct the evolutionary relationships of the extinct Carolina Parakeet.

Molecular Phylogenetics

DNA extraction.—We used sterile scalpel blades and forceps to remove slivers of skin and connective tissue from the toes of six specimens at the New York State Museum (NYSM 9420, no data; NYSM 9421, female, Manatee County, Florida; NYSM 9422, male, Florida; NYSM 9436, female, Florida) and the Elon Howard Eaton Collection at Hobart and William Smith Colleges (E 778, Florida; E 996, Florida). All specimens have extensive yellow plumage on the head and are presumed to be adults. None of the NYSM specimens have reliable dates; the Eaton Collection specimens were both collected in April 1884. Latex gloves were worn while handing specimens and changed between specimens, and instruments were sanitized with 10% bleach between specimens. Toe-pad samples were removed to the dedicated ancient-DNA lab at NYSM, where no previous work on parrots had been performed. All DNA extractions and polymerase chain reaction (PCR) setups from *C. carolinensis* were performed in this lab, following procedures intended to minimize and highlight contamination from exogenous DNA sources, including negative extraction controls (containing no toe pad), negative PCR controls (containing no DNA extract), glove changes before handling each sample, ultraviolet irradiation of all plastics, exclusive use of aerosol-barrier pipette tips, and daily sanitation of all equipment and surfaces with 10% bleach solution.

DNA was extracted from toe pads using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, California), modifying the manufacturer's protocol as follows: toe pads were incubated overnight at 55°C with agitation in double volumes of digest buffer ("ATL" 380 μL) and proteinase K (40 μL). Undigested chunks were ground with micro-pestles, and an additional 20 μL of proteinase K was added for a second night of digestion at 55°C with agitation. Following complete digestion, double volumes of the binding buffer ("AL") and ethanol were added and the resulting mixture was applied to the silica-membrane spin columns in 2 or 3 batches, centrifuging each batch through the membrane to bind the DNA. Following binding, the manufacturer's protocol was followed for two rounds of washing, and DNA was eluted in 75 μL of filtered, autoclaved, DNase-free water. Elution was repeated and both filtrates were combined for a total volume of 150 μL of DNA in water. Extracts from three specimens (NYSM 9421, NYSM 9436, and E 778) yielded PCR products and DNA sequences in initial trials; these specimens were resampled as above and subjected to a second extraction that involved a 2-day washing and hydration procedure[13] followed by the modified DNeasy protocol above. Extracts from this second round yielded additional PCR products and sequences, which confirmed the sequencing results from the first set of extracts.

Extractions, PCR, and sequencing for additional taxa were performed at New Mexico State University, where DNA was extracted from blood and frozen tissue samples from captive birds or museum specimens (Table 1) using the DNeasy Blood and Tissue Kit, following the manufacturer's protocol as described by Wright et al.[14]

Portions of the mitochondrial protein-coding genes cytochrome oxidase I (COI) and NADH-dehydrogenase subunit 2 (ND2) were amplified from nonhistorical samples, using primers and reaction conditions described by Wright et al.[14] These mitochondrial genes were selected because their utility has been well demonstrated in other phylogenetic studies of parrots.[14,15] To obtain homologous sequences from *C. carolinensis*, we used several primer pairs (Table 2)[16,17] to amplify and sequence short, overlapping fragments of both genes. We amplified these fragments in 50-μL PCRs that each contained 5 μL of DNA extract (concentration unquantified), 1.0 unit of HotMaster DNA Polymerase (Eppendorf), 0.10 mM of each dNTP, 0.5 mM of each primer, and 0.025 mg of bovine serum albumin, using a "touchdown" thermal program: five cycles with a primer annealing temperature of 58°C followed by 10 cycles at 56°C, 10 cycles at 54°C, and 25 cycles at 50°C.

Table 1. Samples used in the analysis of Carolina Parakeet phylogenetic relationships.

Species	GenBank accession number		Museum [a]	Specimen number [b]	Locality	Sample type [c]
	COI	ND2				
Alipiopsitta xanthops	GU826172	HQ270479	LSU	B-28972	Captive, USA, Pennsylvania, Kutztown, Rick Jordan Aviary	T
Amazona festiva	GU826171	HQ270478	ANSP	3175	Imuya Cocha, Ecuador	T
A. viridigenalis	EU621595	EU327598	NMNH	B06400	Captive, Miami Zoo, Miami, FL, USA	T
Anodorhynchus hyacinthinus	GU826173	HQ270480	LSU	B100020/ B-13478	Captive, Locality unknown	T
Ara glaucogularis	GU826174	HQ270481	LGEMA	623	Captive, Breeder Mauricio Santos. LP 931622	B
A. macao	EU621596	EU327601	NMNH	B07059	Brazil, Para, Altamira, 52 km SSW, E. Bank Rio Xingu	T
Aratinga aurea	GU826175	HQ270482	NMNH	B07010	Brazil, Para, Altamira, 52 km SSW, E Bank Rio Xingu	T
A. auricapillus	GU826176	HQ270483	LSU	B-29884	Captive, U.S.A., Texas, McKinney, Clarence Killian Aviary	T
A. chloroptera	GU826178	HQ270484	LPF	LP07-26 (UV)	Captive, Spain, Canary Islands, Tenerife, Puerto de la Cruz, Loro Parque	B
A. euops	GU826179	HQ270485	LPF	LP07-25 (UV)	Captive, Spain, Canary Islands, Tenerife, Puerto de la Cruz, Loro Parque	B
A. finschi	GU826180	HQ270486	NMNH	B00402	Panamá, Bocas del Toro, Isla San Cristobal, Bocatorito	T
A. holochlora	GU826181	HQ270487	LSU	B-23651	Captive, USA, Texas	T
A. leucophthalmus	GU826182	HQ270488	NMNH	B05906	Argentina, Corrientes, Corrientes, 45 km S, Manuel Derqui	T
A. mitrata	GU826183	HQ270489	LPF	LP07-12 (UV)	Captive, Spain, Canary Islands, Tenerife, Puerto de la Cruz, Loro Parque	B
A. nana	GU826184	HQ270490	NMNH	B00465	Panamá, Bocas del Toro, Isla San Cristobal, Bocatorito	T
A. pertinax	EU621597	EU327600	NMNH	B04190	Guyana, Berbice, West Bank Berbice River, Dubulay Ranch	T
A. solstitialis	GU826185	HQ270491	NMNH	B06816	Captive, Locality unknown	T
A. wagleri	GU826186	HQ270492	LPF	LP07-14 (UV)	Captive, Spain, Canary Islands, Tenerife, Puerto de la Cruz, Loro Parque	B
Bolborhynchus lineola	GU826187	HQ270493	LSU	B-16252	Costa Rica, San Jose Province	T
Brotogeris versicolurus	GU826188	HQ270494	LSU	B-13057	Bolivia, Santa Cruz Department, Velasco	T
Calyptorhynchus funereus	EU621603	EU327606	NMNH	B06460	Captive, Australia, Queensland, Brisbane, Moggill, Queensland NP&WS	T
Conuropsis carolinensis	GU826189	HQ270495	NYSM	9421	USA, Florida, Manatee County	S
C. carolinensis	GU826190	HQ270496	NYSM	9436	USA, Florida	S
Cyanoliseus patagonus	EU621609	EU327613	AMNH	DOT 10384	Argentina, Neuquen, Departmento Anelo, Sierra Auca Mahuida	T
Cyanopsitta spixii	EU621610	EU327614	LPF	Cyspi01 (UV)	Captive, Spain, Canary Islands, Tenerife, Puerto de la Cruz, Loro Parque	F
Deroptyus accipitrinus	EU621613	EU327617	NMNH	B04256	Guyana, Berbice, Wiruni River	T
Diopsittaca nobilis	EU621614	EU327618	NMNH	B12392	Guyana, Karaudanawa, ca. 4 km S	T

(*continued*)

TABLE 1. continued.

Species	GenBank accession number		Museum [a]	Specimen number [b]	Locality	Sample type [c]
	COI	ND2				
Enicognathus leptorhynchus	EU621616	EU327620	LPF	LP07-17 (UV)	Captive, Spain, Canary Islands, Tenerife, Puerto de la Cruz, Loro Parque	B
Forpus passerinus	EU621621	EU327625	NMNH	B12187	Guyana, Corriverton, South of Along Courantyne River	T
Graydidascalus brachyurus	GU826191	HQ270497	LSUMNS	B-3626	Peru, Loreto Department, along Rio Samiria	T
Guaruba guarouba	EU621624	EU327628	NMNH	B-06578	Locality unknown	T
Hapalopsittaca melanotis	GU826192	HQ270498	LSU	B-1229	Bolivia, La Paz Department	T
Leptosittaca branickii	EU621626	EU327630	ANSP	4966	Ecuador, 25 km SSE Jimbura; E Slope Cord. Lagunillas	T
Melopsittacus undulatus	EU621629	EU327633	NMNH	B06360	Australia, New South Wales, 20 km W Griffith	T
Myiopsitta monachus	EU621631	EU327635	NMNH	B02706	Argentina, Entre Rios, Estancia el Tala, Near Puerto Constanza	T
Nandayus nenday	EU621632	EU327636	LPF	LP07-23 (UV)	Captive, Spain, Canary Islands, Tenerife, Puerto de la Cruz, Loro Parque	B
Nannopsittaca panychlora	EU621633	EU327637	NMNH	B05168	Guyana, Essequibo, Waruma River, E. Bank, ca. 15 River km S Kako River	T
Orthopsittaca manilata	EU621640	EU327644	NMNH	B04393	Guyana, Berbice, West Bank Berbice River, Dubulay Ranch	T
Pionites melanocephala	EU621644	EU327648	NMNH	B09553	Guyana, North West, Baramita	T
Pionus menstruus	EU621646	EU327650	NMNH	B00277	Panama, Bocas del Toro, Isla San Cristobal Bocatorito	T
P. senilis	GU826194	HQ270500	LPF	LP07-07 (UV)	Captive, Spain, Canary Islands, Tenerife, Puerto de la Cruz, Loro Parque	B
Primolius couloni	GU826195	HQ270501	FMNH	395540	Brazil, Acre, Reserva Extravista Alto Jurua, Rio Tejo	T
Psittacus erithacus	EU621657	EU327661	NMSU	987	Captive, USA, Texas, Brownsville, Gladys Porter Zoo	T
Pyrilia vulturina	GU826193	HQ270499	NMNH	B06888	Brazil, Para, Altamira, 52 km SSW, E Bank Rio Xingu	T
Pyrrhura perlata lepida	GU826196	HQ270502	NMNH	B07007	Brazil, Para, Altamira, 52 km SSW, E Bank Rio Xingu	T
P. picta	EU621660	EU327664	NMNH	B06897	Brazil, Para, Altamira, 52 km SSW, E. Bank Rio Xingu	T
Rhynchopsitta pachyrhyncha	EU621661	EU327665	NMNH	B08714	Locality unknown	T
Strigops habroptila	EU621663	EU327667	VU	CD1139	New Zealand, Fiordland	B
Touit batavica	EU621666	EU327670	ANSP	6192	Guyana, Iwokrama Reserve; ca. 16 road mi. SW Kurupukari	T

[a] Abbreviations: AMNH = American Museum of Natural History; ANSP = Academy of Natural Sciences, Philadelphia; LGEMA = Laboratorio de Genetica e Evolucao Molecular, Universidade de São Paulo, Brazil; LPF = Loro Parque Fundación, Tenerife, Spain; LSU = Louisiana State University Museum of Natural Science; NMNH = National Museum of Natural History, Smithsonian Institution; NMSU = New Mexico State University Vertebrate Museum; NYSM = New York State Museum; VU = Victoria University, Wellington, New Zealand.

[b] (UV) designates unvouchered sample.

[c] Sample type: T = frozen tissue; B = blood; F = feather; S = museum skin.

Table 2. PCR primers used to amplify short fragments of DNA from specimens of Carolina Parakeet.

CO I primers	5′ to 3′ sequence	Reference
L7773	GTCTCACAGGRATCGTCC	16
H7879	GAATAGGGGGAATCAGTGGG	16
L7804	AATAGGTGCCGTCTTTGCC	16
HCOIp	GTRTAGGCRTCTGGRTAGTC	14
ND2 primers		
L5216	RGAKGAGAARGCYAGGATTYTTKCG	17
H5370	CGTGGGTGGTGGGRTTTTGA	Present study
L5363	CAACACCYTATCAATCATCCCC	Present study
H5493	GGGTRGGAGAGTTGGGTGAT	Present study
L5485	CCAACGCATGAYACACYGGRCA	Present study
H5613	GTTGARAGGAGTAGGGCTGT	Present study
L5587	CCMTTYCACTTYTGRTTCCC	Present study
H5733	TTTAGYCCTATTCARCCRCC	Present study
L5730	TRTCYAYCAYRTCYATYGCCC	Present study
H5842	TARRYGTAGAAGGYTAGTAGKG	Present study

Amplification success was determined by visualizing 5 μL of each PCR product on 1.5% agarose gels stained with ethidium bromide. We cleaned PCR products using a QiaQuick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. We sequenced each PCR product in both directions using the PCR primers and BIG DYE, version 3.1, Terminator Cycle Sequencing chemistry (Applied Biosystems, Foster City, California) on an ABI 3100 Genetic Analyzer. Raw sequences were edited and assembled into consensus sequences for each specimen in SEQUENCHER, version 4.7 (Gene Codes, Ann Arbor, Michigan). We used MACCLADE, version 4.0,[18] to convert the sequence alignment from all specimens into Nexus format and to establish codon positions.

Phylogenetic analyses.—We included sequences from two specimens of *C. carolinensis* and representatives from 43 extant species of parrots from 28 genera of the Neotropical tribe Arini. We selected single representatives from 23 genera, two representatives from the speciose genera *Amazona*, *Ara*, *Pionus*, and *Pyrrhura*, and 12 representatives from *Aratinga*, the hypothesized sister clade to *Conuropsis* (Table 1). This sample represents all but three genera (*Triclaria*, *Ognorhynchus*, and *Psilopsiagon*) in tribe Arini. Outgroups included one extant species from each of four Old World tribes: *Psittacus erithacus*, of the Afrotropical tribe Psittacini; *Strigops habroptila,* of the New Zealand tribe Strigopini; *Melopsittacus undulatus*, of the Australian and Oceanian tribe Platycercini, and *Calyptorhynchus funereus*, of the Australasian tribe Calyptorhynchini. Our taxonomy followed the South American Classification Committee of the American Ornithologists' Union[19] for Neotropical parrots and Forshaw[2] for other species.

The final alignment of sequences from 47 extant species and 2 representatives of *Conuropsis carolinensis* was analyzed using Bayesian and maximum-likelihood (ML) methods. For Bayesian analysis, the data set was analyzed using several different partitioning strategies. These were (1) a two-partition strategy with separate models for each gene region, (2) a four-partition strategy that further partitioned each gene region with first and second codon positions under one nucleotide substitution model and third codon positions under a different model, and (3) a six-partition strategy with a separate model for each codon position within each gene region. The nucleotide substitution model for each partition was determined in MRMODELTEST, version 2.3,[20] using Akaike's information criterion (AIC). Analysis was conducted in MRBAYES, version 3.1,[21] with 2 runs of 4 chains each (1 cold and 3 heated) for 10 million generations, sampling every 1,000 generations under the default settings. Convergence was assumed when the average deviation of split frequencies was <0.01. Trees and parameters were summarized after discarding the first 25% (2,500) samples. Bayes factor analysis identified the four-partition strategy as optimal, but inspection of the trees produced by each strategy indicated that the six-partition strategy resulted in a better-resolved topology with higher posterior probabilities across the tree (data not shown). Simulations have suggested that, in general, overpartitioning produces fewer errors than underpartitioning[22]; we therefore selected the six-partition strategy as optimal for this data set.

For ML analysis, we ran five independent runs of GARLI[23] using random starting trees, default search parameters, and the GTR + I + G substitution model and parameters selected by the AIC in MODELTEST, version 3.8.[24,25] Nodal support was assessed by 100 ML bootstrap replicates in GARLI using the same model and search parameters.

Results

We obtained sequences totaling 251 nucleotides from COI and 625 nucleotides from ND2 from replicated DNA extracts and PCR amplifications from two specimens of *C. carolinensis* (NYSM 9421 and NYSM 9436). Shorter sequences obtained from an additional specimen (E 778) were not included in phylogenetic analyses; we did not obtain PCR products from the three other specimens we sampled. The sequences from NYSM 9421 and NYSM 9436 differed by three synonymous nucleotide substitutions at third postitions, one in COI and two in ND2. Both sequences were unambiguously aligned with sequences totaling 1,611 nucleotides from 47 other parrot species (GenBank accession numbers in Table 1). Uncorrected pairwise genetic distances (*p*-distances) between *C. carolinensis* and other Arini species ranged from 5.3% to 12.8% at COI and from 9.3% to 23.6% at ND2.

The Bayesian and ML searches recovered trees that showed generally similar topologies. Basal relationships and the most recent nodes in these trees were congruent in the two searches and were well supported by both Bayesian posterior probabilities and ML bootstraps (Fig. 1). All analyses placed the two *C. carolinensis* samples together with strong support and sister to a well-supported clade of three species (*Aratinga solstitialis*, *A. auricapillus*, and *Nandayus nenday*). This entire clade had a posterior probability of 0.99 in the Bayes run and an ML bootstrap support value of 75. The two searches differed in their placement of this *Conuropsis–Nandayus–Aratinga* clade within the tree: the Bayes search placed it sister to a diverse clade containing the macaws of the genera *Ara*, *Primolius*, *Orthopsittaca,* and *Cyanopsitta* with a posterior support value of 0.99, whereas the most likely ML tree (final mL = –20,277.8826) from five independent GARLI runs placed it sister to a clade containing three *Aratinga* species (*A. nana*, *A. aurea*, and *A. pertinax*). The ML bootstrap value, however, showed poor support for this latter relationship, and relationships at the base of the entire long-tailed parrot clade had generally low nodal support. In both Bayes and ML trees, members of the genus *Aratinga* formed multiple distinct and well-supported clades that

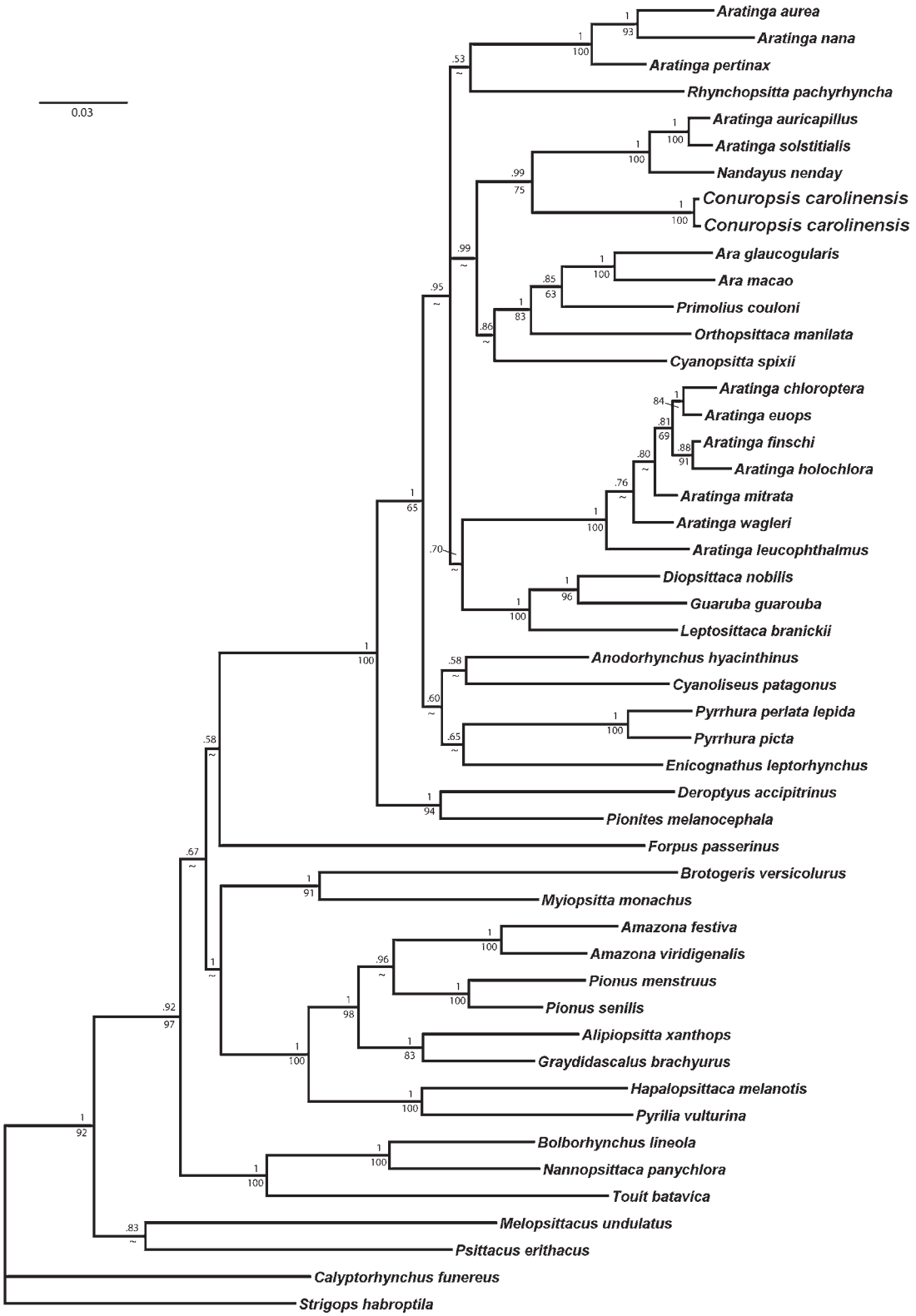

FIG. 1. Phylogram of the relationships among the extinct Carolina Parakeet, 43 extant species of Neotropical parrots, and four Old World parrots, based on a six-partition mixed-model Bayseian analysis of sequences from the mitochondrial COI and ND2 genes. Nodal support values indicate maximum likelihood (ML) bootstraps (below branch) and Bayesian posterior probabilities (above branch). Tildes (~) indicate ML bootstrap support values <50%. Scale bar indicates number of base substitutions per site.

each contained members of other genera (Fig. 1). All analyses also recovered well-supported clades within the Neotropical Arini of (1) short-tailed parrots in the genera *Alipiopsitta*, *Amazona*, *Graydidascalus*, *Hapalopsittaca*, *Pionus*, and *Pyrilia*; (2) parrotlets in the genera *Bolborhynchus*, *Nannopsittaca*, and *Touit*; and (3) parakeets in the genera *Myiopsitta* and *Brotogeris* (Fig. 1).

Discussion

We find robust support for the placement of the extinct Carolina Parakeet within a diverse clade of long-tailed parrots, thus clarifying the evolutionary history of this iconic North American parrot. DNA sequence data from museum specimens of the Carolina Parakeet reveal a strongly supported sister relationship with a clade containing *Nandayus nenday* and two of the 12 sampled species in the genus *Aratinga*, *A. soltitialis* and *A. auricapillus*. As previously hypothesized,[1,2,3,4] *Conuropsis* is closely related to some *Aratinga* species, but it is clear that *Aratinga* itself is highly polyphyletic and in need of revision. Previous analyses have suggested that *Aratinga* is paraphyletic with regard to *Nandayus*[26,27]; our results here suggest that this paraphyly may extend to as many as seven other genera. Ongoing work (by E.E.S.) on a much larger data set of DNA sequences from a comprehensive taxonomic sample of Arini should help resolve relationships in this diverse group.

We find no evidence for the previously hypothesized close relationship with *M. monachus*.[4] That species is native to temperate regions of South America and has established year-round breeding colonies in several large metropolitan areas of North America that experience long, harsh winters.[28] Our data suggest that the shared presence of feathered ceres and other cold tolerances in *Conuropsis* and *Myiopsitta* represent convergent traits in these temperate-dwelling taxa.

By contrast, the bright yellow and orange plumage and blue wing feathers found in *Conuropsis carolinensis* are traits shared by *Aratinga solstitialis* and *A. auricapillus* and a third species, *A. jandaya*, that was not sampled in our study but is generally thought to be closely related to these two *Aratinga* species.[2] These shared similarities suggest that this striking coloration evolved in the shared ancestor of this clade and was partially lost in *Nandayus nenday*, which has blue primaries but lacks any yellow plumage on its black head. It should be noted that one other Neotropical species with bright yellow plumage, *Guaruba guarouba*, did not group closely with this clade in our trees and thus likely evolved its yellow plumage independently. Intriguingly, there are also reports that *A. solstitialis* will roost communally within tree cavities both at night and during the day,[29] as reported for the Carolina Parakeet.[4]

High genetic distances and long branch lengths support the continued recognition of the genus *Conuropsis*. In addition to *C. carolinensis*, the genus includes only one other extinct species, *C. fratercula*, which was described by Alexander Wetmore[30] on the basis of a single complete humerus from the middle Miocene lower–Sheep Creek beds of the Snake Creek Quaries, Sioux County, Nebraska. This humerus is about three-quarters the size of that of *C. carolinensis* (29.5 mm vs. 36.2 mm) but is otherwise so similar that Wetmore considered "that the two would be inseparable were it not for their disparity in size."[30] Regarding *C. fratercula*, Olson did "not regard its generic affinities as having been positively established"[31] but did not question its placement within the Psittacidae.

Historical biogeography.—The Psittaciformes are hypothesized to have arisen in Gondwana in the late Cretaceous.[14,26,32,33] The earliest undisputed fossil psittacines are from several Paleogene sites, including some in Europe.[34] However, to our knowledge, the only reliable fossil representative of this order known from North America is *C. fratercula*—this despite the rich assemblages of avian fossils, including many Neotropical taxa, from the Pliocene and Pleistocene of Florida.[35] The colonization of temperate North America by *Conuropsis* therefore represents a biogeographic puzzle. We find that *C. carolinensis* is more closely related to species with broad distributions in temperate and tropical South America than it is to several species of *Aratinga* currently found in Mexico, Central America, or the Greater Antilles. If Neotropical parrots indeed had their original diversification in South America after colonization from Antarctica, as is generally agreed,[14,33] this result suggests that there have been several distinct colonizations of Central and North America by different lineages.

Future directions.—Our mitochondrial DNA sequences may be useful as "barcodes" against which controversial remains of putative Carolina Parakeets can be evaluated. Of particular interest to historians are two egg sets in the collection of the Florida Museum of Natural History (UF 87234, 3 eggs; UF 89434, 2 eggs) that were collected by Charles Doe on 30 April 1927, several years after the last uncontroversial sightings. McKinley doubted their validity because of their small size and early date of collection,[11] but Snyder believed that they were genuine.[4] Obtaining COI or ND2 sequences using recently established protocols for extraction of DNA from centuries-old egg specimens[36] could establish these sets as the latest specimen records of Carolina Parakeets. Genotyping other putative Carolina Parakeet egg sets and museum specimens with these markers would help resolve lingering mysteries regarding the reproductive ecology and phylogeography of this once widespread but enigmatic species.

Note Added In Proofs

A recent revision of the nomenclature of the parrots[37] inadvertently omitted *Conuropsis* from its list of named genera. Following this new taxonomic classification, *Conuropsis* should be placed in the Superfamily Psittacoidea, Family Psittacidae, Subfamily Arinae, and Tribe Arini with its closest extant relatives in the genera *Nandayus* and *Aratinga*.

Acknowledgments

We are grateful to the following organizations and people for providing samples: New York State Museum, Hobart and William Smith Colleges (M. Deutschlander), Smithsonian National Museum of Natural History (G. Graves and C. Huddleston), American Museum of Natural History (P. Sweet), Field Museum of Natural History (D. Willard), Academy of Natural Sciences of Philadelphia (L. Joseph), Universidade de São Paulo's Laboratório de Genética e Evolução Molecular de Aves (C. Miyaki), Loro Parque Fundácion (D. Waugh, S. Capelli, H. Müller, J. Scharpegge), and New Mexico State University Vertebrate Museum (P. Houde). J. Sanchez and A. Hernandez of the National Institute of Toxicology and Forensic Science, Tenerife, Spain, and T. Matsumoto and C. Miyaki of the Universidade de São Paulo graciously provided space, supplies, and assistance to E.E.S. during some lab work. Funding was provided by the New York State Museum, National Institutes of Health grant S06 GM008136 (T.F.W.), and National Science Foundation grant

IOS-0725032 (T.F.W.). This study was approved by the Institutional Animal Care and Use Committee of New Mexico State University.

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Associate Editor: M. T. Murphy