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PHYLOGENY AND BIOGEOGRAPHY OF THE *AMAZONA OCHROCEPHALA* (AVES: PSITTACIDAE) COMPLEX

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Abstract.—We present a phylogenetic analysis of relationships among members of the *Amazona ochrocephala* species complex of parrots, a broadly distributed group in Middle and South America that has been a "taxonomic headache." Mitochondrial DNA sequence data are used to infer phylogenetic relationships among most of the named subspecies in the complex. Sequence-based phylogenies show that Middle American subspecies included in the analysis are reciprocally monophyletic, but subspecies described for South America do not reflect patterns of genetic variation. Samples from the lower Amazon cluster with samples collected in western Amazonia—not with samples from Colombia and Venezuela, as was predicted by subspecies classification. All subspecies of the complex are more closely related to one another than to other *Amazona* species, and division of the complex into three species (*A. ochrocephala*, *A. auropalliata*, and *A. oratrix*) is not supported by our data. Divergence-date estimates suggest that these parrots arrived in Middle America after the Panama land-bridge formed, and then expanded and diversified rapidly. As in Middle America, diversification of the group in South America occurred during the Pleistocene, possibly driven by changes in distribution of forest habitat. *Received 20 January 2003, accepted 3 December 2003.*

Resumen.—Presentamos un análisis de las relaciones filogenéticas entre miembros del complejo de loros *Amazona ochrocephala*, un grupo ampliamente distribuido en Mesoamérica y Suramérica, y que ha sido un "dolor de cabeza taxonómico." Utilizamos secuencias de ADN mitocondrial para reconstruir la relaciones filogenéticas entre la mayoría de las subespecies nombradas del complejo. Las filogenias basadas en estas secuencias muestran que las subespecies mesoamericanas incluidas en el análisis son recíprocamente monofiléticas, pero las subespecies descritas para Suramérica no reflejan patrones de variación genética. Muestras de la baja Amazonía se agrupan con muestras de la Amazonía occidental, en vez de agruparse con las muestras de Colombia y Venezuela, como se esperaba con base en la clasificación actual de subespecies. Todas las subespecies del complejo están estrechamente relacionadas entre sí, separadas por distancias menores que las distancias entre miembros del complejo y otras especies de *Amazona*, y la división del complejo en tres especies (*A. ochrocephala*, *A. auropalliata*, y *A. oratrix*) no es apoyada por nuestros datos. Las fechas de divergencia estimadas con los datos moleculares sugieren que estos loros llegaron a Mesoamérica después de la formación del istmo de Panamá y luego expandieron su distribución y se diversificaron rápidamente. Como en Mesoamérica, la diversificación del grupo en Suramérica occurió durante el Pleistoceno, posiblemente como resultado de cambios en la distribución de hábitats forestales.

Biogeographical studies of Neotropical birds have been of central importance in development of models aimed at explaining the high species diversity of the Neotropics (e.g. Cracraft 1985, Haffer 1985). An obvious example is the Forest Refuge hypothesis initially outlined by Haffer (1969, 1974), which suggests that isolation of remnant patches of rainforest during dry glacial periods fostered diversification of tropical flora and fauna. Other important vicariant models of speciation that have been supported by studies of the diversification of Neotropical avifauna include the Andean uplift (Chapman 1917, Cracraft and Prum 1988), formation of river systems in the Amazon basin (Wallace 1853, Simpson and Haffer 1978, Capparella 1988), formation of the Panama land bridge (Cracraft 1985, Cracraft and Prum 1988), and marine incursions (Nores 1999).

It is within this rich ornithological tradition that we present results of a phylogenetic analysis of the *Amazona ochrocephala* complex of parrots, a group of biogeographic interest because of its broad Neotropical distribution.

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A historical perspective on relationships among these parrots serves as a baseline from which to study the interaction of history and ecology that has led to their contemporary diversity and distribution. Furthermore, a molecular systematic analysis of the group is of taxonomic interest, because classification of the complex using morphological characters has been a "taxonomic headache" (Howell and Webb 1995). Finally, identification of conservation units, particularly of the Mesoamerican subspecies, is important because their populations have suffered precipitous declines due to habitat loss and the pet trade (Collar et al. 1994).

Study species.—The *A. ochrocephala* complex includes eleven named subspecies that are distributed from Mexico to the Amazon basin (Fig. 1). Characters used to identify the various subspecies include plumage (in particular, extent and position of yellow on head and thighs, and coloration at the bend of the wing), bill and foot pigmentation, and body size (Monroe and Howell 1966, Forshaw 1989, Juniper and Parr 1998). However, those characters can vary significantly, even among individuals from the same locality (Howell and Webb 1995, Lousada and Howell 1996, Juniper and Parr 1998, J. R. Eberhard pers. obs.), in part because of age-related variation (Howell and Webb 1995, Lousada and Howell 1996). Some taxonomists have considered the entire complex to constitute a single species, *A. ochrocephala* (e.g. Monroe and Howell 1966, Forshaw 1989), but others (e.g. Sibley and Monroe 1990, American Ornithologists' Union 1998, Juniper and Parr 1998) divide the complex into three species: the Yellow-crowned Amazon (*A. ochrocephala* [*A. o. ochrocephala*, *A. o. xantholaema*, *A. o. nattereri*, and *A. o. panamensis*]); the Yellow-naped Amazon (*A. auropalliata* [*A. a. auropalliata*, *A. a. parvipes*, and *A. a. caribaea*]); and the Yellow-headed Amazon (*A. oratrix* [*A. o. oratrix, A. o. tresmariae*, *A. o. belizensis*, and *A. o. hondurensis*]). Two other races of *oratrix* are mentioned in the literature: "*magna*," from the Caribbean slope of Mexico, is not considered valid (Juniper and Parr 1998); and "*guatemalensis*" has not been formally described and is included in *belizensis* by Juniper and Parr (1998).

FIG. 1. Distribution of the *Amazona ochrocephala* complex (after Juniper and Parr 1998); taxa sampled for the present study are indicated in bold type. Distribution of *A. aestiva* is outlined with the dashed line. Sample locations are indicated by points, and are numbered to correspond with the sample listing in Table 1.

Parrots of the *ochrocephala* complex are generally found below 750 m (Forshaw 1989, Juniper and Parr 1998), inhabiting deciduous woodland, gallery forest, savannah woodland, dry forest, secondary growth along major rivers, and seasonally flooded forests (Forshaw 1989, Juniper and Parr 1998). In Middle America, most of the subspecies appear to be allopatric, though the biological barriers (if any) that separate the ranges are not obvious (Juniper and Parr 1998). A zone of contact among yellow-headed, yellow-naped, and yellow-crowned forms may occur along the Atlantic slope of Central America from Belize to Nicaragua (Lousada and Howell 1996). Unfortunately, free-flying birds are very rare in that region, and we were unable to secure representative samples for inclusion here. Given currently available information on distribution of *ochrocephala* subspecies in South America, no range discontinuity is known between *A. o. ochrocephala* (eastern Colombia, Venezuela, Trinidad, Guianas, and northern Brazil) and *A. o. nattereri* (southern Colombia, eastern Ecuador and Peru, western Brazil, and northern Bolivia) (Juniper and Parr 1998). The range of *A. o. panamensis* is mostly separated from other yellow-crowned forms by the Andes, though it may be continuous with *A. o. ochrocephala* in northwestern Venezuela (Juniper and Parr 1998).

Four congeneric species were included as outgroups in our phylogenetic analyses: *A. aestiva*, *A. amazonica*, *A. farinosa*, and *A. autumnalis*.

Two of those species, *A. aestiva* and *A. amazonica*, are consistently listed next to the *A. ochrocephala* complex in linear taxonomies (Forshaw 1989, Sibley and Monroe 1990, Juniper and Parr 1998). The other two were included because samples were obtained opportunistically. An additional outgroup species, *A. barbadensis*, was included in analysis of cytochrome oxidase I (COI) sequences. That species was of particular interest because—like members of the *ochrocephala* complex, *A. aestiva*, and *A. amazonica*—it is characterized by yellow plumage on parts of the head.

METHODS

Samples.—Where possible, we used vouchered tissue samples from museum frozen-tissue collections. However, because the *ochrocephala* complex is poorly represented in those collections, many of the samples were obtained from field workers, captive breeding facilities, and pet owners (Table 1). Use of material from captive birds was contingent on availability of information on the sampled individuals' geographic origins. Source material included frozen tissues (muscle, liver), blood, feathers (both emerging and full-grown), and small pieces of museum skins (toe and body skin). Three of the outgroup samples (representing *A. aestiva* and *A. barbadensis*) were from captive birds of unknown origin. Collection locations for the *ochrocephala* samples included here are shown in Figure 1.

Laboratory procedures.—Total cellular DNA extractions from frozen tissue, blood, and feather samples were done by incubating samples overnight in CTAB buffer (Murray and Thompson 1980) and proteinase K, followed by a standard phenol-chloroform extraction and dialysis. Extractions from museum skin samples were done using the Qiamp kit (Qiagen, Valencia, California), following the protocol outlined by Mundy et al. (1997). Three mitochondrial DNA (mtDNA) fragments—the complete ATP synthase 6 and 8 genes (ATPase6,8), a 622-bp portion of COI, and the complete NADH dehydrogenase 2 (ND2) gene—were amplified via the polymerase chain reaction (PCR) for most samples; only COI was analyzed for museum skin samples. In addition, a 694-bp fragment of the cytochrome-*b* (cyt *b*) gene was sequenced for a subset of the samples (see Table 1) selected to include one member of each of the clades identified using the other three coding regions.

To amplify and sequence the ATPase6, ATPase8, and ND2 genes, we used primers originally designed by G. Seutin for studies of Neotropical passerine birds. Primer sequences are given (5′ to 3′) followed by the base position of the primer's 3′ base relative to the domestic chicken's (*Gallus gallus*) mtDNA sequence (Desjardins and Morais 1990); the H or L indicates whether the primer is located on the heavy or light strand, respectively. The primers CO2GQL (GGACAATGCTCAGAAATCTGCGG, L8929) and CO3HMH (CATGGGCTGGGGTCRACTATGTG, H9947) were used to amplify a 1,074-bp fragment that included the full ATPase6 and ATPase8 genes; along with those, the internal A6PWL (CCTGAACCTGACCATGAAC, L9245) was used for sequencing the fragment. For one sample (a molted feather stored at room temperature for about eight years), the ATPase region was amplified in two overlapping pieces, using CO2GQL with A6VALH (AGAATTAGGGCTCATTTGTGRC, H9436), and A6PWL with CO3HMH. The ND2 gene was amplified with primer pairs METB (CGAAAATGATGGTTTAACCCCTTCC, L5233) and TRPC (CGGACTTTAGCAGAAACTAAGAG, H6343), and METB with ND2LSH(GGAGGTAGAAGAATAGGCYTAG, H6102). The COI fragment was amplified using primers COIa and COIf (Palumbi 1996), and the cyt-*b* fragment was amplified and sequenced using primers CB1 and CB3 (Palumbi 1996). Except for those involving museum skins, PCRs were done using AmpliTaq (Perkin-Elmer, Wellesley, Massachusetts) and five cycles with an annealing temperature of 50°C followed by 30 cycles at 56°C.

A set of additional COI primers was designed to amplify and sequence a series of five overlapping fragments ranging in size from 106 to 196 bp. Sequences (5′ to 3′) of those primers, named according to the position of the primer's 5′ end, are as follows: L7506 (TAGGGTTYATCGTATGGGCC), H7523 (ACTGTGAATATGTGGTGGGC), L7628 (GACTCGCCACACTACACGG), H7642 (CTCATTTGATGGTCCCTCCG), L7773 (GTCTCACAGGRATCGTCC), H7813 (GTATGTGTCGTGTAGGGCA), L7804 (AATAGGTGCCGTCTTTGCC), and H7879 (GAATAGGGGGAATCAGTGGG).

That primer set was used in conjunction with primers COIa and COIf to amplify and sequence DNA extracted from museum skin samples. Polymerase chain reaction amplifications of museum skin extracts were done using AmpliTaq Gold (Perkin-Elmer) in 25-μl reactions and 40 cycles with an annealing temperature of 60°C. Those reactions were set up in a UV hood to avoid contamination.

Amplification products were visualized in agarose gels, and then cleaned and purified using GELase (Epicentre Technologies, Madison, Wisconsin) following the manufacturer's protocol. PCR fragments were then sequenced using either Dyedeoxy or dRhodamine (Applied Biosystems, Foster City, California; Perkin-Elmer) cycle sequencing reactions and an ABI 377 automated sequencer. Amplification primers were used for sequencing both the heavy and light strands of PCR fragments, and an additional internal primer, A6PWL, was used to sequence the ATPase region.

Three samples—two *A. o. ochrocephala* samples from Venezuela and the *A. barbadensis* sample—were sequenced by M. Rusello (Columbia University, New

TABLE 1. List of *Amazona* parrot samples sequenced in the present study. The numbers in the first column correspond to numbered locations on the map. Sample numbers correspond to those shown on the phylogenetic trees. Museum or collection abbreviations are as follows: AMNH (American Museum of Natural History), ANSP (Philadelphia Academy of Natural Sciences), LSU (Louisiana State University Museum of Natural Sciences), NMNH (U.S. National Museum of Natural History), and STRI (Smithsonian Tropical Research Institute Molecular Labs). Tissue or skin numbers listed are accession numbers assigned to each sample or specimen by the corresponding museum or collection. Sample types listed include: tissue (frozen muscle or liver), feather (both full-grown and pin-feathers were used), skin (taken from either the foot or body of a museum study skin), and blood (preserved in a lysis buffer). The following abbreviations are used to indicate the regions sequenced: A6 (ATPase 6 gene), A8 (ATPase8 gene), COI (COI fragment), ND2 (ND2 gene), cytb (cytochrome *b*), G (Gapdh). An asterisk (*) indicates that the sample was included in the PAUP* maximum-likelihood analyses. Numbers along the left margin refer to map locations in Figure 1.

	Species	Sample name	Museum or collection	Tissue or skin number	Locality	Sample type	Regions sequenced
1	*A. o. ochrocephala*	ochro1	NMNH	B06867	Brazil: Para; Altamira, 52 km SSW, east bank Rio Xingu	Tissue	A6,A8,COI,ND2,cytb,G *
1	*A. o. ochrocephala*	ochro2	NMNH	B07034	Brazil: Para; Altamira, 52 km SSW, east bank Rio Xingu	Tissue	A6,A8,COI,ND2
2	*A. o. ochrocephala*	ochro3	STRI	stri-x-61	Colombia: Depto. Meta; Carimagua	Feather	A6,A8,COI,ND2,cytb *
3	*A. o. ochrocephala*	ochro4	ANSP	168178	Colombia: Depto. Meta; La Macarena	Skin	COI
4	*A. o. ochrocephala*	ochro5	AMNH	177109	Venezuela: Caicara; R bank of Rio Orinoco, 290 km west of Ciudad Bolivar	Skin	COI
5	*A. o. ochrocephala*	ochro6	AMNH	437237	Venezuela: Maipures; along rapids on middle of Rio Orinoco, 50 km southwest of Puerto Ayacucho	Skin	COI
6	*A. o. nattereri*	nater1	LSU	B-9409	Bolivia: Pando; Nicolas Suarez, 12k by road S of Cobija, 8 km west on road to Mucden	Tissue	A6,A8,COI,ND2 *
7	*A. o. nattereri*	nater2	LSU	B-12973	Bolivia: Santa Cruz; Velasco, west bank Rio Paucerna, 4 km upstream from Rio Itenez	Tissue	A6,A8,COI,ND2 *
8	*A. o. nattereri*	nater3	LSU	B-25220	Bolivia: Beni; 6 km southeast of Trinidad	Tissue	A6,A8,COI,ND2
9	*A. o. xantholaema*	xanth1	STRI	LP1	Loro Parque, band# NB-91-4ESY3 [a]	Blood	A6,A8,COI,ND2,cytb *
10	*A. o. panamensis*	pana1	STRI	stri-x-26	Panama: Coclé; El Coco, south of Penonomé	Blood	A6,A8,COI,ND2,cytb *
11	*A. o. panamensis*	pana2	STRI	stri-x-27	Panama: Chiriquí; La Zeledonia	Blood	A6,A8,COI,ND2,G *
11	*A. o. panamensis*	pana3	STRI	stri-x-30	Panama: Chiriquí; San Lorenzo, Playa Nanzal	Blood	A6,A8,COI,ND2
11	*A. o. panamensis*	pana4	STRI	stri-x-34	Panama: Chiriquí; Pedregal	Blood	A6,A8,COI,ND2
12	*A. o. auropalliata*	auro1	STRI	stri-x-98	Costa Rica: Guanacaste; Finca Tempisquito, 4 km southeast of entrance to Parque Nac. Santa Rosa	Feather	A6,A8,COI,ND2,G *
13	*A. o. auropalliata*	auro2	STRI	stri-x-56	Mexico: Chiapas; Marqués de Comillas	Feather	A6,A8,COI,ND2,cytb *
14	*A. o. oratrix*	orat1	STRI	stri-x-44	Mexico: Tamaulipas; Los Colorados	Blood	A6,A8,COI,ND2
14	*A. o. oratrix*	orat2	STRI	stri-x-45	Mexico: Tamaulipas; Los Colorados	Blood	A6,A8,COI,ND2
14	*A. o. oratrix*	orat3	STRI	stri-x-46	Mexico: Tamaulipas; Los Colorados	Blood	A6,A8,COI,ND2
14	*A. o. oratrix*	orat4	STRI	stri-x-47	Mexico: Tamaulipas; Los Colorados	Blood	A6,A8,COI,ND2 *
14	*A. o. oratrix*	orat5	STRI	stri-x-48	Mexico: Tamaulipas; Los Colorados	Blood	A6,A8,COI,ND2,cytb *
15	*A. o. oratrix*	orat6	STRI	stri-x-54	Mexico: Veracruz; Tempoal	Feather	A6,A8,COI,ND2
15	*A. o. oratrix*	orat7	STRI	stri-x-55	Mexico: Veracruz; Tempoal	Feather	A6,A8,COI,ND2

Table 1. Continued.

	Species	Sample name	Museum or collection	Tissue or skin number	Locality	Sample type	Regions sequenced
16	*A. o. belizensis*	beliz1	STRI	stri-x-37	Belize Zoo, band # 90185 [b]	Feather	A6,A8,COI,ND2
16	*A. o. belizensis*	beliz2	STRI	stri-x-38	Belize Zoo, band # 90144	Feather	A6,A8,COI,ND2,cytb,G *
16	*A. o. belizensis*	beliz3	STRI	stri-x-39	Belize Zoo, band # 90118	Feather	A6,A8,COI,ND2 *
16	*A. o. belizensis*	beliz4	STRI	stri-x-41	Belize Zoo, band # 90197	Feather	A6,A8,COI,ND2
17	*A. o. tresmariae*	tres1	STRI	stri-x-49	Mexico: Nayarit; Isla María Madre [c]	Blood	A6,A8,COI,ND2
17	*A. o. tresmariae*	tres2	STRI	stri-x-50	Mexico: Nayarit; Isla María Madre [c]	Blood	A6,A8,COI,ND2 *
17	*A. o. tresmariae*	tres3	STRI	stri-x-51	Mexico: Nayarit; Isla María Madre [c]	Blood	A6,A8,COI,ND2,cytb *
17	*A. o. tresmariae*	tres4	STRI	stri-x-52	Mexico: Nayarit; Isla María Madre [c]	Blood	A6,A8,COI,ND2
17	*A. o. tresmariae*	tres5	STRI	stri-x-53	Mexico: Nayarit; Isla María Madre [c]	Blood	A6,A8,COI,ND2
	A. aestiva	A.aest1	STRI	stri-x-97	Unknown (captive bird)	Feather	A6,A8,COI,ND2,cytb
	A. aestiva	A.aest2	NMNH	B06584	Unknown (captive bird)	Tissue	A6,A8,COI,ND2
	A. aestiva	A.aest3	STRI	stri-x-173	Unknown (captive bird), sample taken at UGA vet school; bird's ID# BLFR (78-73)	Blood	G
	A. amazonica		ANSP	3307	Ecuador: Sucumbios; Imuya Cocha	Tissue	A6,A8,COI,ND2,cytb,G *
	A. autumnalis		STRI	stri-x-42	Mexico: Tamaulipas; Los Colorados	Blood	A6,A8,COI,ND2,G *
	A. barbadensis				Unknown (captive bird, Wildlife Survival Center on St. Catherine's Island, Georgia)	Feather	COI
	A. farinosa		STRI	stri-x-21	Unknown (captive bird from Chiriquí, Panama)	Blood	A6,A8,COI,ND2,G *
	A. viridigenalis		STRI	stri-x-43	Mexico: Tamaulipas; Los Colorados	Blood	G

[a]Sample taken from captive bird at Loro Parque, Canary Islands; parents were wild-caught birds obtained in 1984 by R. van Dieten on western Marajó Island, Brazil (R. van Dieten pers. comm.)

[b]All of the *belizensis* samples from the Belize Zoo birds are from wild-caught birds, but no data are available on their capture locations. One sample (band #90185) consistently clustered with the *oratrix* samples, and is presumed to be a misidentified bird.

[c]The *tresmariae* samples are from birds taken from Isla María Madre in 1997 for a captive-breeding program at Fundación ARA, Monterrey, Mexico. The birds were donated to ARA by convicts at the prison on María Madre, and had originally been captured at Balleto, Campamento Rehilete, and Campamento Aserradero, all on Isla María Madre.

York), using museum skin samples from the American Museum of Natural History. Those sequences were obtained using the primer set listed above.

To obtain an independent molecule-based estimate of the relationship between *A. aestiva* and the *ochrocephala* complex (see below), a nuclear intron fragment from the glyceraldehyde-3-phosphate dehydrogenase (Gapdh) gene was sequenced for a subset of samples. The nuclear sequence for *A. aestiva* was obtained using a DNA extract taken by P. Wainright from a blood sample (A.aest3; attempts to obtain nuclear sequences using extracts of A.aest1 and A.aest2 were unsuccessful). Primers GapdL890 and GapdH950 (Friesen et al. 1997) were used for amplification and sequencing of the nuclear fragment. Polymerase chain reactions were done using AmpliTaq or TaqGold (Perkin-Elmer), beginning with 5 min at 94°C, and then five cycles with an annealing temperature of 50°C followed by 30 cycles at 56°C. In some cases, the initial PCR product had to be re-amplified (30 cycles at 56°C) prior to sequencing.

All sequences have been deposited in GenBank, under accession numbers AY194295–AY194327 (ATPase6), AY194328–AY194360 (ATPase8), AY194367–AY194403 (COI), AY194434–AY194466 (ND2), AY194404–AY194413 (cyt *b*), and AY194425–AY194433 (Gapdh).

Sequence analysis.—Sequences generated by the automated sequencer were aligned and proofread using SEQUENCHER (version 3.1.1; GeneCodes, Ann Arbor, Michigan). The ATPase6,8, COI, and ND2 sequences were then concatenated for most subsequent phylogenetic analyses, which were done using PAUP* (version 4.0b8; Swofford 1999). Sequences were combined because the mitochondrial gene regions are fully linked and thus represent a single phylogenetic marker (a partition-homogeneity test showed that the gene regions were not significantly heterogeneous [$P > 0.50$]). The PAUP* and SEQUENCER 5.0 programs (see Acknowledgments) were used to calculate descriptive statistics about nucleotide variation. Analyses that included the museum skin specimens were based on COI sequences, because we attempted amplifications of that gene region only from the DNA extracted from skin samples. The cyt-*b* sequences, which were obtained for a subset of the samples, were used only for estimates of divergence dates (see below).

Phylogenies were reconstructed using neighbor-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) algorithms in PAUP*, and a Bayesian approach as implemented in MRBAYES (Huelsenbeck and Ronquist 2001). Outgroup rooting was used to root trees. The MP and NJ analyses were done with all characters weighted equally; MP searches were also done with weights of 1 and 18 assigned to transitions (Ti) and transversions (Tv), respectively. For analysis of COI sequences, the Ti:Tv weighting was 1:17. Those weightings reflect the Ti:Tv ratios determined empirically from the data. Parsimony trees were found using heuristic searches and random branch addition. Neighbor-joining trees were obtained using Tamura-Nei distances (Tamura and Nei 1993).

Substitution model parameters for ML analyses in PAUP* were found using MODELTEST 3.1 (Posada and Crandall 1998), which uses hierarchical likelihood-ratio tests to compare the fit of different nested models of DNA substitution to the data matrix. For the ATPase+COI+ND2 data set, MODELTEST supported the Tamura-Nei model with I = 0.7666 and equal rates at all variable sites. To reduce computing time, the ML analyses of ATPase+COI+ND2 in PAUP* were done using a reduced data set of 18 taxa (see Table 1) that included representatives of all major clades identified by MP and NJ analyses. For the COI data set, the best fit found by MODELTEST was an HKY model with a Ti:Tv ratio of 30.9904, the proportion of invariable sites set to 0.6426, and a gamma shape parameter of 0.4975. Those parameters were specified in PAUP* for heuristic ML tree searches and bootstrapping analyses.

For the Bayesian Markov chain Monte Carlo (MCMC) searches, a general time-reversible model was specified, with site-specific variation partitioned by codon position. Four chains were run for 500,000 generations and sampled every 1,000 generations. In the ATPase+COI+ND2 analysis, because stationarity was reached by 15,000 generations, the first 20,000 generations were discarded, and the remaining trees were used to obtain a majority-rule consensus. For analysis of the COI data set, trees from the first 35,000 generations were discarded prior to generating the consensus tree.

Nodal support was assessed by bootstrap analysis in the MP, NJ, and ML analyses (1,000, 1,000, and 125 replicates, respectively), and by posterior probabilities in the Bayesian analyses. Posterior probabilities indicate percentage of the time that a given clade occurs among trees sampled in the Bayesian analyses (Huelsenbeck and Ronquist 2001).

Divergence times among clades in the *ochrocephala* complex were estimated using two different molecular clock calibrations (no specific calibration exists for the Psittaciformes). A 2% sequence divergence per million years (my) calibration (based on restriction-site variation across the mitochondrial genome; see Shields and Wilson 1987, Tarr and Fleischer 1993) was used with the ATPase+COI+ND2 data set. Another set of divergence time estimates was calculated using the parrot cyt-*b* data and a fossil-based molecular clock calibration for cyt *b* in cranes (Krajewski and King 1996). That second calibration uses cyt-*b* maximum-likelihood distances (as calculated using the DNADIST program in PHYLIP; Felsenstein 1995), which in cranes diverge by 0.7%–1.7% my^{-1}. For both of those data sets, the assumption of clock-like sequence change was first tested by using a likelihood ratio test (LRT; Felsenstein 1988) to compare likelihood scores of ML

trees found by heuristic searches in PAUP* with a molecular clock enforced versus not enforced. The LRTs for both the ATPase+COI+ND2 and the cyt-*b* data sets showed no statistical difference between trees found with or without a clock enforced ($P = 0.2286$ and $P = 0.2032$, respectively).

To determine whether our data support division of the *ochrocephala* complex into three species, alternative tree topologies were compared using the nonparametric Shimodaira-Hasegawa (S-H) test (Shimodaira and Hasegawa 1999) in PAUP*, using RELL bootstrapping. As pointed out by Goldman et al. (2000), that test is applicable when one of the trees being compared is one selected with reference to the same data being used in the test. Using both the ATPase+COI+ND2 and the COI data sets, we compared the Bayesian tree with a test tree composed of three clades: an *oratrix* clade (*oratrix* + *belizensis* + *tresmariae*), an *auropalliata* clade, and an *ochrocephala* clade (*panamensis* + *ochrocephala* + *nattereri* + *xantholaema*). Within each of those clades, relationships among subspecies were not resolved; the A.ae1 and beliz1 samples were omitted from the analysis (see below). The S-H test was also used to compare the Gapdh tree obtained in the parsimony analysis with a tree in which the *A. aestiva* sample is forced within the *ochrocephala* clade. For that test, we used likelihood parameters suggested by MODELTEST (Posada and Crandall 1998) for the Gapdh data (F81, with equal rates for all sites and no invariable sites).

RESULTS

A total of 2,515 bp of coding sequence (ATPase6, 684 bp; ATPase8, 168 bp; COI, 622 bp; and ND2, 1,041 bp) was obtained for each parrot individual, except museum skin specimens, for which only the COI fragment was sequenced. We also sequenced a 694-bp fragment of cyt *b* for 10 of the parrots (see Table 1). Overlap between sequences generated using heavy- and light-strand primers averaged approximately 72% for the ATPase coding region, 86% for the COI fragment, 58% for the ND2 gene, and 90% for the cyt-*b* fragment; no nucleotide differences were found between overlapping complementary sequences.

No indels or stop codons were observed, as expected for protein-coding mitochondrial regions. In the ATPase+COI+ND2 data set, 378 (15.0%) base positions are variable and 205 (8.2%) are parsimony-informative. Sequence variability differed across codons (Table 2). Third-position changes are the most common (49.5% of variable sites), whereas 38.9% of changes occur at first position, and 11.6% at the second codon position. Transitions outnumber transversions by 18.6 to 1, averaging over all pairwise comparisons. In the COI data set, which includes the additional skin samples, 95 (15.3%) characters are variable, and 49 (7.9%) are parsimony informative. In that region, the vast majority (92.6%) of changes occur at third position, and the remainder occur at first position. All base changes in COI are synonymous. In the cyt-*b* fragment, most changes (83.5%) occur at third position, with 15.2% at first position, and the remaining 1.3% at second position.

TABLE 2. Nucleotide variability at different codon positions in the ATPase6, ATPase8, COI, and ND2 genes of *Amazona*. Both ingroup and outgroup species were included in the calculations.

Region	Codon position	Total bp	Number variable	Percentage variable
ATPase6	all	684	100	14.6
	1	228	25	11.0
	2	228	7	3.1
	3	228	68	29.9
ATPase8	all	168	29	17.3
	1	56	7	12.5
	2	56	5	8.9
	3	56	17	30.4
COI	all	622	95	15.3
	1	208	7	7.4
	2	207	0	0.0
	3	207	88	92.6
ND2	all	1041	167	16.0
	1	347	32	9.2
	2	347	25	7.2
	3	347	110	31.7

According to the phylogenies generated by all of the tree-reconstruction algorithms using the ATPase+COI+ND2 sequences, the *ochrocephala* complex forms a well-supported clade (bootstrap values of 100% in MP, NJ, and ML analyses, and 100% posterior probability value in the Bayesian tree). Topology of the Bayesian tree (Fig. 2) is nearly identical to that of the trees found by PAUP* using parsimony, distance, and maximum-likelihood algorithms. The MP and NJ trees differ only slightly in the arrangement of the South American clade that includes samples A.aest1, ochro1, ochro2, and nater3. The Middle American subspecies (*oratrix*, *tresmariae*, *belizensis*, *auropalliata*, and *panamensis*) consistently form reciprocally monophyletic clades that are strongly supported by bootstrap analysis, with bootstrap values ≥99% in the MP

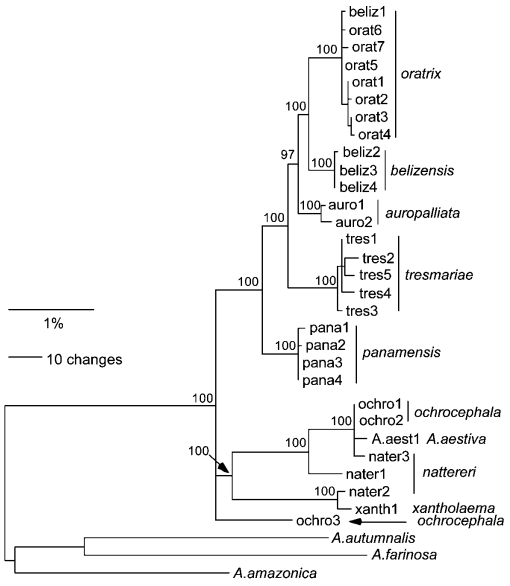

FIG. 2. Phylogram of the *Amazona ochrocephala* complex obtained in a Bayesian analysis of the mitochondrial ATPase6,8, COI, and ND2 sequences (2,514 bp) obtained in the present study. The tree represents a 50% majority-rule consensus of 479 trees generated by MRBAYES during an MCMC search (see text), and numbers at nodes are posterior probabilities. The tree was rooted using *A. amazonica*, *A. autumnalis*, and *A. farinosa* as outgroups. Current subspecies groupings (e.g. Forshaw 1989, Juniper and Parr 1998) are indicated to the right of sample names.

and NJ trees, and ≥95% in the ML tree, and with posterior probability values of 100% in the Bayesian analysis. Down-weighting transitions in the MP and NJ analyses of the complete data set resulted in trees with almost no resolution within the *ochrocephala* complex clade. Transversion weighting of the COI data set also resulted in trees with decreased resolution, but no results were in conflict with tree topologies found using the equally weighted data. A single *belizensis* sample (beliz1) consistently clustered with the *oratrix* samples, but because the *belizensis* samples were from wild-caught captive birds for which exact location data were not available, and because morphological differences used to distinguish *oratrix* and *belizensis* are fairly subtle, we hesitate to overinterpret that result, assuming instead that the bird was misidentified.

The samples representing named subspecies from South America do not form clades (see Fig. 2). Individuals representing *nattereri* (three localities in Bolivia), *xantholaema* (Ilha de Marajó, at the mouth of the Amazon River), and *ochrocephala* (Xingu River, Brazil) intermingle to form a well-supported clade that does not include the remaining *ochrocephala* sample, which is from Colombia. The Colombian *ochrocephala* sample falls at the base of the complex and is separated from other South American samples by almost 2% (uncorrected) sequence divergence. The separation of the Colombian *ochrocephala* sample from the other South American samples was further investigated by sequencing the COI fragment for three additional samples taken from museum skins: one from a second locality in Colombia, and two others from different localities in Venezuela (see Table 1 and Fig. 1). The COI sequence was also obtained for an *A. barbadensis* sample. That was done in response to placement of *A. aestiva* within the *ochrocephala* complex (see below). Like *A. aestiva*, *A. barbadensis* has yellow plumage on the head that is similar in hue and extent to that found in members of the *ochrocephala* complex.

Phylogenetic analysis of the COI sequences, which includes the additional samples from Colombia and Venezuela, again demonstrates a clear phylogenetic separation of *ochrocephala* samples from northern South America versus those from central South America (Fig. 3). The Colombian and Venezuelan samples form an mtDNA clade that is sister to the remaining South American individuals from Brazil and Bolivia, with moderate (79%) support in the Bayesian analysis (Fig. 3). Bootstrap values for the same node were 88% in an NJ tree, 67% in an MP tree, and 64% in an ML tree (trees not shown). Overall, the COI tree (Fig. 3) is not as well resolved as one based on the full mitochondrial data set (Fig. 2), presumably because the COI data set is much smaller. Together, the two trees indicate that parrots from the Amazon, northern South America, and Mesoamerica form a polytomy uniting three lineages of equivalent evolutionary distinctiveness. The COI data also show that *A. barbadensis* is a distinct species, and not particularly closely related to the *ochrocephala* complex.

The unexpected placement of *A. aestiva* within the *ochrocephala* complex was confirmed using a second extraction from a different feather of the same bird, and using a second sample (A.aest2) obtained from the NMNH (the

COI sequences of A.aest1 and A.aest2 differed by a single base substitution). We are confident that those results are not due to species misidentification, because *A. aestiva* can be unambiguously distinguished from *A. ochrocephala* by the presence of blue plumage on the forehead (species identity of A.aest1 was confirmed with photographs, A.aest2 was identified by NMNH personnel, and A.aest3 was identified by staff at the University of Georgia Veterinary School). The relationship between *A. aestiva* and the *ochrocephala* group was explored further using the Gapdh nuclear sequences. A total of 404 bp were sequenced for an *A. aestiva* sample, four members of the *ochrocephala* complex (representing *belizensis*, *panamensis*, *ochrocephala*, and *auropalliata*), as well as four other *Amazona* species (*A. farinosa*, *A. amazonica*, *A. viridigenalis*, and *A. autumnalis*). The Gapdh sequences were easily aligned, with only a single one-nucleotide indel shared by the *A. autumnalis* and *A. viridigenalis* samples. Of the 404 bp in the Gapdh data set, 28 are variable and only two are parsimony-informative; 2 bp changes separate *Amazona aestiva* and the *ochrocephala* complex. Parsimony and distance-based analyses of the nuclear data produced trees with consistent topologies in which *A. aestiva* falls outside of the *ochrocephala* clade (parsimony tree shown in Fig. 4), but an S-H test shows that those topologies are not significantly different from one in which *A. aestiva* is forced within the *ochrocephala* clade ($P = 0.15$).

Short internode distances uniting Middle American subspecies indicate a rapid geographic expansion across Mesoamerica and recent diversification. The genetic distances (uncorrected *p* distance, calculated using the ATPase+COI+ND2 data set) between individuals of different named Mesoamerican subspecies in the *ochrocephala* complex are small, ranging from 0.007 (*belizensis* vs. *auropalliata*) to 0.016

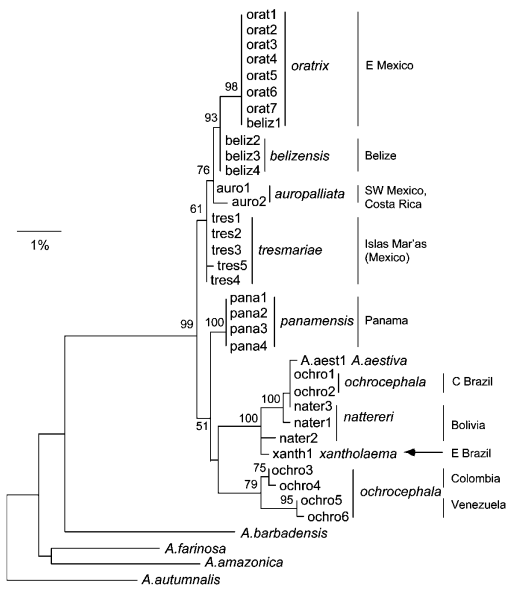

FIG. 3. Phylogram of the *Amazona ochrocephala* complex obtained in a Bayesian analysis of the COI dataset (622 bp). The tree represents a 50% majority-rule consensus of 464 trees generated by MRBAYES during an MCMC search (see text), and numbers at nodes are posterior probabilities. The tree was rooted using *A. amazonica*, *A. autumnalis*, *A. barbadensis*, and *A. farinosa* as outgroups. Current subspecies groupings (e.g. Forshaw 1989, Juniper and Parr 1998) and geographic origin of samples are indicated to the right of sample names.

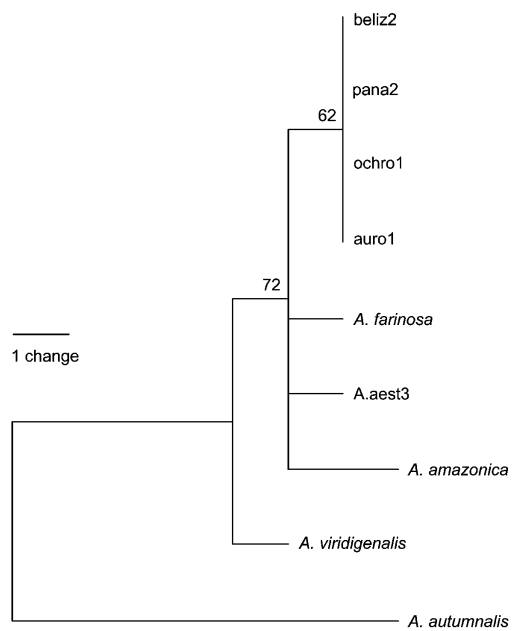

FIG. 4. Phylogeny showing the relationship between four members of the *Amazona ochrocephala* complex and several *Amazona* species based on nuclear Gapdh sequences (404 bp). The tree shown is one of the two most parsimonious trees found in an exhaustive maximum parsimony search by PAUP*, and is identical to the strict consensus of the two most parsimonious trees. The phylogeny was rooted using *A. autumnalis* as the outgroup; the same topology is obtained using midpoint rooting. Numbers at the nodes indicate bootstrap values obtained in 1,000 bootstrap replicates.

(*panamensis* vs. *tresmariae*). Genetic distances between Middle American and South American *ochrocephala* subspecies average 0.014, whereas distances between members of the *ochrocephala* group and outgroup species range from 0.059 (*A. amazonica* vs. *nattereri*) to 0.076 (*A. farinosa* vs. *nattereri*). Assuming that *Amazona* mtDNA's rate of sequence divergence is approximately 2% Ma^{-1}, as in a variety of other birds, such as geese (Shields and Wilson 1987) and honeycreepers (Tarr and Fleischer 1993), the above distances indicate that lineages sampled within the *ochrocephala* complex shared a common ancestor 1.2 million years ago (mya).

Maximum-likelihood distances calculated using DNADIST and the cyt-*b* data range from 0.003 (*oratrix* vs. *auropalliata*) to 0.039 (*tresmariae* vs. *xantholaema*) within the *ochrocephala* complex, whereas distances to *A. amazonica* range from 0.088 (*A. amazonica* vs. *belizensis*) to 0.100 (*A. amazonica* vs. *tresmariae*). According to the crane calibration of Krajewski and King (1996), the cyt-*b* ML distances among taxa in the *ochrocephala* complex suggest that diversification of the group occurred during the past 0.2–5.6 Ma. That interval is broad, but consistent with calculations based on the ATPase+COI+ND2 data; taken together, estimates indicate that the *ochrocephala* complex diversified recently, probably within the past 2 Ma.

The ATPase+COI+ND2 and COI data sets strongly support reclassification of the South American *ochrocephala* subspecies. South American *ochrocephala* parrots separate along geographic rather than currently described subspecies lines. The mean genetic distance between the northern and central South American birds that we have examined is 0.02 (uncorrected *p* distance), indicating a split 1.0 mya under the 2% divergence per my calibration (the mean cyt-*b* ML distance is 0.026, yielding an estimate of 1.5–3.7 mya, with the cyt *b* 0.7%–1.7% Ma^{-1} calibration). Results of the comparison of alternative tree topologies do not support division of the *ochrocephala* complex into three species. According to the S-H test, the three-species tree is rejected at the $P < 0.001$ level when compared to the log-likelihood of the Bayesian tree (Fig. 2).

Discussion

Molecular systematics of the ochrocephala *complex*.—The molecular data presented here provide an informative counterpoint to the morphological characters that have previously been used to classify members of the *ochrocephala* parrot complex. Consistent with taxonomic arrangements that group all of the subspecies under a single species name (e.g. Forshaw 1989), sequence data indicate that members of the complex are very closely related, and much more closely related to one another than to other surveyed *Amazona* species. Molecular phylogenetic analysis supports the monophyly of named subspecies from Middle America (*oratrix*, *tresmariae*, *belizensis*, *auropalliata,* and *panamensis*), but not of the South American ones (*ochrocephala*, *nattereri*, *xantholaema*). We note that our analysis did not include samples from the Caribbean slope of Honduras and Nicaragua, where there may be a contact zone between several different subspecies (Lousada and Howell 1996).

Subdivision of the *ochrocephala* complex into three species is not supported by phylogenetic analysis of parrot mtDNA genes. In the comparison of alternative tree topologies, the ATPase+COI+ND2 and COI data sets rejected the three-species topology that reflects division of the complex into *A. ochrocephala*, *A. auropalliata*, and *A. oratrix*. Although plumage characters support subdivision of the *ochrocephala* complex into three species, plumage patterns are quite variable and appear to be very labile. For example, an examination of museum skins at the American Museum of Natural History by J.R.E. found an *ochrocephala* specimen (AMNH 133032) with a yellow feather on its nape, and a *nattereri* skin (AMNH 255153) with a yellow feather on its throat; in both of those subspecies, yellow feathers are typically confined to the forehead and crown (Forshaw 1989, Juniper and Parr 1998). Similarly, Monroe and Howell (1966) note a well-documented instance of an individual captive parrot that had a yellow-crowned plumage pattern for 10 years, and afterward developed a yellow nape in addition to the crown.

Under the phylogenetic species concept (Cracraft 1983), the mtDNA sequence data would support elevating the Middle American subspecies included here to species status, and regrouping the South American taxa into two species, *A. ochrocephala* (*ochrocephala* from northern South America) and *A. nattereri* (*ochrocephala* from Amazonia, plus the currently recognized

nattereri and *xantholaema*), representing the mtDNA clades from northern and central South America. Alternatively, the data support recognition of a single monophyletic species, *A. ochrocephala* (with South American subspecies revised as described here), which conforms to the opinions of Monroe and Howell (1966) and Forshaw (1989).

Because of the low levels of nuclear sequence divergence among members of the *ochrocephala* complex, reciprocal monophyly of the mtDNA lineages that form the Middle American subspecies included here is not sufficient to conclusively identify them as evolutionarily significant units (ESUs), following criteria proposed by Moritz (1994). However, the morphological differences (e.g. yellow nape and lack of red at the bend of the wing in *auropalliata*, or extensive yellow on the head in *oratrix* and *tresmariae*) probably reflect meaningful divergences at nuclear loci, even if such variation alone is not diagnostic for some of the subspecies. Regardless of the taxonomic nomenclature adopted for the *ochrocephala* complex, the combination of mtDNA data and plumage variation among the Mesoamerican members indicates that each subspecies should be considered distinct units for conservation purposes.

Analyses of the mitochondrial and nuclear sequence data produce conflicting results with respect to the position of *A. aestiva* relative to the *ochrocephala* complex. The Gapdh phylogeny (Fig. 4), in which *A. aestiva* falls outside of the *ochrocephala* clade, is in agreement with the morphological characters that can be used to distinguish the two. However, low levels of variation in the Gapdh sequence data make it impossible to conclusively reject the hypothesis supported by mtDNA analysis, which places *A. aestiva* within the *ochrocephala* complex. Agreement between the Gapdh analysis and the morphological distinctiveness of *A. aestiva* suggests to us that the mtDNA data do not accurately reflect *A. aestiva*'s phylogenetic history, but additional nuclear sequence data are necessary to resolve that issue. Our mtDNA data placing *A. aestiva* within the *ochrocephala* clade are consistent with those of Rusello and Amato (2004), who used both mitochondrial and nuclear sequences in a phylogenetic analysis of *Amazona*. However, they also found *A. barbadensis* to fall within the *ochrocephala* complex, whereas we did not. When Rusello and Amato's (2004) nuclear data were analyzed separately, they recovered a "yellow-headed" clade, which presumably included *A. aestiva*, though a tree is not shown. The distributions of *A. ochrocephala* and *A. aestiva* are largely separate, although there is some overlap in their ranges (see Fig. 1). That region of overlap could permit hybridization between *A. ochrocephala* and *A. aestiva*, possibly leading to differential mtDNA introgression, which could explain the mitochondrial sequence data. Such introgression has been reported in studies of a range of animal taxa (Ferris et al. 1983, Powell 1983, Tegelstrom 1987, Dowling et al. 1989, Lehman et al. 1991, Boyce et al. 1994, Quesada et al. 1995, Rohwer et al. 2001). Although no hybridization between those species has been noted in the wild, *Amazona* species are known to hybridize in captivity (Nichols 1980). Another possible explanation is that mitochondrial primers preferentially amplified an ancestral mitochondrial pseudogene "frozen" in the nuclear genome of the *A. aestiva* samples. However, that does not seem likely, given the size of the pseudogene—or the repeated nature of translocation—required to explain the coincident pattern observed in the four different mitochondrial gene regions.

Biogeography of the ochrocephala *complex*.—Recent work by Rusello and Amato (2004) indicates that the *ochrocephala* complex arose from a South American ancestor, and our phylogenetic hypothesis (Fig. 2) is consistent with their analysis. Two of the three principal *ochrocephala* mtDNA clades are confined to South America, and the level of intraclade mtDNA divergence observed in the Amazonian lineages (Brazilian and Bolivian parrots) is greater than the genetic distances observed between individuals representing the relatively large and taxonomically diverse sample of Mesoamerican parrots. Failure to reject a molecular clock prompted our application of available avian mtDNA clock calibrations, which indicated that the three principal *ochrocephala* mtDNA clades formed contemporaneously in the Pliocene or early Pleistocene.

The short genetic distances among Middle American subspecies, and the close relationship between the Middle and South American lineages, suggests that Middle America was colonized by *ochrocephala* parrots well after the rise of the isthmus of Panama 3.5 mya (Coates 1997). Both the short internodes and the short terminal

branch lengths of Middle American subspecies indicate that the area was colonized relatively quickly and recently. A similar pattern of rapid expansion across the Mesoamerican landscape, followed by *in situ* phylogenetic diversification, has been demonstrated for freshwater fish (Bermingham and Martin 1998, Perdices et al. 2002, G. Reeves and E. Bermingham unpubl. data) and howler monkeys (Cortés-Ortiz et al. 2003). The branching order of the tree in Figure 2 is consistent with a south-to-north stepping-stone pattern of colonization for Middle America, a dispersal pattern that has been recognized in a number of organisms (see Savage 1982).

The allopatry of Middle American subspecies, and their diversification, may be attributable to habitat preferences. These parrots are lowland birds, generally found in relatively dry or deciduous forests, forest edges and gallery forest, and savannahs; in South America, they appear to avoid continuous moist forest, perhaps being replaced by *A. amazonica* in those habitats (Juniper and Parr 1998). Expansion of *ochrocephala* parrots across Middle America may have occurred during glacial periods of the Pleistocene, when dry forest and savannah vegetation were probably more continuous over much of the region (Colinvaux 1997). Subsequent warmer and wetter periods would have permitted an extension of wet forests (Colinvaux 1997), possibly leading to fragmentation of the drier habitat preferred by parrots of the *ochrocephala* complex. In addition, G. Reeves and E. Bermingham (unpubl. data) have produced a model suggesting that phylogenetic breaks between lineages can be maintained in the absence of discrete barriers to gene flow, owing to inertia resulting from behavioral interactions (repulsion) or demographic interactions resulting from differences in population sizes of resident and immigrant populations.

The strong phylogeographic structure in Mesoamerican *ochrocephala* parrots stands in contrast to the apparent lack of geographic structure in parrots collected across a region extending from the mouth of the Amazon to Bolivia and Peru, a distance of >2,000 km. Contrary to current subspecies descriptions, our results clearly show that parrots described as *A. o. ochrocephala* and *A. o. xantholaema*, from Rio Xingu and Ilha de Marajó in the the lower Amazon River, are closely allied with parrots recognized as *A. o. nattereri* from western Brazil, Bolivia, and Peru. Recent population-genetic analysis of mahogany (*Swietenia macrophylla*) distributed along the southern arc of the Amazon also failed to demonstrate strong geographic subdivision in the region, in contrast to Mesoamerican *Swietenia macrophylla*, which was strongly structured into four regional population groups (Novick et al. 2003). The compelling data for geographically structured populations of Middle American freshwater fish, howler monkeys, and mahogany may indicate a geographic history of population expansion and subdivision for many groups that is considerably more dynamic than that of *Amazona* parrots. Differences in regional histories such as the one documented here for South American *ochrocephala* parrots versus their Middle American counterparts might also provide a partial explanation for the reduction in beta diversity (species turnover) that characterizes western Amazonian rainforest tree communities in comparison to those in Panama (Condit et al. 2002).

The phylogenetic break between *ochrocephala* parrots from northern and central South America has not been suggested by previous taxonomic work but is well supported by the molecular data (see Figs. 2 and 3). The most obvious landscape feature that coincides with divergence between the two South American lineages is the Amazon River, which runs between the geographic areas represented by the samples in the two South American clades. This is consistent with the riverine barrier hypothesis (Wallace 1853, Capparella 1988), which argues that large river courses impede gene flow between populations on opposite banks, leading to speciation. Nevertheless, our support for the riverine barrier hypothesis is weak, and further sampling on both the north and south banks along the Amazon River would permit a much stronger test of the hypothesis. Alternatively, the genetic break could reflect past habitat discontinuities, such as changes in forest cover resulting from climatological cycles (Haffer 1969) or isolation of Guiana Shield populations due to sea-level changes (Nores 1999).

Alternatively, the genetic break could reflect past habitat discontinuities (e.g. changes in forest cover resulting from Pleistocene glacial cycles; Haffer 1969), with the Amazon being a secondary barrier, halting the expansion of lineages from their centers of origin. Observed

genetic distances between members of the two South American clades, which imply that divergences occurred during the Pleistocene, would be consistent with that hypothesis. The northern samples could also reflect isolation of an *ochrocephala* lineage on the Guiana shield of northwestern South America, which may have been isolated during ≈100 m sea-level rises that occurred during the late Tertiary and the Pleistocene (Nores 1999).

Of the three named South American subspecies, *xantholaema* is the most different morphologically (more extensive yellow on the head), has vocalizations that differ from mainland *ochrocephala* (C. Yamashita and P. Martuschelli pers. comm.), and is somewhat isolated on Ilha de Marajó. However, according to our sequence data, *xantholaema* clusters closely with a *nattereri* sample from Bolivia. That lack of divergence is also shown by phylogenetic analysis of sequence data from the rapidly evolving control region, using samples from the present study (J. R. Eberhard and E. Bermingham unpubl. data) and additional samples from wild-caught *xantholaema* (C. Y. Miyaki pers. comm.). There may be sufficient gene flow between island and mainland populations to prevent genetic divergence of *xantholaema* from its relatives. Alternatively, *xantholaema* may have diverged too recently for mtDNA to be a useful marker, but sufficiently long ago to permit divergence in plumage and vocalization patterns.

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