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Phylogeny and biogeography of Yellow-headed and Blue-fronted Parrots (*Amazona ochrocephala* and *Amazona aestiva*) with special reference to the South American taxa

CAMILA C. RIBAS,[1]* ERIKA S. TAVARES,[2] CELINA YOSHIHARA[3] & CRISTINA Y. MIYAKI[3]
[1]*Department of Ornithology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA*
[2]*Department of Natural History, Royal Ontario Museum, 100 Queen's Park, M5S 2C6, Toronto, ON, Canada*
[3]*Instituto de Biociências, Universidade de São Paulo, R. do Matão, 277, 05508-090, São Paulo, SP, Brazil*

The Yellow-headed Parrot (*Amazona ochrocephala*) has a broad Neotropical distribution, ranging from Mexico to the Amazon Basin, and a history of complex taxonomy and controversial species limits. Recent molecular analyses have started to clarify the taxonomic arrangement of the complex, but have not included a representative geographical sampling from South America. These studies have shown that the Yellow-headed complex can be divided into three main lineages, and seems to be paraphyletic, due to the inclusion of the Blue-fronted Parrot (*Amazona aestiva*) that occurs in central South America. Here we present a phylogenetic analysis based on mitochondrial DNA sequences of 45 representatives of the Yellow-headed complex from South and Central America, plus 13 Blue-fronted individuals from different localities in South America. Our analyses recover the three primary lineages found previously in the Yellow-headed complex, show that there is genetic structure in the South American lineage, which can be divided into two well-supported, closely related clades, and demonstrate that Blue-fronted samples are distributed in both clades. Differentiation of South American Blue-fronted and Yellow-headed Parrot populations does not correspond to the plumage differences used to distinguish the Blue-fronted Parrot from the Yellow-headed Parrot, nor to plumage differences used to distinguish among South American Yellow-headed subspecies. This suggests that traditional taxonomy based on plumage characters needs revision, and that this may be an interesting example of ongoing divergence-with-gene-flow related to the forest/open area ecotone in southern Amazonia.

The Neotropical biota encompasses a high diversity of species with common patterns of endemism (Haffer 1969, Cracraft 1985). The assemblage of this complex biota may be better understood through the study of the patterns of diversification of its component taxa (Prance 1982). Due to their patterns of diversity and distribution (Forshaw 1989, Juniper & Parr 1998), the study of the evolutionary history of Neotropical parrots may be particularly informative for understanding the biogeographical history of this region. Recent molecular studies of this group suggest that the current taxonomic arrangement often does not reflect phylogenetic relationships (Eberhard & Bermingham 2004, Ribas & Miyaki 2004, Russello & Amato 2004, Tavares *et al.* 2004, Ribas *et al.* 2005, 2006). A good correspondence between phylogenetic history and systematics is needed in order to reveal biogeographical patterns, as well as for conservation purposes, as parrots are among the most threatened birds in the Neotropics.

Russello and Amato (2004) presented a phylogenetic hypothesis for the genus *Amazona* based on mitochondrial and nuclear data. Although their phylogeny corroborated the majority of the traditional taxonomic arrangement of the group, an important exception was among members of the Yellow-headed species complex. This complex occurs in lowlands

*Corresponding author.
Email: ribas@amnh.org

from Mexico to the Amazon Basin and is considered by some authors as comprising only one species (*A. ochrocephala*) with nine (Forshaw 1989) or ten (Collar 1997) subspecies, defined on the basis of the distribution of yellow on the head and bill coloration. Other authors recognize three species and 11 subspecies in the complex: *A. ochrocephala* (includes the three South American subspecies – *ochrocephala*, *xantholaema* and *nattereri* – plus *panamensis* from Central America), *A. auropalliata* (with three Central American subspecies – *auropalliata*, *parvipes* and *caribaea*), and *A. oratrix* (composed of the other four Central American subspecies – *oratrix*, *tresmariae*, *belizensis* and *hondurensis*) (AOU 1998, Juniper & Parr 1998). Russello and Amato's (2004) phylogenetic results did not corroborate the subdivision of the complex into the three proposed species, but rather suggested paraphyly of the Yellow-headed complex as a result of *A. aestiva* and *A. barbadensis* being nested within it. Their findings strongly suggested that the current taxonomy is in need of revision.

Eberhard and Bermingham (2004) presented a more detailed study of the Yellow-headed complex by including five Central American and three South American subspecies. They found that the complex is divided into three primary lineages that disagree with the arrangements previously proposed. The 'Central American' lineage comprises all Central American taxa, the 'Northern South American' lineage comprises *A. o. ochrocephala* from Colombia and Venezuela, and the 'South American' lineage comprises all other South American samples (including *A. o. ochrocephala* from Brazil). Like Russello and Amato (2004), they found that the Blue-fronted Parrot (*A. aestiva*) is nested within the Yellow-headed complex, grouping with the South American lineage, whereas the Yellow-shouldered Parrot (*A. barbadensis*) appears to be the sister group to the complex. According to their results there is strong phylogenetic structure in the Central American lineage contrasting with a lack of structure in the South American lineage.

Although Eberhard and Bermingham's (2004) sampling of Central American taxa was quite complete, sampling of South American taxa was sparse: they included in their analyses individuals from only four localities representing a lineage that is distributed across the whole Amazon Basin. Furthermore, only one Blue-fronted Parrot individual was included, without information with respect to its geographical origin.

Here we present a molecular phylogenetic analysis including 22 representatives of the South American lineage of the Yellow-headed complex, one representative of the Northern South American lineage, 22 representatives of the Central American lineage, plus 13 Blue-fronted Parrot individuals from different localities, with the objective of better understanding the geographical patterns of diversity in this group. We use the resulting phylogeny to test: (1) if the lack of structure among South American taxa proposed by Eberhard and Bermingham (2004) holds even when more individuals are included in the analyses; (2) if the Amazon River may be an important barrier separating the South American from the Northern South American lineage, as proposed by Eberhard and Bermingham (2004); and (3) how Blue-fronted Parrot individuals from different localities relate to the Yellow-headed Parrot complex.

METHODS

Taxon sampling

Blood samples were obtained from 12 individual Blue-fronted Parrots and 16 South American Yellow-headed Parrots, all with known geographical origins (Appendix 1). Sequences of the following were obtained from GenBank (Appendix 1; Eberhard & Bermingham 2004): one Blue-fronted and seven South American Yellow-headed individuals; of individuals representing all the Central American taxa belonging to the Yellow-headed complex; and of the outgroups Orange-winged (*Amazona amazonica*), Red-lored (*Amazona autumnalis*) and Mealy (*Amazona farinosa*) Parrots.

DNA extraction and sequencing

DNA was extracted from blood samples through incubation overnight at 55 °C in a solution containing 0.1% SDS, 100 mM Tris-HCl (pH 8.0), 10 mM NaCl, 10 mM EDTA and 10 mg/mL proteinase K, and subsequently purified using the standard phenol-chloroform-isoamyl alcohol method (Bruford *et al.* 1992).

The primers used for amplification and sequencing of segments of four mitochondrial genes were: LMet (5′-GGCCCATACCCCGAAAATGA-3′; J. Groth pers. comm.) and H5766 (5′-GAGAAGCTAG-GATTTTTCGTG-3′; P. Brito pers. comm.) for NADH Dehydrogenase Subunit 2 (ND2); CO2GQL (Eberhard & Bermingham 2004) and CO3HMH

(Eberhard & Bermingham 2004) for a segment including the ATPase 8 and ATPase 6 genes, and COIF (Palumbi 1996) and COIA (Palumbi 1996) for Cytochrome Oxidase Subunit I (COI).

PCR amplifications were performed in 25 μL reactions with 1× buffer, 40 μM of each dNTP, 0.2 μM of each primer, 1 U *Taq* polymerase (GE Healthcare) and 25–50 ng of DNA. The thermocycling procedure was an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 53–55 °C for 30 s and 72 °C for 1 min, and a final extension of 72 °C for 7 min. A touchdown with the same incubation times was applied to amplify the ATPases 8 and 6 segment, with the annealing temperature dropping from 65 to 55 °C, one degree per cycle, followed by 23 cycles at 55 °C. PCR products were purified by incubation with 5 U of exonuclease I (USB), and 0.5 U of shrimp alkaline phosphatase (USB) at 37 °C for 1 h, followed by 10 min at 80 °C. The purified products were used as templates in sequencing reactions performed with Big Dye Terminator chemistry (Applied Biosystems) and the same primers used for the PCR amplifications. Ethanol precipitation was performed and the sequences were obtained either on an ABI377 or on an ABI3100 automated DNA sequencer (Applied Biosystems).

Sequence alignment and analysis

Sequences were assembled and checked for ambiguities using Sequencher 4.1.2 (GeneCodes Corp., Ann Arbor, MI, USA). Alignments were verified visually in MacClade 4.0 (Maddison & Maddison 2005). Base composition and transition/transversion rates were calculated in PAUP* 4.0b10 (Swofford 2002). The best-fit models of nucleotide evolution for each partition and for the combined dataset were determined through a hierarchical likelihood-ratio test in Modeltest 3.5 (Posada & Crandall 1998). Corrected genetic distances and standard errors (based on 500 bootstrap replicates) were calculated using MEGA 3.1 (Kumar *et al.* 2004). The Partition-Homogeneity Test was conducted in PAUP* to check for evidence of any conflicting phylogenetic signal among the four gene regions.

Phylogenetic tree reconstruction under maximum parsimony (MP) was performed using PAUP* 4.0b10 (Swofford 2002) for each gene independently and for the combined dataset using heuristic tree search, and tree-bisection-reconnection branch-swapping algorithm with 100 random addition sequence replicates. All characters were equally weighted. The support for each node was estimated using 1000 bootstrap replicates.

Bayesian analysis (BI) with Markov Chain Monte Carlo (MCMC) sampling was performed in MRBAYES 3.1 (Ronquist & Huelsenbeck 2003) for the combined dataset using a partitioned likelihood approach (one partition for each gene), in which parameters were estimated separately for each data partition (nst = 6, rates = gamma). Two independent runs were executed, each for ten million generations, with one cold and four heated chains, sampling once every 1000 generations and with a burn-in time determined by the time to convergence of the likelihood scores. The posterior probabilities of each node were computed by combining the trees sampled (after burn-in, a total of 9000 trees from each run) in both runs. Another run was performed with the same parameters, but for only two million generations, unlinking the topologies of the four partitions to test if the four genes recovered the same clades that were recovered in the combined analysis.

RESULTS

Sequences of the genes ND2 (533 bp), COI (513 bp) and ATPase 6 and 8 (764 bp) were obtained from 28 individuals (Appendix 1 and Table 1). There were no indels or stop codons, and the base composition was typical of mtDNA for all regions (Table 1). When our data were analysed together with the sequences obtained from GenBank, ND2 had the highest number of informative characters for the ingroup, but for the South American taxa alone, ATPase 6 had the highest number of informative characters (Table 1). Most variation was at third codon positions in all gene regions, and the transition/transversion rate varied from 10.5 to 29.7 for the ingroup (Table 1).

Independent analysis of each gene region using both MP and BI methods recovered trees similar to those obtained in the combined analysis, but with less resolution. The Partition-Homogeneity Test failed to detect any conflict in the phylogenetic information among the four datasets ($P = 0.72$). Based on these results all subsequent analyses were conducted using the combined dataset, consisting of a matrix with 61 taxa (including three outgroups) and 1820 bp.

MP analysis recovered ten trees of 399 steps (consistency index = 0.8, retention index = 0.9). The trees differed only in the relationships among individuals within well-supported clades. The strict

Table 1. Number of base pairs sequenced, variable and informative sites, base composition and Ti/Tv ratios for each gene region analysed.

Gene	ND2	COI	ATPase 6	ATPase 8
No. of base pairs sequenced	533	513	606	168
All *ochrocephala*/*aestiva*				
Variable	49	31	44	12
Informative	35	19	30	8
%A	34.1	26.6	34.3	24.4
%C	35.7	28.8	38.2	36.9
%G	10.3	19.0	6.2	11.3
%T	19.9	25.6	21.3	27.4
Ti/Tv ratio	14.1	29.7	20.2	10.5
South American clade only				
Variable	13	12	21	5
Informative	9	7	13	1
%A	34.3	26.7	34.2	24.2
%C	35.7	28.8	38.1	36.9
%G	10.1	18.9	6.2	11.5
%T	19.9	25.6	21.5	27.4
Ti/Tv ratio	10.9	18.8	8.9	N/A

consensus of all MP trees resulted in a topology almost identical to the BI topology (Fig. 1), differing only in the position of some terminal taxa within the two South American clades.

Both analyses recovered the three primary lineages found previously by Eberhard and Bermingham (2004): the Central American (CA), South American (SA) and Northern South American lineages (NSA) (Fig. 1). The SA lineage is sister to the CA lineage with good support in both analyses (84% bootstrap support and posterior probability of 100). Within the CA lineage, several strongly supported groups were recovered (bootstrap values ranging from 91 to 100%, posterior probability values of 100), and the relationships among them are resolved (Fig. 1) and agree with the relationships found previously by Eberhard and Bermingham (2004). All Blue-fronted Parrot samples were recovered within the SA lineage, which was divided into two well-supported clades: clade 1, comprising *A. o. xantholaema*, *A. a. aestiva* from Miranda and Chapada Gaúcha (central Brazil), and *A. o. nattereri* from Santa Cruz (western Bolivia) (Appendix 1, Fig. 1), and clade 2, comprising *A. o. nattereri* from Acre (western Amazonia), Pando (northern Bolivia), Jacareacanga (southern Amazonia) and Beni (western Bolivia), *A. a. aestiva* from Miranda and Chapada Gaúcha, *A. a. xanthopteryx* and *A. o. ochrocephala* (Appendix 1, Fig. 1). These two clades were recovered with high support in the combined analyses (bootstrap support of 91 and 100%, for clades 1 and 2, respectively, and posterior probabilities of 100) and were also recovered in the analyses of each gene independently. In the molecular data matrix, five diagnostic characters were sampled for clade 1, and six for clade 2. Within clade 2, there are four diagnostic characters for the clade containing individuals from Macapá. Corrected genetic distances (using the Tamura–Nei model, as selected by the hierarchical likelihood-ratio tests performed in Modeltest for the ingroup only) between the two SA clades were lower (0.9%) than the genetic distances between the two SA clades and the CA clade (2.1%; Table 2). The only representative of the NSA lineage was 2.3 and 2.2% divergent compared with the SA and CA clades, respectively (Table 2).

DISCUSSION

Our results corroborate the division of the Yellow-headed Parrot complex into three independent evolutionary lineages (Eberhard & Bermingham 2004, Russello & Amato 2004). Whereas Eberhard and Bermingham (2004) recovered a polytomy uniting these three lineages, in our analyses the SA lineage is sister to the CA lineage with relatively good support (bootstrap of 84% and posterior probability of 100), leaving the only representative of the NSA lineage in a basal position.

The NSA lineage, represented in our study by the Colombian sample, occurs between the ranges of the SA and CA lineages (Fig. 2). Eberhard and

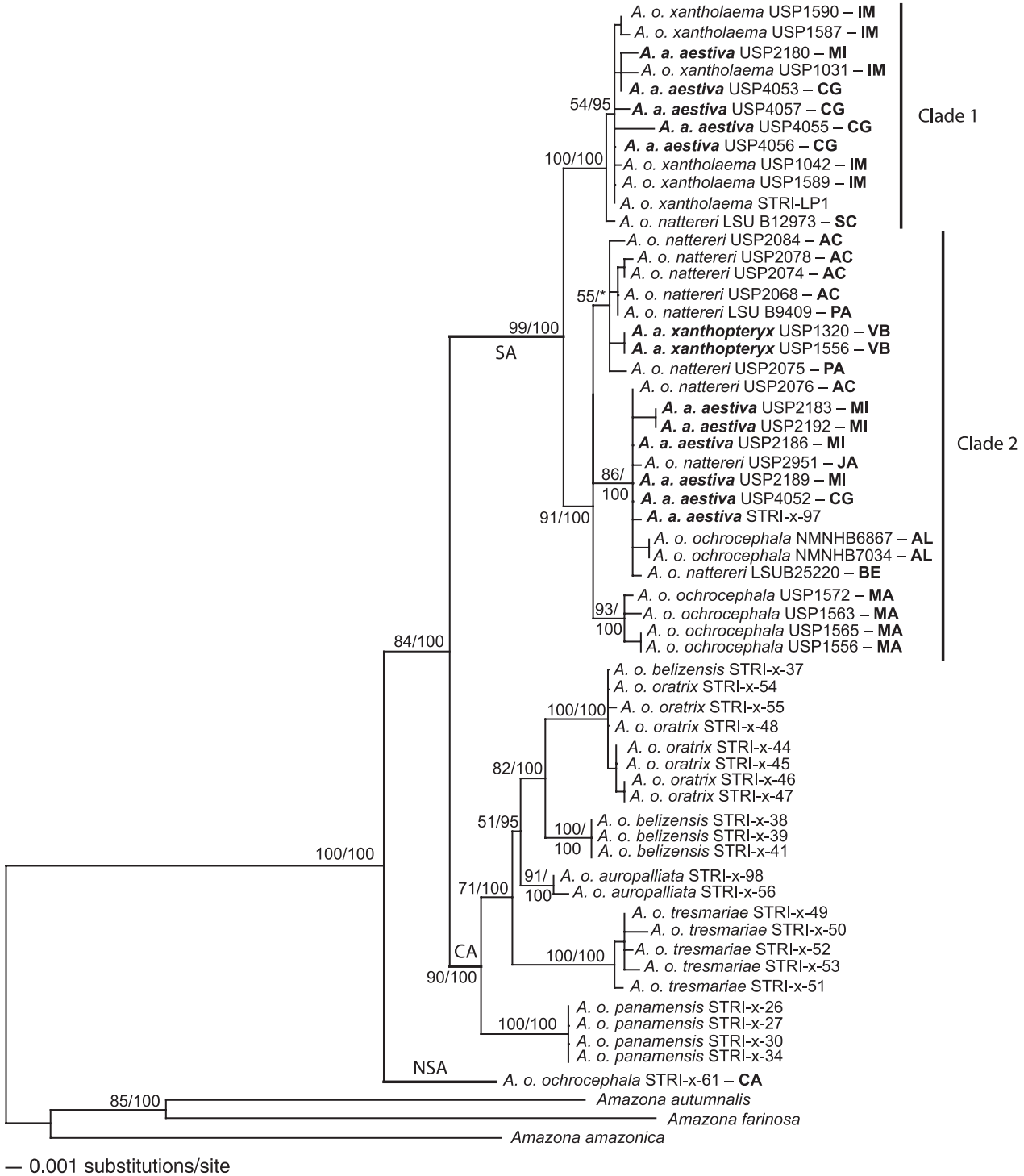

Figure 1. Topology derived from Bayesian analyses based on 1820 bp of mitochondrial sequences. Numbers on the nodes are maximum parsimony bootstrap percentages and Bayesian posterior probabilities, respectively. *Indicates posterior probability smaller than 85%. Clades 1 and 2 are indicated by vertical lines. South American (SA), Central American (CA) and Northern South American (NSA) lineages are indicated by bold branches. Voucher numbers and locality codes are indicated after each taxon name (see Fig. 2 and Appendix 1).

Table 2. Genetic distances between and within groups.

	Between groups					Within groups		
	SA1 × SA2	SA1 × CA	SA2 × CA	NSA × SA	NSA × CA	SA1	SA2	CA
All genes	0.9 (0.2)	2.1 (0.3)	2.1 (0.3)	2.3 (0.3)	2.2 (0.3)	0.2 (0.0)	0.4 (0.1)	1.0 (0.2)
ND2	0.8 (0.3)	2.3 (0.5)	2.4 (0.5)	1.7 (0.5)	2.6 (0.6)	0.2 (0.1)	0.5 (0.2)	1.8 (0.3)
COI	0.6 (0.3)	2.0 (0.5)	2.0 (0.5)	2.7 (0.7)	2.0 (0.6)	0.2 (0.1)	0.3 (0.1)	0.7 (0.2)
ATPase 6/8	1.2 (0.3)	2.0 (0.4)	2.0 (0.4)	2.5 (0.5)	2.0 (0.5)	0.2 (0.1)	0.4 (0.1)	0.8 (0.2)

Values are percentage Tamura–Nei distances (standard errors, 500 bootstrap replicates).
SA, South American lineage; SA1, South American clade 1; SA2, South American clade 2; CA, Central American lineage; NSA, Northern South American lineage.

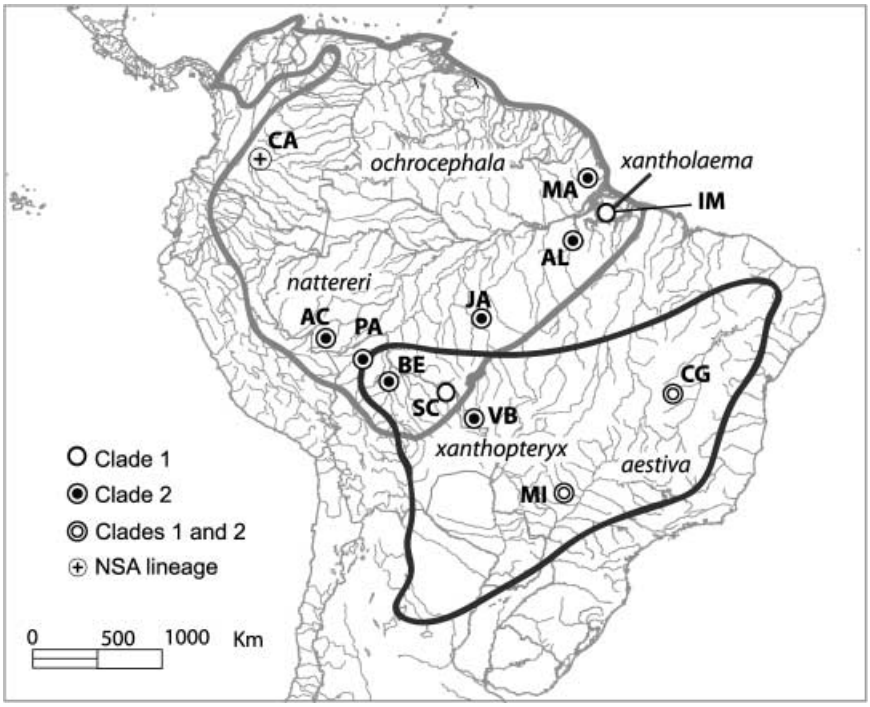

Figure 2. Collection localities of South American samples. Two letter codes correspond to localities specified in Appendix 1 and Figure 1. Different circles refer to clades 1 and 2, and to the NSA lineage (see Fig. 1). The grey line delimits the distribution of Yellow-headed Parrot (*Amazona ochrocephala*). The black line delimits the distribution of Blue-fronted Parrots (*Amazona aestiva*). Distributions of subspecies are indicated with their corresponding names.

Bermingham (2004) obtained sequences of the COI gene from an additional skin from Colombia and two from Venezuela. These individuals group with the Colombian sample included in our study. This suggests an ancestral distribution of the group in northern South America with subsequent diversification to the north and to the south, with different patterns of diversification in each direction. This is consistent with *A. o. panamensis* being basal in the CA clade. Interestingly, the CA lineage shows greater geographical mtDNA differentiation than the SA lineage, despite the larger area occupied by the latter, and the patterns of clear geographical differentiation found for other groups of Amazonian parrots (Ribas *et al.* 2005, 2006).

According to Russello and Amato (2004) and Eberhard and Bermingham (2004), the most closely related taxa to the Yellow-headed Parrot complex are *A. aurasiaca*, *A. versicolor* and *A. barbadensis*. Samples from these taxa were not available for the present study. Thus, it is important to note that although the basal nodes in our phylogeny are well

supported, the outgroups used are not the most closely related taxa to the ingroup. More closely related outgroups and additional individuals representing the NSA lineage will have to be studied in order to understand better its phylogenetic relationships and evolutionary origins.

Contrary to previous suggestions (Eberhard & Bermingham 2004) our results show a subdivision of the SA lineage into two clades. Despite the small genetic distances within the SA lineage, the two clades are well supported in all analyses with several diagnostic characters defining each one, and do not correspond to the subspecies previously recognized based on plumage coloration.

The individuals from Macapá (eastern Amazon Basin, north of the Amazon River, Fig. 2) form a well-supported clade, which is included in SA clade 2, whereas *A. o. xantholaema* from the neighbouring Marajó Island are all included in SA clade 1. This result suggests that the Amazon River may act as a barrier within the SA lineage, but is not the northern limit of the distribution of the SA lineage, as proposed by Eberhard and Bermingham (2004). More detailed sampling is needed in northern Amazonia in order to understand the geographical limits of the NSA and SA lineages.

South American clade 1 is mainly composed of individuals that occur on Marajó Island. This may indicate that these individuals originated recently from different populations on the mainland, or as a consequence of past isolation of a population on the island and subsequent introgression with mainland populations. However, this second scenario is less likely due to the very low genetic distances between SA clades 1 and 2 and marine introgressions that have probably affected Marajó island in the recent past (Klammer 1984, Marroig & Cerqueira 1997, Nores 1999). This implies that the genetic break between the two clades is a result of some other isolation event unrelated to isolation on the island. The mean genetic distance between the two clades is approximately 1%. If the evolutionary rate of 2% divergence per million years (Shields & Wilson 1987, Randi 1996) is adopted, this differentiation occurred about 500 000 years ago in the mid-Pleistocene. Glacial cycles are thought to have affected the distribution of habitats in South America during the Pleistocene (Haffer 1969, Bush 1994), and this genetic discontinuity could have originated as a consequence of barriers that are not evident today.

The lack of reciprocal monophyly between Yellow-headed and Blue-fronted Parrots has been suggested in previous studies (Eberhard & Bermingham 2004, Russello & Amato 2004), but neither of these studies included sufficient samples of Blue-fronted Parrots to be able to detect the complex pattern that we observed here. Blue-fronted individuals from two different localities have haplotypes that are included within both clades of the SA lineage (Figs 1 & 2).

Species-level polyphyly may occur due to a number of different causes, but can be divided into two main kinds: misinterpretation of the morphological variation that gave rise to the delimitation of the species, or population-level phenomena such as incomplete lineage sorting or introgression (Funk & Omland 2003). Taxonomy of Blue-fronted and Yellow-headed Parrots is based mainly on the colour patterns of the head. In the Yellow-headed Parrot the amount of yellow on the head is the primary character used to identify the subspecies (Forshaw 1989, Lousada & Howell 1996, Juniper & Parr 1998). Some authors have reported that the amount of yellow decreases from northwest to southeast throughout the distribution range (Lousada & Howell 1996). The primary character that distinguishes the Yellow-headed Parrot from the Blue-fronted Parrot is that the latter has blue on the forehead, although the extent of the blue patch varies among individuals even within the same locality (G. Seixas pers. comm.). There are two subspecies described for the Blue-fronted Parrot: *Amazona aestiva aestiva* and *Amazona aestiva xanthopteryx*, the primary difference between them being the coloration of the shoulder, which changes from red in eastern populations to yellow in western populations, but this character also seems to vary among individuals at any particular locality (Darrieu 1983, Forshaw 1989).

In addition to the plumage characters, Blue-fronted and Yellow-headed Parrots are considered to be mostly allopatric, with just a small region of sympatry in western Brazil and northeastern Bolivia (Fig. 2). All Blue-fronted samples included in our analyses are from regions that are outside the traditionally recognized range of Yellow-headed Parrots, so that the observed mixture of haplotypes cannot be attributed to misidentification of specimens. The pattern recovered may occur because traditional taxonomy does not reflect the evolution of the group. This would mean that variation in plumage may be the result of changes related to habitat differences that are not reflected among the mtDNA lineages, or that the current interpretation of plumage characters should be reviewed, as they are highly variable in this group of parrots.

Another possibility is that the pattern recovered (gene tree) is different from the species tree due to phenomena such as introgression or incomplete lineage sorting. To test this possibility, it would be necessary to obtain sequences from nuclear genes that are inherited independently from the mitochondrial genome. The problem with this approach is the very low level of variation in nuclear genes among these closely related taxa. Eberhard and Bermingham (2004) generated a nuclear DNA (Intron XI of GADPH, 404 bp) phylogeny including one Blue-fronted and four Yellow-headed individuals. The four Yellow-headed individuals form a clade with low support (62% bootstrap support in a maximum parsimony analysis) that excludes the Blue-fronted individual, but the GADPH dataset had very low levels of variation (Eberhard & Bermingham 2004). By contrast, Russello and Amato (2004) used both mitochondrial (COI, 12S rRNA and 16S rRNA) and nuclear (β-fibrinogen Intron 7, Ribosomal Protein 40 Intron 5, and Tropomyosin α-subunit Intron 5) sequences, and in their analysis the Blue-fronted Parrot was placed within the Yellow-headed Parrot clade, even when only nuclear sequences were included. In addition, the fact that Blue-fronted individuals occur in both SA clades in our mtDNA analysis points to a genetic structure in the group that is different from the one that would be expected from morphological variation.

It is interesting to note that, whereas both Yellow-headed and Blue-fronted Parrots occupy open areas adjacent to wooded habitats, South American Yellow-headed Parrots occur in the Amazon Basin, while Blue-fronted Parrots occur in more dry and open habitats, including Cerrado, Caatinga and Chaco. If their plumage differences do reflect incipient differentiation that is not yet detectable in the mtDNA, this may be one interesting example of ongoing divergence-with-gene-flow related to the forest/open areas ecotone in southern Amazonia (Rice & Hostert 1993, Smith *et al.* 1997, Ogden & Thorpe 2002). A morphological revision of the group is needed in order to understand better the variation in plumage patterns that diagnose the two taxa.

Eberhard and Bermingham (2004) proposed two possible taxonomic arrangements for South American Yellow-headed Parrots: (1) to recognize two species, *A. ochrocephala*, including *ochrocephala* from northern South America (corresponding to the NSA lineage), and *A. nattereri*, including *ochrocephala* from Amazonia, *nattereri* and *xantholaema* (corresponding to the SA lineage); or (2) to recognize only one species in South America, *A. ochrocephala*. The second alternative would not correspond to the phylogenetic relationships presented here, as the NSA and SA lineages are not each other's closest relatives. Our data support recognizing *ochrocephala* from Colombia and Venezuela as a separate species, *A. ochrocephala*, but the limits of its geographical distribution remain to be determined. The situation in the SA lineage is more complicated, as the three traditionally recognized subspecies are not recovered in the analysis, and are not monophyletic relative to *A. aestiva*. Additional studies are needed to determine what taxonomic arrangement should be adopted.

This study is another example of a common problem that is becoming increasingly evident with the growing number of molecular phylogenetic studies in birds: the taxonomic delimitation of taxa has to be carefully reviewed before any conclusions can be drawn with respect to biogeographical history or population dynamics. This indicates that it is not advisable to assume that nominal species or subspecies are monophyletic, and phylogenetic and phylogeographical studies must always include as many representatives of closely related taxa as possible.

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Appendix 1. Taxa sampled, sample locality, codes of the localities within South America, voucher numbers and GenBank accession numbers.

Taxon	Locality	Locality code	Institution/ voucher	ND2	ATP8	ATP6	COI
A. a. aestiva	Chapada Gaúcha, MG, Brazil	CG	USP 4057	DQ453646	DQ453674	DQ453674	DQ453618
A. a. aestiva	Chapada Gaúcha, MG, Brazil	CG	USP 4055	DQ453648	DQ453676	DQ453676	DQ453620
A. a. aestiva	Chapada Gaúcha, MG, Brazil	CG	USP 4056	DQ453650	DQ453678	DQ453678	DQ453622
A. a. aestiva	Chapada Gaúcha, MG, Brazil	CG	USP 4052	DQ453647	DQ453675	DQ453675	DQ453619
A. a. aestiva	Chapada Gaúcha, MG, Brazil	CG	USP 4053	DQ453657	DQ453685	DQ453685	DQ453621
A. a. aestiva	Miranda, MS, Brazil	MI	USP 2180	DQ453639	DQ453667	DQ453667	DQ453611
A. a. aestiva	Miranda, MS, Brazil	MI	USP 2183	DQ453636	DQ453664	DQ453664	DQ453608
A. a. aestiva	Miranda, MS, Brazil	MI	USP 2186	DQ453638	DQ453666	DQ453666	DQ453610
A. a. aestiva	Miranda, MS, Brazil	MI	USP 2189	DQ453645	DQ453673	DQ453673	DQ453617
A. a. aestiva	Miranda, MS, Brazil	MI	USP 2192	DQ453656	DQ453684	DQ453684	DQ453628
A. aestiva	Captive		STRI-x-97*	AY194434	AY194328	AY194295	AY194367
A. a. xanthopteryx	Vila Bela da Santíssima Trindade, MT, Brazil	VB	USP 1319	DQ453652	DQ453680	DQ453680	DQ453624
A. a. xanthopteryx	Vila Bela da Santíssima Trindade, MT, Brazil	VB	USP 1320	DQ453643	DQ453671	DQ453671	DQ453615
A. o. ochrocephala	Macapá, AP, Brazil	MA	USP 1556	DQ453659	DQ453687	DQ453687	DQ453631
A. o. ochrocephala	Macapá, AP, Brazil	MA	USP 1563	DQ453649	DQ453677	DQ453677	DQ453621
A. o. ochrocephala	Macapá, AP, Brazil	MA	USP 1565	DQ453653	DQ453681	DQ453681	DQ453625
A. o. ochrocephala	Macapá, AP, Brazil	MA	USP 1572	DQ453635	DQ453663	DQ453663	DQ453607
A. o. ochrocephala	Rio Xingú, Altamira, PA, Brazil	AL	NMNH B06867*	AY194435	AY194329	AY194296	AY194368
A. o. ochrocephala	Rio Xingú, Altamira, PA, Brazil	AL	NMNH B07034*	AY194436	AY194330	AY194297	AY194369
A. o. ochrocephala	Carimaguá, Colombia	CA	STRI-x-61*	AY194460	AY194354	AY194321	AY194393
A. o. xantholaema	Ilha do Marajó, PA, Brazil	IM	USP 1587	DQ453640	DQ453668	DQ453668	DQ453612
A. o. xantholaema	Ilha do Marajó, PA, Brazil	IM	USP 1589	DQ453655	DQ453683	DQ453683	DQ453627
A. o. xantholaema	Ilha do Marajó, PA, Brazil	IM	USP 1590	DQ453632	DQ453660	DQ453660	DQ453604
A. o. xantholaema	Ilha do Marajó, PA, Brazil	IM	USP 1031	DQ453651	DQ453679	DQ453679	DQ453623
A. o. xantholaema	Ilha do Marajó, PA, Brazil	IM	USP 1042	DQ453654	DQ453682	DQ453682	DQ453626
A. o. xantholaema	Captive		STRI-LP1*	AY194445	AY194339	AY194306	AY194378
A. o. nattereri	Jacareacanga, PA, Brazil	JA	USP 2951	DQ453642	DQ453670	DQ453670	DQ453614
A. o. nattereri	Rio Acre, Xapurí, AC, Brazil	AC	USP 2078	DQ453637	DQ453665	DQ453665	DQ453609
A. o. nattereri	Rio Iaco, AC, Brazil	AC	USP 2068	DQ453644	DQ453672	DQ453672	DQ453616
A. o. nattereri	Rio Acre, Basiléia, AC, Brazil	AC	USP 2074	DQ453641	DQ453669	DQ453669	DQ453613
A. o. nattereri	Assis, AC, Brazil	AC	USP 2076	DQ453634	DQ453662	DQ453662	DQ453606
A. o. nattereri	Rio Itimarí, AC, Brazil	AC	USP 2084	DQ453633	DQ453661	DQ453661	DQ453605
A. o. nattereri	Pando Department, Bolivia	PA	USP 2075	DQ453658	DQ453686	DQ453686	DQ453630
A. o. nattereri	Pando Department, Bolivia	PA	LSU B9409*	AY194439	AY194333	AY194300	AY194372
A. o. nattereri	Santa Cruz Department, Bolivia	SC	LSU B12973*	AY194437	AY194331	AY194298	AY194370
A. o. nattereri	Beni, Bolivia	BE	LSU B-25220*	AY194438	AY194332	AY194299	AY194371
A. o. panamensis	Coclé, Panama		STRI-x-26*	AY194462	AY194356	AY194323	AY194395
A. o. panamensis	Chiriqui, Panama		STRI-x-27*	AY194463	AY194357	AY194324	AY194396
A. o. panamensis	Chiriqui, Panama		STRI-x-30*	AY194464	AY194358	AY194325	AY194397
A. o. panamensis	Chiriqui, Panama		STRI-x-34*	AY194465	AY194359	AY194326	AY194398
A. o. auropalliata	Guanacaste, Costa Rica		STRI-x-98*	AY194444	AY194338	AY194305	AY194377
A. o. auropalliata	Chiapas, Mexico		STRI-x-56*	AY194449	AY194343	AY194310	AY194382

Appendix 1. *Continued*.

Taxon	Locality	Locality code	Institution/ voucher	ND2	ATP8	ATP6	COI
A. o. oratrix	Tamaulipas, Los Colorados, Mexico		STRI-x-44*	AY194451	AY194345	AY194312	AY194384
A. o. oratrix	Tamaulipas, Los Colorados, Mexico		STRI-x-45*	AY194452	AY194346	AY194313	AY194385
A. o. oratrix	Tamaulipas, Los Colorados, Mexico		STRI-x-46*	AY194453	AY194347	AY194314	AY194386
A. o. oratrix	Tamaulipas, Los Colorados, Mexico		STRI-x-47*	AY194457	AY194351	AY194318	AY194390
A. o. oratrix	Tamaulipas, Los Colorados, Mexico		STRI-x-48*	AY194450	AY194344	AY194311	AY194383
A. o. oratrix	Veracruz, Tempoal, Mexico		STRI-x-54*	AY194447	AY194341	AY194308	AY194380
A. o. oratrix	Veracruz, Tempoal, Mexico		STRI-x-55*	AY194448	AY194342	AY194309	AY194381
A. o. belizensis	Belize Zoo		STRI-x-37*	AY194440	AY194334	AY194301	AY194373
A. o. belizensis	Belize Zoo		STRI-x-38*	AY194441	AY194335	AY194302	AY194374
A. o. belizensis	Belize Zoo		STRI-x-39*	AY194442	AY194336	AY194303	AY194375
A. o. belizensis	Belize Zoo		STRI-x-41*	AY194443	AY194337	AY194304	AY194376
A. o. tresmariae	Nayarit, Isla Maria Madre, Mexico		STRI-x-49*	AY194454	AY194348	AY194315	AY194387
A. o. tresmariae	Nayarit, Isla Maria Madre, Mexico		STRI-x-50*	AY194455	AY194349	AY194316	AY194388
A. o. tresmariae	Nayarit, Isla Maria Madre, Mexico		STRI-x-51*	AY194456	AY194350	AY194317	AY194389
A. o. tresmariae	Nayarit, Isla Maria Madre, Mexico		STRI-x-52*	AY194458	AY194352	AY194319	AY194391
A. o. tresmariae	Nayarit, Isla Maria Madre, Mexico		STRI-x-53*	AY194459	AY194353	AY194320	AY194392
A. amazonica	Sucumbios, Equador		ANSP 3307*	AY194466	AY194360	AY194327	AY194399
A. autumnalis	Tamaulipas, Los Colorados, México		STRI-x-42*	AY194446	AY194340	AY194307	AY194379
A. farinosa	Captive		STRI-x-21*	AY194461	AY194355	AY194322	AY194394

USP, Universidade de São Paulo; STRI, Smithsonian Tropical Research Institute; NMNH, National Museum of Natural History; LSU, Louisiana State University; ANSP, Academy of Natural Sciences of Philadelphia.
*Sequences from Eberhard and Bermingham (2004).