

GEOGRAPHIC VARIATION IN MITOCHONDRIAL DNA SEQUENCES OF AN AMAZONIAN NONPASSERINE: THE BLACK-SPOTTED BARBET COMPLEX

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Abstract. We reconstructed the phylogeny and estimated mitochondrial DNA diversity to assess levels of divergence and population structure in the Black-spotted Barbet complex. This complex consists of three Amazonian taxa: *Capito niger*, *C. auratus*, and *C. brunneipectus*. Sequence divergence between these taxa is relatively high and all are monophyletic, which is consistent with the classification of these taxa as distinct species. Within *C. auratus*, a relatively deep split in the gene tree yields two reciprocally monophyletic groups separated by the Amazon, Solimões, and Ucayali Rivers. Apparently, these rivers serve as an effective barrier to mtDNA gene flow between these populations of *C. auratus*. Carotenoid plumage coloration did not contain phylogenetic information for this group and several subspecies of *C. auratus* named on the basis of such coloration were not monophyletic based on mtDNA comparisons. However, mtDNA variation suggests that *C. auratus* contains at least two evolutionarily significant units which might indicate two geographically separate species-level taxa.

Key words: Amazon, barbet, Capitoninae, carotenoid coloration, cryptic species, river barriers.

Variación Geográfica en las Secuencias de ADN Mitocondrial de un Ave no Paserina Amazónica: el Complejo de *Capito niger*

Resumen. Reconstruimos la filogenia y estimamos la diversidad de ADN mitocondrial del complejo de *Capito niger* para evaluar los niveles de divergencia y la estructura poblacional. Este complejo consiste en tres taxa amazónicos: *Capito niger*, *C. auratus* y *C. brunneipectus*. La divergencia en las secuencias entre estos taxa es relativamente alta y todos son monofiléticos, lo cual concuerda con la clasificación de estos taxa como especies distintas. Dentro de *C. auratus*, el árbol génico tiene una división relativamente profunda en dos grupos recíprocamente monofiléticos que están separados por los ríos Amazonas, Solimões y Ucayali. Aparentemente, estos ríos sirven como una barrera eficaz que impiden el flujo genético de ADN mitocondrial entre estas poblaciones de *C. auratus*. La coloración carotenóide del plumaje no proveyó información filogenética para este grupo, y varias subespecies de *C. auratus* nombradas con base en este tipo de coloración no fueron monofiléticas según comparaciones de ADN mitocondrial. Sin embargo, la variación de ADN mitocondrial sugiere que *C. auratus* contiene por lo menos dos unidades evolutivas, las cuales podrían ser dos especies geográficamente separadas.

INTRODUCTION

Studies of geographic variation in mitochondrial DNA (mtDNA) sequences from Amazonian birds show that Amazonian biogeographic history is complex and that no single pattern, and probably no single mechanism, underlies the di-

versity of birds in this region (Marks et al. 2002, Aleixo 2002a). Marks et al. (2002) noted that each new study of geographic variation in Amazonia seems to have its own idiosyncrasies, with patterns of genetic variation differing from those found in other studies. Although comparisons of widespread, sympatric taxa should help us to gain a better understanding of the general biogeographic patterns in the Amazon, all currently published studies of mtDNA geographic variation in Amazonian birds have concentrated on suboscine passerines including *Drymophila* and *Hypocnemis* (Thamnophilidae, Bates et al. 1999), *Hemitriccus*

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(Tyrannidae, Cohn-Haft 2000), *Glyphorynchus* (Dendrocolaptidae, Marks et al. 2002), and *Xiphorhynchus* (Dendrocolaptidae, Aleixo 2002a, 2002b). Such studies have shown that within several biological species of Amazonian suboscine passerines multiple distinct evolutionary lineages occur, suggesting that current species-level taxonomy has underestimated the diversity of these Amazonian birds (Bates et al. 1999). However, population-level genetic variation in Amazonian nonpasserine birds has not been described previously. Here, we present one of the first assessments of genetic variation in a nonpasserine species complex that inhabits Amazonian forests.

The Black-spotted Barbet complex (Ramphastidae: Capitoninae) consists of a set of allopatric taxa that inhabit humid lowland terra firme forests of the Amazon Basin (Chapman 1928, Haffer 1997, Lane 1999). This group was originally described as three different species, the Black-spotted Barbet (*Capito niger*; Müller 1776), the polytypic Gilded Barbet (*C. auratus*; Dumont 1816), and the Cinnamon-breasted Barbet (*C. brunneipectus*; Chapman 1921), but later were combined into a single, polytypic species (*Capito niger*) due to similarities in plumage and an allopatric distribution of the different forms (Ripley 1945, Peters 1948, Meyer de Schauensee 1966). More recently, Short and Horne (2001, 2002) recognized *C. brunneipectus* as a distinct species based on plumage and vocalization differences. However, they hesitated to split *C. auratus* from *C. niger* because of the similarity of plumage in these two taxa. Others, however, have recommended splitting *C. auratus* and *C. niger* because their vocalizations are distinctly different, whereas within *C. auratus*, vocalizations are consistent throughout its wide geographic range (Haffer 1997, Lane 1999). In addition, *C. niger* can be distinguished readily from all forms of *C. auratus* by the presence of a discrete red forehead, lighter underparts, and weaker flank spotting.

Capito auratus varies in throat color within and among localities, from orange to red, with many intermediate forms (Chapman 1928, Haffer 1997, Lane 1999). Subspecies within *C. auratus* have been defined largely on the extent and color of these plumage patches (Chapman 1928). Although barbets' plumages have not been tested explicitly, this red and orange throat coloration is most likely due to carotenoids

(Brush 1978; but see McGraw et al. 2004a, 2004b). However, it is now well known that birds cannot synthesize carotenoids and must obtain them from food (Goodwin 1950, Brush 1978). As a result, patches of carotenoid coloration can vary within and among populations of some species, due to differential intake and regional availability of dietary carotenoids (Hill 1992, 1993a, 1993b). The taxonomic value of a character so heavily influenced by environmental variables is questionable (Troy and Brush 1983).

We undertook this phylogenetic study using mtDNA sequence data to 1) investigate the relationships between *C. niger*, *C. auratus*, and *C. brunneipectus*, 2) reconstruct the relationships and assess genetic diversity among populations within *C. auratus*, and 3) evaluate carotenoid coloration as a taxonomic character for the group.

METHODS

PCR AMPLIFICATION AND SEQUENCING

We isolated DNA from both frozen tissues ($n = 18$) and skin samples ($n = 3$) from recently collected museum specimens representing nine of fifteen subspecies of the *C. niger* complex (Table 1; Chapman 1928, Peters 1948). We also isolated DNA from muscle tissue of seven outgroup taxa including samples from six of the seven other *Capito* species (Table 1). For all in-group taxa we sampled individuals from the extremes of their geographic distributions when available (Fig. 1). We were unable to obtain tissue samples for six subspecies of *C. auratus*. The taxonomic validity of most of these subspecies is doubtful (Lane 1999) and they are not represented in modern tissue collections. Also, we do not assess patterns of intraspecific variation within *C. niger* and *C. brunneipectus* because of the limited number of samples available.

We extracted DNA from all muscle tissue samples using a DNeasy extraction kit (Qiagen, Valencia, California) following its protocol for animal tissues. For DNA extraction from skin samples, we used 50 μ L of eluting buffer instead of the recommended 200 μ L. Negative controls were included during extraction from skin samples to check for contamination.

For each sample, we sequenced a total of 1427 base pairs of mitochondrial DNA, consist-

ing of 1048 base pairs from Cytochrome *b* and 379 base pairs from Cytochrome oxidase I (COI). For PCR amplification of Cytochrome *b*, we used primers L14841 (Kocher et al. 1989), BARBCBH (5'-GAGAAGTANGGGTGGAAKGG-3'), BARBCBL (5'-CTTCCTCTNCCATTYCTAATCRCAGG-3') and H16065 (Helm-Bychowski and Cracraft 1993) with the following thermal cycling profile: 30 cycles of 90°C for 40 sec, 50°C for 40 sec, 72°C for 40 sec, followed by 72°C for 5 min. To amplify COI, we used primers L6625 and H7005 (Hafner et al. 1994) and the following thermal cycling profile: 94°C for 2 min, 35 cycles of 94°C for 30 sec, 46°C for 30 sec, 72°C for 30 sec, and 72°C for 7 min. We verified all PCR products and negative controls on a 1% agarose gel and purified PCR products using a QIAquick PCR Purification kit (Qiagen, Valencia, California). Cycle sequencing reactions were performed using an ABI Big Dye kit (Applied Biosystems, Foster City, California), the above-listed primers, and approximately 75 ng of purified PCR product. We removed unincorporated dyes from sequencing reaction products using Centriseq columns (Princeton Separations, Adelphia, New Jersey) repacked with Sephadex G-50. Sequences were collected using an ABI 377 automated DNA sequencer (Applied Biosystems). We used Sequencher (V. 3.1, GeneCodes Co., Ann Arbor, Michigan) to reconcile double-stranded sequences and to align sequences. All sequences used in this study are deposited in GenBank (accession numbers AY897014–AY897069).

PHYLOGENETIC AND STATISTICAL ANALYSES

We performed a partition homogeneity test as implemented in PAUP* (Farris et al. 1994, Swofford 2001) to test for incongruence between Cytochrome *b* and COI data sets. We then used the combined data set to construct phylogenetic trees using maximum parsimony, maximum likelihood, and Bayesian analyses. Maximum parsimony and maximum likelihood analyses were conducted using PAUP* (V. 4.0 beta10, Swofford 2001). For maximum parsimony analysis we performed 100 bootstrap replicates using a heuristic search with 10 random additions per replicate and tree-bisection-and-reconnection branch swapping. We also performed 100 bootstrap replicates for the maximum likelihood analysis using a heuristic

search, one random addition per replicate, and tree-bisection-and-reconnection branch swapping. We used Modeltest (V. 3.06, Posada and Crandall 1998) to obtain the best-fit model (Cunningham et al. 1998), the rate substitution parameters, and the gamma distribution shape parameter for maximum likelihood analyses. For maximum likelihood analyses we used the model TrN+G (Tamura and Nei 1993). Bayesian analyses were conducted with MrBayes (V. 2.01, Huelsenbeck and Ronquist 2001) using the site-specific gamma model, which we ran for 4 000 000 generations. We discarded the first 50 000 generations as “burn-in” (Leache and Reeder 2002).

We calculated uncorrected sequence divergence (*p*-distance) using PAUP* (Swofford 2001). We estimated levels of population structure by calculating F_{ST} values and performing Analysis of Molecular Variance (AMOVA) using Arlequin (Schneider et al. 2000). We used MacClade (V. 3.07, Maddison and Maddison 1992) to perform Maddison and Slatkin's (1991) test of phylogenetic signal to assess whether throat coloration contains significant phylogenetic signal. The Maddison and Slatkin test included only ingroup taxa as most outgroup taxa lack corresponding carotenoid throat patches. Throat coloration (orange, red, or brown) was assessed from the museum voucher skins corresponding to the tissue samples used in this study. Where appropriate, statistical results are presented as mean \pm SD. We considered results statistically significant if $P \leq 0.05$.

Using PASSAGE (V. 1.1, Rosenberg 2001), we performed two Mantel tests (Mantel 1967) with 10 000 iterations. First, to assess whether uncorrected genetic distances are statistically associated with geographic distances for the *C. auratus* group we compared a matrix of uncorrected *p*-distances to a matrix of geographic distances. Second, to assess whether major rivers form significant genetic barriers we compared the same matrix of uncorrected *p*-distances to a pair wise riverbank matrix in which each pair from the *C. auratus* group was coded as having come from the same or different river bank. In this case, for example, we considered birds north of the Amazon and Solimões and west of the Ucayali Rivers as being on the same bank.

RESULTS

Using the partition homogeneity test, we did not detect incongruence between Cytochrome *b* and

TABLE 1. Species and subspecies analyzed, including localities, specimen voucher information, and GenBank accession numbers. Locality number refers to the numbers found on the map in Figure 1. Taxon names are based on locality and assessment of museum voucher skins.

Species and subspecies ^a	Locality	Locality number	Tissue specimen number ^b	GenBank accession numbers
<i>Capito niger</i>	Guyana: Northwest District, Baramita	1	USNM B-09373	AY897032, AY897060
<i>C. niger</i>	Guyana: Rupununi District, Sipu River	2	USNM B-10393	AY897033, AY897061
<i>C. brunneipectus</i>	Brazil: Pará, W bank Rio Tapajós, ca. 18 km W Alterdo Chão	3	LSU B-25499	AY897030, AY897058
<i>C. brunneipectus</i>	Brazil: Pará, Alto Rio Arapiuns, ca. 139 km WSW Santarem	4	LSU B-35612	AY897031, AY897059
<i>C. auratus auratus</i>	Peru: Loreto Dpto., ca. 10 km SSW mouth Rio Napo on E bank Quebrada Vainilla	5	LSU B-4805	AY897021, AY897049
<i>C. a. auratus</i>	Peru: Loreto Dpto., Quebrada Oran, ca. 85 km NE Iquitos	6	LSU B-6969	AY897028, AY897056
<i>C. a. auratus</i>	Peru: Loreto Dpto., Quebrada Oran, ca. 85 km NE Iquitos	6	LSU B-6947	AY897027, AY897055
<i>C. a. amazonicus</i>	Brazil: Amazonas, Mun Caítau Uara	7	MPEG M-49882	AY897022, AY897050
<i>C. a. aurantiicinctus</i>	Brazil: Roraima, Rio Inajá, Serra Parima	8	MPEG M-49289	AY897035, AY897063
<i>C. a. aurantiicinctus</i>	Brazil: Roraima, Mucajui	9	MPEG M-45737	AY897034, AY897062
<i>C. a. insperatus</i>	Peru: Ucayali Dpto., SE slope Cerro Tahuayo, ENE Pucallpa	10	LSU B-11206	AY897025, AY897053
<i>C. a. insperatus</i>	Bolivia: Beni Dpto., ca. 50 km N Yacumo	11	LSU B-6798	AY897026, AY897054
<i>C. a. orosae</i>	Brazil: Amazonas, 13 km S Benjamin Constant	12	LSU B-25503	AY897023, AY897051
<i>C. a. orosae</i>	Brazil: Amazonas, S Rio Solimões, 13.5 km E São Paulo de Olivença	13	LSU B-35719	AY897024, AY897052
<i>C. a. punctatus</i>	Peru: Loreto Dpto., NE bank Rio Cushabatay, 84 km WNW Contamana	14	LSU B-27403	AY897040, AY897068
<i>C. a. punctatus</i>	Peru: Loreto Dpto., Rio Cushabatay, 79 km WNW Contamana	15	LSU B-27553	AY897029, AY897057
<i>C. a. transilens</i>	Brazil: Amazonas, São Gabriel da Cacheoera, Comunidade Tapajós	16	LSU B-25572	AY897037, AY897065
<i>C. a. transilens</i>	Venezuela: Amazonas, Cerro de la Neblina	17	LSU B-7567	AY897036, AY897064
<i>C. a. transilens</i>	Venezuela: Amazonas, Cerro de la Neblina	17	LSU B-7549	AY897041, AY897069
<i>C. a. auratus</i> > <i>C. a. punctatus</i> ^c <i>intergrade</i>	Peru: Loreto Dpto., lower Rio Napo, E bank Rio Yanayacu, ca. 90 km N Iquitos	18	LSU B-4430	AY897038, AY897066
<i>C. a. auratus</i> > <i>C. a. punctatus</i> ^c <i>intergrade</i>	Peru: Loreto Dpto., lower Rio Napo, E bank Rio Yanayacu, ca. 90 km N Iquitos	18	LSU B-4448	AY897039, AY897067

TABLE 1. Continued.

Species and subspecies ^a	Locality	Locality number	Tissue specimen number ^b	GenBank accession numbers
<i>C. aurovirens</i>	Peru: Loreto Dpto.	—	LSU B-7344	AY897014, AY897042
<i>C. dayi</i>	Bolivia: Santa Cruz Dpto.	—	LSU B-18305	AY897017, AY897045
<i>C. maculicoronatus maculicoronatus</i>	Panama: Colon Prov.	—	LSU B-28524	AY897019, AY897047
<i>C. m. rubrilateralis</i>	Panama: Darien Prov.	—	LSU B-2178	AY897020, AY897048
<i>C. quinticolor</i>	Ecuador: Esmeraldas Prov.	—	LSU B-29985	AY897018, AY897046
<i>C. squamatus</i>	Ecuador: Bolivar Prov.	—	LSU B-7738	AY897016, AY897044
<i>C. wallacei</i>	Peru: Loreto Dpto.	—	LSU B-27757	AY897015, AY897043

^a Classification is based on Lane (1999).

^b Abbreviations: LSU, Louisiana State University Museum of Natural Science, Baton Rouge; MPEG, Museu Paraense Emílio Goeldi, Belém, Pará, Brazil; USNM, National Museum of Natural History, Smithsonian Institution.

^c *C. a. auratus* > *punctatus* denotes a morphological intergrade between these two subspecies.

COI partitions ($P = 0.92$). Also, when analyzed separately, Cytochrome *b* and COI gene trees (not shown) were completely compatible with each other. Therefore, we combined these partitions for subsequent analyses. When both genes were combined, every individual sequenced had a unique haplotype.

All tree topologies reconstructed by maximum parsimony, maximum likelihood, and Bayesian analyses were essentially identical with differing amounts of resolution (Fig. 2, Fig. 3). The relationships among *C. niger*, *C. auratus*, and *C. brunneipectus* were unresolved. For example, only 56% of the trees sampled in the

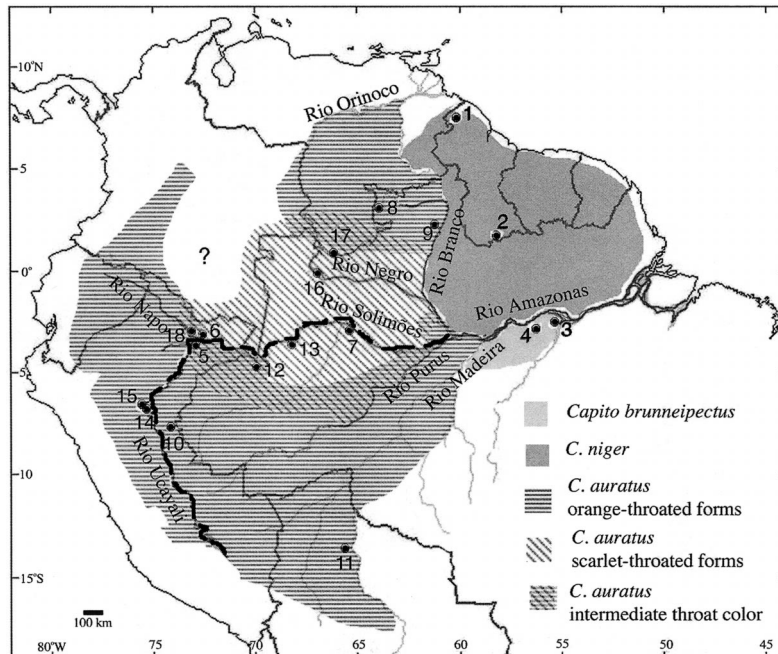


FIGURE 1. Localities of specimens of *Capito niger* complex. Numbers on the map refer to localities in Table 1. Shading pattern reflects species and plumage type. The question mark indicates areas of uncertain *C. auratus* distribution. The dark line indicates the major break seen in the phylogeny (Figs. 2 and 3) separating birds found north and west of the Amazon, Solimões, and Ucayali Rivers from birds located south and east of these rivers.

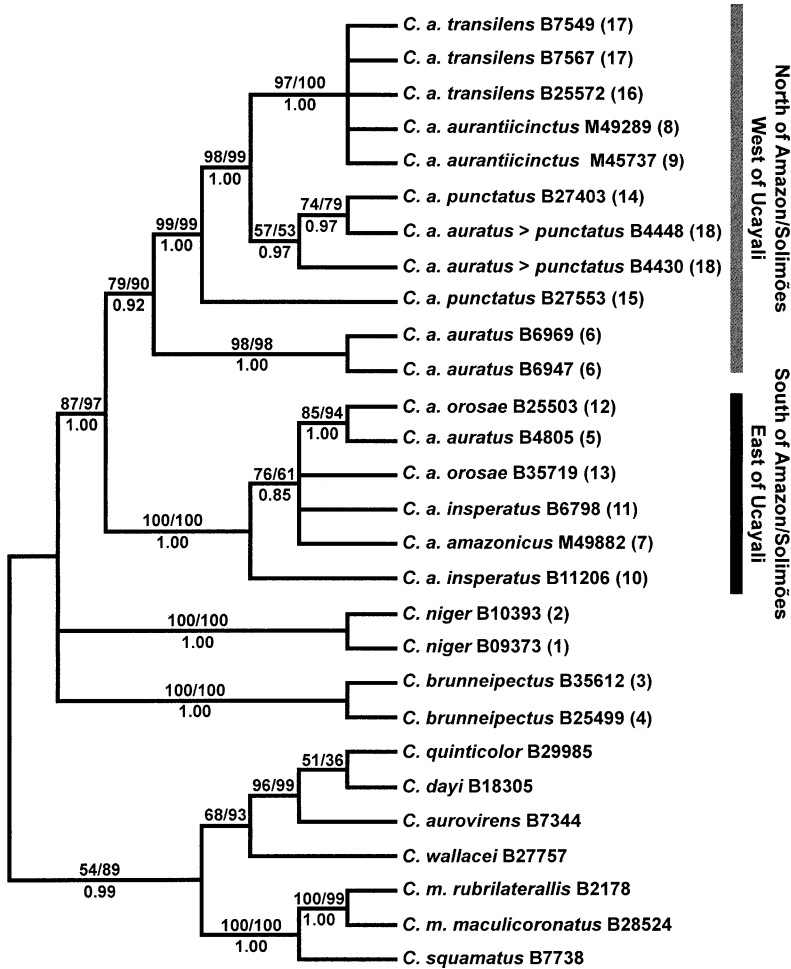


FIGURE 2. Maximum likelihood bootstrap consensus (L = 5788,409) showing the phylogenetic relationships of species and populations within the *Capito niger* superspecies complex. Numbers above the branches indicate maximum likelihood/maximum parsimony bootstrap support (only >50% are shown). Model used in maximum likelihood analysis includes substitution types (A-C, A-T, C-G, and G-T = 1.000; A-G = 23.758, C-T = 28.580), unequal base frequencies (A = 0.247, C = 0.333, G = 0.142, T = 0.278), and rate heterogeneity according to a gamma distribution (shape parameter = 0.196). Numbers below the branches indicate Bayesian posterior probability values. Immediately following the species names are the specimen numbers for the tissue samples as shown in Table 1. Numbers in parentheses to the right of the specimen numbers refer to localities labeled on Figure 1. The taxon name *C. a. auratus* > *punctatus* denotes a morphological intergrade between these two subspecies.

Bayesian site-specific gamma search placed *C. niger* and *C. brunneipectus* as sister taxa (Fig. 2 and 3). However, the monophyly of *C. auratus* is well supported. Within *C. auratus*, two reciprocally monophyletic groups are separated by the Amazon, Solimões, and Ucayali Rivers. These clades differ by 5% uncorrected sequence divergence on average. *C. auratus auratus* (sensu Chapman 1928) is polyphyletic because it appears in both *C. auratus* clades. Furthermore, *C.*

a. punctatus and *C. a. insperatus* are paraphyletic. Throat color changes numerous times on the gene tree (Fig. 3) and lacks significant phylogenetic signal ($P = 0.25$, Maddison and Slatkin 1991).

The mean sequence divergence of all individuals sequenced is 0.042 ± 0.021 . The mean pairwise sequence divergence between *C. niger*, *C. auratus*, and *C. brunneipectus* groups ranges from 0.061 ± 0.002 (comparing *C. niger* and *C.*

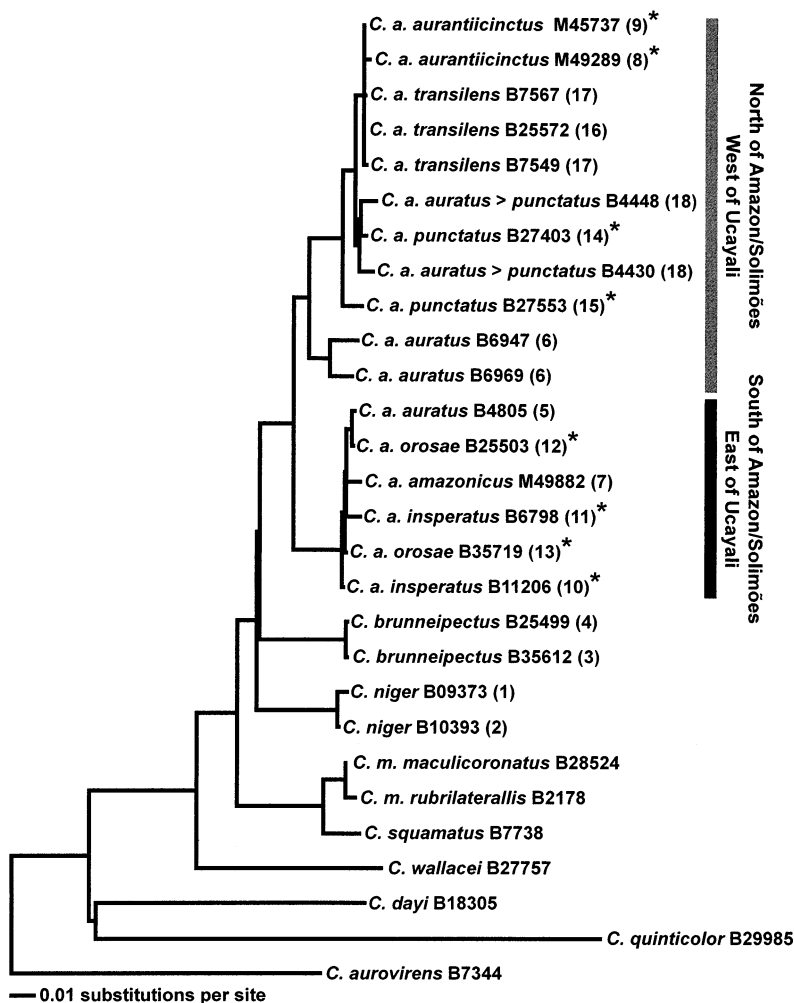


FIGURE 3. Maximum likelihood phylogram ($L = 5788.361$) of the phylogenetic relationships of species and populations within the *Capito niger* superspecies complex. The corrected branch lengths, which are proportional to the number of substitutions per site, were calculated under the TrN+G model with the same parameter values and settings reported for Figure 2. Numbers immediately following the species names refer to the specimen numbers for the tissue samples as shown in Table 1. Numbers in parentheses to the right of the specimen numbers refer to localities labeled on Figure 1. *C. auratus* specimens with orange throat coloration are denoted with an asterisk, whereas *C. auratus* specimens with red throat coloration are not marked with an asterisk. The taxon name *C. a. auratus > punctatus* denotes a morphological intergrade between these two subspecies.

brunneipectus) to 0.062 ± 0.004 (comparing *C. auratus* and *C. brunneipectus*).

Within the *C. auratus* group, 71% ($F_{ST} = 0.71$, $df = 16$, $P < 0.001$) of the sequence variation is due to variation between the two main clades. Sequence divergence between the *C. auratus* populations on either side of the Amazon, Solimões, and Ucayali Rivers is 0.049 ± 0.003 . Average sequence divergence within each clade is much lower: 0.020 ± 0.015 in the clade found

north of the Amazon and Solimões Rivers and west of the Ucayali River and 0.007 ± 0.003 in the clade found south of the Amazon and Solimões and east of the Ucayali. Mantel test results suggest no significant association ($P = 0.21$) between the uncorrected genetic distance matrix and the geographic distance matrix. However, the comparison of the uncorrected genetic distance matrix to the riverbank matrix indicates that high genetic distances are significantly as-

sociated with pairs being from opposite river banks ($P < 0.001$).

DISCUSSION

This study indicates that *C. auratus*, *C. niger*, and *C. brunneipectus* should be considered distinct species. Although they are each other's closest relatives, each is separated from the other by at least 6% mitochondrial sequence divergence. Relatively high divergences and monophyly of the *C. auratus* group suggest that it has been evolving independently from *C. niger* and *C. brunneipectus* for a considerable period of time. Although plumage differentiation between *C. niger* and *C. auratus* is minimal, these two taxa are both distinct from *C. brunneipectus* in plumage. No zone of contact or hybridization is known to exist between *C. niger* and *C. auratus*, and the songs of the two species differ from each other (Haffer 1997, Lane 1999). Some phylogenetic reconstructions even suggest that *C. niger* may be more closely related to *C. brunneipectus* than to *C. auratus*. However, more data are needed to resolve the relationships among these three groups.

Population genetics, phylogenetic analysis and geography all suggest that *C. auratus* north and west of the Ucayali, Amazon, and Solimões Rivers and south and east of the Ucayali, Amazon, and Solimões Rivers are distinct. We calculate an approximate mitochondrial sequence divergence of 5% between these two clades. These analyses suggest at least two evolutionarily significant units within *C. auratus* (Moritz 1994) and might indicate the existence of two geographically separated species-level taxa.

This level of divergence is similar to the relatively high levels of mitochondrial differentiation found within Amazonian suboscine birds. For example, uncorrected sequence divergences range from 5%–6% between populations of *Hypocnemis cantator* on opposite banks of the Amazon River (Bates et al. 1999), and they range between 1%–8% for *Glyphorhynchus spirurus* populations (Marks et al. 2002). Within the genus *Xiphorhynchus*, uncorrected divergences between populations of *X. guttatus*, *X. ocellatus*, and *X. spixii* range from 2%–5%, 1%–6% and 2%–4%, respectively (Aleixo 2002b). Divergence within *C. auratus* was also similar to that between populations of *Hemitriccus minor*, which ranged from 0%–6% (Cohn-Haft 2000). Similar to the patterns found in suboscine

passerines, our results suggest that the current species-level taxonomy may underestimate the genetic diversity found in Amazonia.

The Mantel test indicates that there is not a significant relationship between geographic distance and uncorrected genetic distance. However, the Mantel test comparing uncorrected genetic distances with the riverbank matrix indicates that high genetic distances are significantly associated with pairs being from opposite river banks. In this case, the Amazon, Solimões, and Ucayali Rivers appear to be effective barriers to dispersal for *C. auratus*, at least for females. The reciprocal monophyly of *C. auratus* clades from opposite banks of the Amazon, Solimões, and Ucayali Rivers also indicates that among the birds sampled, closest mitochondrial relatives are not necessarily close geographically. Apparently, the most closely related individuals are often found hundreds of miles away but on the same side of the river, whereas those directly across the river are more distantly related.

Relatively high levels of divergence within taxa across the Amazon, Solimões, and Ucayali Rivers has been observed in only two other species studied, *Hemitriccus zosterops* (Cohn-Haft 2000) and *Xiphorhynchus elegans* (Aleixo 2002a). In *H. zosterops*, clades were separated by only 2%–4% sequence divergence, and two *X. elegans* subspecies, *X. e. ornatus* and *X. e. juruanus*, were separated by only 2% sequence divergence. These lower divergences perhaps indicate a more recent cessation of mitochondrial gene flow or a different rate of evolution within these groups as compared to the *C. auratus* populations separated by these same barriers. Several taxa, including *Hypocnemis cantator* (Bates et al. 1999) and *Glyphorhynchus spirurus* (Marks et al. 2002) exhibit high levels of divergence across the Amazon. The geographic location of barriers to gene flow for each taxon will depend partly upon the taxon's distribution. In other suboscines, the Madeira (Bates et al. 1999, Cohn-Haft 2000), Negro (Cohn-Haft 2000), and Tapajós (Aleixo 2002a) Rivers act as barriers to gene flow. Additional sampling is needed to determine whether other large rivers, such as the Rio Napo, act as barriers to gene flow between *C. auratus* populations.

Several *C. auratus* subspecies are non-monophyletic in their mtDNA gene trees. *C. auratus auratus* (sensu Chapman 1928) is polyphyletic, with *C. a. auratus* from the north bank of the

Amazon (locality 6, Fig. 1) and *C. a. auratus* from the south bank of the Amazon (locality 5, Fig. 1) in two different clades, despite the fact that the specimens were separated by less than 30 km. Other subspecies, including *C. a. punctatus*, *C. a. insperatus*, and *C. a. orosae* are paraphyletic. For *C. auratus* subspecies this non-monophyly might result from gene flow, incomplete lineage sorting, or imperfect taxonomy based on throat coloration (see Funk and Omland 2003).

C. auratus subspecies were previously defined primarily on the basis of throat coloration. However, when we mapped throat coloration onto the *C. auratus* gene tree, throat coloration did not appear to contain significant phylogenetic signal. Marks et al. (2002) also found that differences in plumage coloration between subspecific taxa of *Glyphorhynchus spirurus* were not consistent with patterns of mtDNA variation. This result is not surprising given that some forms of coloration are heavily influenced by environmental variables. Furthermore, several studies have called into question the use of carotenoid-based coloration as a defining systematic or taxonomic character (Troy and Brush 1983, Hill 1993a, 1993b, Omland and Lanyon 2000, Mahler et al. 2003). However, we cannot rule out the possibility that there may be a genetic component involved in the process of sequestering and depositing carotenoid pigments. It is also possible that sexual selection on throat color is not reflected in neutral mtDNA. However, we believe that for *C. auratus*, the non-monophyly of subspecific taxa and the lack of concordance in phylogenetic signal between throat coloration and mtDNA indicate the need for taxonomic revision using additional, independent genetic markers, morphological characters, and larger sampling of individuals.

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