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Avifaunal interchange across the Panamanian isthmus: insights from *Campylorhynchus* wrens

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The fossil record of mammals records a major interchange of northern and southern faunas in the New World, upon closure of the Panamanian isthmus approximately 3 Mya, termed the Great American Biotic Interchange (GABI). Due to their poor preservation in the fossil record, the degree of participation of birds in this interchange remains largely unknown. A phylogeny for wrens of the genus *Campylorhynchus* (Aves: Passeriformes) was reconstructed using DNA sequences from the mitochondrial control region and cytochrome *b* gene. This phylogeny, in combination with biogeographical inference and molecular clock methods, allows estimates of the importance of Late Pliocene interchange to the history of the group. Biogeographical reconstructions and divergence date estimates suggest that the genus began diversification in North America prior to closure of the Panamanian isthmus, consistent with a hypothesized North American origin for the family Troglodytidae. These reconstructions are consistent with pre-GABI dispersal of at most a single *Campylorhynchus* lineage into South America, with subsequent dispersal of additional lineages, probably across the fully formed isthmus. Increased sampling of avian taxa with widespread New World distributions will continue to clarify the timing and direction of continental interchange. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, **90**, 687–702.

ADDITIONAL KEYWORDS: dispersal – Great American Biotic Interchange – molecular clock – Troglodytidae.

INTRODUCTION

The faunas of North and South America, largely isolated from one another for most of the Cenozoic, were profoundly impacted by closure of the Panamanian isthmus in the late Pliocene (Stehli & Webb, 1985). The extensive dispersal of taxa across this terrestrial corridor has been termed the Great American Biotic Interchange (GABI; Stehli & Webb, 1985), and represents one of the greatest natural ecological experiments recorded in the fossil record (Simpson, 1980; Marshall *et al*., 1982; Vrba, 1992). Unfortunately, the degree to which many groups of terrestrial animals participated in this interchange remains largely speculative because most of the data on continental origins, dispersal direction, and timing derive from mammalian fossils (Stehli & Webb, 1985). For example, some groups, such as reptiles and amphibians (Savage, 1982; Vanzolini & Heyer, 1985), as well as insects (Simpson & Neff, 1985), may have been much less limited by the water gaps between New World land masses due to their ability to survive oceanic rafting or to fly. Even within mammals, the available fossil data suggest preisthmian dispersal of strictly terrestrial animals such as ground sloths (Megalonychinae), some carnivores (Procyonidae), and possibly rodents (Sigmodontinae; Hershkovitz, 1969; Marshall, 1979; Webb, 1985). These early dispersals may have been mediated by eustatic sea level changes, which occasionally exposed large terrestrial areas in the Caribbean and proto-Central America (Marshall, 1979).

Birds fly. Therefore, it has been argued that dispersal (i.e. across water barriers or otherwise) must be explicitly incorporated into hypotheses of avian biogeographical history (Voelker, 1999; Zink, Blackwell-Rago & Ronquist, 2000). However, there is tantalizing evidence that water gaps may present significant barriers to dispersal in some birds as a function of morphological or behavioural limitations (Capparella,

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1988). Nonetheless, the degree to which avian lineages participated in the GABI largely remains an open question. Vuilleumier (1985) reviewed the little fossil evidence for Pliocene interchange of birds, which suggested that the sampled groups (larger birds tending to lacustrine or estuarine associations) may have participated in isthmian exchange. Mayr (1946, 1964) reviewed the history of the New World avifauna, and suggested that many bird groups dispersed between North and South America prior to the existence of a land bridge, as demonstrated by extant distributions of his 'pan-American' or 'readily colonizing' component (e.g. Tyrannidae, Thraupidae, and Icteridae) whereas he attributed the distribution of other groups to more recent (possibly Pliocene) interchange (from the north, Momotidae, Troglodytidae, etc.; from the south, Ramphastidae, Furnariidae, etc.). Although these and other qualitative descriptions (Howell, 1969) offer hints of interchange history, the questions of direction and timing of avian dispersal are only now being addressed quantitatively for most bird groups.

The lack of a well-sampled fossil record for birds, and for passerines in particular, limits inferences regarding their biogeographical history to those based on phylogenetic relationships and genetic divergences of extant taxa. Densely-sampled phylogenies of groups distributed on either side of a particular barrier, in combination with some simplifying assumptions, allow reconstruction of dispersals across that barrier (Bremer, 1992; Ronquist, 1997), and molecular clock analyses of appropriate divergences can constrain the timing of inferred dispersals. For example, if inferred dispersals across the Bolivar trough (where final closure of the isthmus likely occurred; Coates & Obando, 1996) significantly predate opening of a terrestrial corridor (~3.5–2.5 Mya; Coates & Obando, 1996), then dispersal across a water barrier would be inferred. Otherwise, habitat-mediated explanations of dispersal might be sufficient (Webb, 1991; Vrba, 1992).

Wrens of the genus *Campylorhynchus* offer an exemplary opportunity to test alternative hypotheses regarding dispersal across the isthmus of Panama. The 13 species of the genus (Selander, 1964) are distributed from the south-western USA through Paraguay and south-eastern Brazil, in a variety of habitats ranging from desert scrub to tropical evergreen forest. These wrens are generally highly social and sedentary, and do not perform long-distance migrations. There are only three significant regions of sympatry among congeners, in Chiapas (*Campylorhynchus chiapensis* and *Campylorhynchus rufinucha*), northern Colombia (*Campylorhynchus zonatus* and *Campylorhynchus nuchalis*), and Venezuela (*Campylorhynchus griseus* and *C. nuchalis*), minimizing distributional redundancy, and the relationship of the genus to other wrens is well resolved (Barker, 2004), simplifying inferences regarding its ancestral distribution. Here, I reconstruct phylogenetic relationships among species of *Campylorhynchus* using mitochondrial DNA (mtDNA) sequences from two gene regions, infer the biogeographical history of the genus, and estimate the dates of inferred dispersal events to test the hypothesis of preisthmian dispersal of the genus. These analyses suggest that the genus had its origin in North America, and that at least one lineage may have dispersed into South America prior to formation of a terrestrial corridor between the continents.

MATERIAL AND METHODS

Taxon and sequence sampling

Taxon sampling strategy

Samples were obtained from all currently recognized species and morphologically distinct subspecies (when available) of the genus *Campylorhynchus* (Table 1). Distinctive populations that were unsampled in the present study, which may represent phylogenetic species (based upon morphological data in Selander, 1964; as well as geographical discontinuities, i.e. disjunct allopatric populations), include: *C. rufinucha rufinucha* (Navarro-Sigüenza & Peterson, 2004), *Campylorhynchus brunneicapillus affinis* (Zink *et al*., 2001), *Campylorhynchus zonatus costaricensis/panamensis*, *Campylorhynchus zonatus brevirostris* – Colombia, *C. zonatus brevirostris* – Ecuador (but see below), and *Campylorhynchus megalopterus nelsoni*. Outgroups chosen for the present study included two species in the sister-group to *Campylorhynchus* (*Thryothorus ludovicianus* and *Thryomanes bewickii*; Barker, 2004), as well as one representative of the next most distant sister-clade (*Thryothorus leucotis*; Barker, 2004). Choice of the particular taxa used to represent the more distant outgroup did not affect inferred hypotheses of ingroup relationships.

Special comment should be made regarding the samples in Table 1 assigned to *Campylorhynchus albobrunneus* '*aenigmaticus*'. These specimens correspond in plumage pattern to *Campylorhynchus albobrunneus aenigmaticus* (de Schauensee, 1948) from Nariño state in southern Colombia. De Schauensee originally interpreted the plumages in his cotypes to represent possible hybridization between *C. albobrunneus* and *C. turdinus* because they approach *C. turdinus* in their spotted underparts and darkened pileum. This was accepted by Selander (1964), although he noted that regular dispersal of *turdinus* across the Andes was unlikely. Haffer (1975) offered a more likely explanation for this variable population, suggesting that it represents hybridization between *C. albobrunneus* and the adjacent population of *C. zonatus brevirostris* in northern Ecuador. If true,

Table 1. Taxonomic assignment, locality, and voucher information for specimens of *Campylorhynchus* and outgroups used in the present study

Subgenus	Species	Subspecies	Locality	Tissue number	Voucher number
Heleodytes	*chiapensis*		México: Chiapas; Arriaga	MZFC*	MZFC*
	griseus	*minor*	Brazil: Roraima; Río Surumu, 4 km west of Vila Surumu	FMNH 5251	MZUSP 73427
	rufinucha	*humilis*	México: Oaxaca; 10 km West, 4 km south of San Francisco Ixhuacan	LSUMZ B18080	MZFC 9762
		capistratus	Costa Rica: Puntarenas; Río Agujas, 1 km from mouth	LSUMZ B16085	LSUMZ 138915
	jocosus		México: Morelos; Cañon de Lobos, east of Cuernavaca	MZFC*	CIBUAEM 1665
	gularis		México: Nayarit; Sierra de Nayarit	FMNH 1800	MZFC 7872
	yucatanicus		México: Yucatán; 18 km east of Dzilam de Bravo	KU B593	KU 89468
				KU B594	MZFC*
	brunneicapillus	*anthonyi*	USA: California; San Diego City	FMNH 5542	FMNH 342076
		guttatus	México: Querétaro; Tequisquiapan, 2 km south of Estación Bernal	FMNH [BEHB016]	MZFC 9648
Campylorhynchus	*zonatus*	*zonatus*	México: Veracruz; Sierra de Santa Marta	FMNH 1805	MZFC 7795
		vulcanius	México: Chiapas; 3 km south of San Cristobal de las Casas	FMNH 2228	MZFC 8459
	megalopterus	*megalopterus*	México: Michoacan; Pico de Tancitaro	FMNH 2175	MZFC 8711
	fasciatus	*pallescens*	Ecuador: Manabi/Guayas; Rio Ayampe	MZUC O2445	MECN 3623
			Ecuador: Loja; 10 km east of Mangaurcu	ANSP 4598	ANSP 185701
	nuchalis	*brevipennis*	Venezuela: Guárico; Hato Masaguaral, 45 km south of Calabozo		Unvouchered
	turdinus	*hypostictus*	Peru: Madre de Díos; Hacienda Amazonía	FMNH 1790	FMNH 323409
		unicolor	Bolivia: El Beni; Laguna Suarez, 5 km south west of Trinidad	FMNH 1816	FMNH 334537
	albobrunneus	*harterti*	Panama: Darién; Cana, on east slope of Cerro Pirré	LSUMZ B2321	LSUMZ 108533
		'*aenigmaticus*'	Ecuador: Esmeraldas; El Placer	LSUMZ B11886	ANSP 180431
				LSUMZ B11826	ANSP 180432
Thryothorus	*ludovicianus*		USA: New York	AMNH [PRS063]	AMNH 20929
	leucotis		Brazil: Roraima; Santa Cecilia, east margin of Río Branco	FMNH 5217	MZUSP 73431
Thryomanes	*bewickii*		México: Querétaro; west of Peña Bernal	FMNH 5743	MZFC 9734

Taxa indicated in italics are genera.

ANSP, Academy of Natural Sciences, Philadelphia; CIBUAEM, Collección del Instituto de Biología, Universidad Autonoma del Estado de Morelos; FMNH, The Field Museum; KU, University of Kansas Museum of Natural History; LSUMZ, Lousiana State University Museum of Zoology; MECN, Museo Ecuadoriano de Ciencias Naturales; MZFC, Museo de Zoología 'Alfonso L. Herrera', Facultad de Ciencias, Universidad Nacionál Autonoma de México; MZUC, Museum of Zoology, University of Copenhagen; MZUSP, Museu de Zoologia, Universidad de Saõ Paolo. Numbers in brackets are collectors numbers.

*Uncatalogued.

mitochondrial haplotypes isolated from these samples should represent either maternal *C. albobrunneus* or maternal *C. zonatus*.

Amplification and sequencing of mitochondrial cytochrome b and control region right domain (RD)

Total genomic DNA was extracted from tissue samples (muscle or liver, except the sample of *C. nuchalis*, which was derived from blood) as previously described (Barker, 2004). Amplification and sequencing of cytochrome *b* and a short flanking-3′ segment (positions 14991–16064 of the *Gallus* mitochondrial genome; Desjardins & Morais, 1990) was performed using primers and procedures as described previously (Barker, 2004). Amplifications of the RD of the mitochondrial control region were accomplished using the F304/H1261 primer pair and identical protocols (Baker & Marshall, 1997). All nucleotide positions were sequenced from both strands. Appropriate coding of cytochrome *b* sequences was verified by translation into amino acids using the avian mitochondrial code (Desjardins & Morais, 1990; implemented in MacClade, version 3.07, Maddison & Maddison, 2000). Additionally, sequence electropherograms were closely examined for evidence (e.g. double banding) of coamplification of nuclear pseudogenes.

Phylogenetic analyses

Sequence alignment and treatment of alignment ambiguity

Cytochrome *b* sequences were aligned by eye. Sequences from the RD of the control region were initially aligned with Clustal W (Thompson, Higgins & Gibson, 1994), using multiple alignment parameters of transition/transversion ratio (TI/TV) = 10, gap insertion penalty = 15, and gap extension penalty = 6.7. The first parameter is a reasonable approximation of the TI/TV ratio for mitochondrial DNA of vertebrates (Wakeley, 1994, 1996; Purvis & Bromham, 1997; Yang & Yoder, 1999), whereas the insertion and extension penalties were initially set at a level which minimized indel events. The alignment obtained with these parameters (EMBL Accession ALIGN_000863) was evaluated for regions of instability by sequentially reducing gap insertion (minimum of 5) and extension (minimum of 1) penalties, and examining the alignments produced. Five regions of alignment instability (regions where the alignment changed with relatively small changes in alignment parameters) were identified (EMBL Accession ALIGN_000863). These regions were excluded from phylogenetic analyses presented here, as inclusion of these regions using the coding methods of Lutzoni *et al*. (2000) did not significantly affect the results (not shown). In addition to these five alignment-ambiguous regions, three phylogenetically informative but unambiguously aligned indels were coded as characters (treated as missing data within the matrix).

Tree inference

Sequences were examined for evidence of saturation and for departures from base compositional stationarity prior to phylogenetic analysis (Griffiths, 1997). Data from both gene regions, and the two combined, were analysed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian methods. All MP analyses were implemented in PAUP* 4.0b10 (Swofford, 1998) using heuristic searches, with at least 50 random taxon-addition replicates and tree-bisection-reconnection (TBR) branch swapping. Support for individual nodes was evaluated via nonparametric bootstrap resampling (Felsenstein, 1985; 500 pseudoreplicates with ten heuristic search replicates). Where several equally parsimonious trees were found, but a single tree was required for comparison with results of other methods or statistical tests, subsequent searches were performed using successive approximation character weights until a single tree was obtained (Farris, 1969).

Assumptions about molecular evolutionary process might significantly impact phylogeny reconstruction in cases where processes vary among sets of characters (i.e. process partitions, *sensu* Bull *et al*., 1993; Miyamoto & Fitch, 1995). Therefore, I evaluated whether substitution parameters differed significantly between the two gene regions using methods first described by Yang (1996), and subsequently applied by Barker (2004). In addition, the most appropriate substitution model for each gene region separately (and for both combined) was evaluated using hierarchical likelihood ratio tests. All likelihood calculations for a given gene region (or regions) were made using the shortest tree (or trees) from an equally weighted parsimony search for that region. The ambiguously aligned regions of the RD were excluded from all maximum likelihood analyses, and remaining gaps retained as missing data.

For ML analyses, the two gene regions were each analysed separately under their respective best-fit models, and the concatenated gene matrix was analysed under its best-fit model (heuristic search with PAUP* 4.0b10, with ten replicates and random addition of taxa, TBR branch swapping; all model parameter values fixed during searches). Support for nodes in these trees was estimated via the bootstrap with 100 pseudoreplicates, using TBR branch swapping on starting trees calculated via Neighbour-joining (Saitou & Nei, 1987) with ML distances. Additionally, an attempt was made to find a ML tree while allowing for heterogeneity in model parameters. Implementation of efficient heuristic searching under a heterogeneous

ML model is not yet publicly available; therefore, a candidate tree approach was used (Wilgenbusch & de Queiroz, 2000). All trees within 1% of the length of the shortest MP trees provided the set of trees used for comparison. Joint likelihoods of these trees were calculated using the *baseml* program of PAML, with the least parameter-rich model appropriate for the data as indicated by the initial model evaluation. This candidate tree approach is not guaranteed to find the globally most-likely tree; nevertheless, it provides a first approximation that can be used to evaluate whether ignoring process heterogeneity in ML analysis impacts the resulting phylogeny.

Bayesian analyses of the data were performed using both homogeneous and heterogeneous model parameterization. The density of posterior probability distributions was estimated by Metropolis-coupled Markov chain Monte Carlo (MC3), using four parallel incrementally heated chains (MrBayes, version 3.0 β3; Ronquist & Huelsenbeck, 2003; Altekar *et al*., 2004). For each parameter set, multiple runs of 10^6 generations (sampling every 100) were performed, and the estimated distributions of parameter and nodal probabilities compared for stability. (Nylander *et al*., 2004; Ronquist & Huelsenbeck, 2003).

Biogeographical and molecular clock analyses

The geographical distributions of *Campylorhynchus* species were compiled from standard sources (Selander, 1964; Wetmore, Pasquier & Olson, 1984; Ridgely & Tudor, 1989). Because the present study addresses the importance of Late Pliocene dispersal between North and South America in explaining *Campylorhynchus* distribution, all taxa were coded as either present in the north (defined as north of the approximate position of the Bolivar Trough; Coates and Obando, 1996) or in the south (distributed south of the Trough; only the distribution of *C. albobrunneus* crossed this region). Ancestral areas were inferred using dispersal-vicariance analysis (Ronquist, 1997), as implemented in DIVA, version 1.1 (Ronquist, 1996).

To evaluate the timing of inferred dispersal events within *Campylorhynchus*, the cytochrome *b* data were analysed using molecular clock methods. Absolute dating of the cytochrome *b* tree was possible due to the availability of the gene-specific calibration of Fleischer, McIntosh & Tarr (1998), estimated for Hawaiian honeycreepers (1.6% pairwise divergence/Mya). The value of Fleischer *et al.* (1998) is based on multiple divergence times, and is derived from taxa in the same avian order as wrens (the Passeriformes). The hypothesis of rate homogeneity between the honeycreepers and *Campylorhynchus* wrens was explicitly tested using a likelihood ratio (as above), including sequences from this genus and its close relatives (Table 1), publicly available honeycreeper sequences (R. A. Feldman, L. A. Freed, J. G. Groth & R. L. Cann, unpubl. data, GenBank Accessions AF015754–AF015763), and a single outgroup (the dawn robin *Tregellasia leucops*, AY443259). The variance associated with the stochastic substitution process was approximated by parametric bootstrapping (Huelsenbeck, Hillis & Jones, 1996; Huelsenbeck & Crandall, 1997). This was accomplished using branch lengths and maximum likelihood parameter estimates for cytochrome *b* on the best-fit ML tree for the combined data, with 1000 replicates generated in Seq-Gen, version 1.1 (Rambaut & Grassly, 1997), and re-fit to the ML topology in PAUP*.

RESULTS

Sequence characteristics

Alignments of 1074 bases of the cytochrome *b* gene (including a three base spacer and the 25 3′ bases of $tRNA^{Thr}$), and 395 bases of the control region right domain (including the 31 5′ bases of $tRNA^{Phe}$) were obtained for all samples listed in Table 1 (GenBank Accessions DQ004856-DQ004898; updates to AY352520, AY352521, AY352541, AY352544, and AY352545; EMBL Accession ALIGN_000863). Cytochrome *b* sequences were length-invariant, whereas the RD fragment varied from 332 to 390 bases, which included one indel event in the DHU loop of the $tRNA^{Phe}$. Cytochrome *b* nucleotide base composition was typical for avian taxa (Table 2; Kocher *et al.*, 1989;

Table 2. Mean proportion of bases across taxa, by codon position and region of mitochondrial DNA sequenced

Category	A	C	G	T	Bias*
Cytochrome *b* first position	0.225 (0.006)	0.307 (0.005)	0.250 (0.005)	0.218 (0.006)	0.076
Cytochrome *b* second position	0.193 (0.001)	0.261 (0.004)	0.129 (0.001)	0.417 (0.004)	0.237
Cytochrome *b* third position	0.354 (0.013)	0.495 (0.017)	0.057 (0.008)	0.095 (0.015)	0.465
All cytochrome *b*	0.257 (0.005)	0.355 (0.007)	0.145 (0.003)	0.243 (0.007)	0.149
Right domain	0.333 (0.007)	0.284 (0.009)	0.079 (0.006)	0.305 (0.009)	0.229

Data in parenthesis are standard deviations.

*Bias $C = 2/3 \cdot \Sigma_i \, |P_i - 0.25|$; P_i = proportion of the ith base.

Edwards, Arctander & Wilson, 1991; Kornegay *et al.*, 1993; Hackett, 1996; Nunn & Cracraft, 1996), as was composition of the RD sequences (Table 2; Baker & Marshall, 1997). Of 1045 positions of cytochrome *b*, 343 were variable (32.8%) and 256 were parsimony informative (24.5%). Of the variable sites, 79% were third coding positions, 18% were first positions, and 3% were second positions. Within the RD alignment, 343 positions were considered unambiguously aligned, and of these 123 were variable (35.9%), and 73 (21.3%) parsimony informative (including five variable sites in the $tRNA^{Phe}$, two of which were parsimony informative). Overall sequence divergence between samples ranged from 0–12.5% (uncorrected for multiple substitutions/site) for cytochrome *b* and 0–18% for RD. Graphical comparison of gene specific divergence patterns (not shown) indicated that the RD continued to accumulate substitutions at the highest cytochrome *b* divergences, whereas cytochrome *b* divergence reached a plateau beyond $p \approx 0.13$ (uncorrected sequence divergence), suggesting the occurrence of multiple substitutions per site for this gene.

Phylogenetic analyses

Phylogenetic analysis under the parsimony criterion

Application of the incongruence length difference test (Farris *et al.*, 1995) to these data, treating cytochrome *b* and the RD as partitions, failed to reject the null hypothesis of homogeneity ($P = 0.82$, 100 permutation replicates), consistent with their shared evolutionary history as members of a nonrecombining linkage group. In addition, separate analyses of the two data sets yielded broadly congruent estimates of relationship, with conflicting nodes lacking substantial support (results not shown). Parsimony analysis of the combined dataset resulted in four minimum-length trees (L = 1079 steps, CI = 0.435, RI = 0.600). Successive character weighting by the rescaled consistency index converged in one round on one of these four trees (Fig. 1A). Support for the majority of nodes in this tree was fair to strong (Fig. 1A), with most ambiguity associated with the relative placement of *Campylorhynchus gularis*, *C. jocosus*, *C. rufinucha*, and a clade containing *C. chiapensis* and *C. griseus*. The strict consensus of the four shortest trees without reweighting yielded a polytomy for relationships among these lineages.

ML model evaluation

Separate analysis of the most appropriate substitution models for cytochrome *b* and the RD yielded divergent results for the two regions. For cytochrome *b*, the relatively complex GTR + I + Γ model fit the data better than less complicated alternatives; however, the simplifying assumption of a molecular clock could not be rejected ($-2 \ln\Lambda = 21.6$, d.f. = 22, $P > 0.25$). For the RD data, a relatively simple HKY85 + Γ model provided the best fit: the RD data also did not reject a molecular clock ($-2 \ln\Lambda = 22.06$, d.f. = 22, $P > 0.05$). A model evaluation of the two data sets combined gave results identical to those for cytochrome *b* alone, significantly preferring the GTR + I + Γ model (with a clock) over less complex models.

Although independent assessment of the two data sets yielded different models, this approach cannot evaluate the statistical significance of the observed differences. To this end, 15 specific comparisons among 11 partitioned likelihood models were made (Barker, 2004: tables 5, 6 for model definitions and meaning of comparisons). Tests of branch length equality under a single model of substitution and of branch length proportionality with heterogeneous substitution models were both insignificant, indicating similar rates of evolution of the two gene regions, although the estimated proportionality constant ($c = 0.779$, SE = 0.11) suggested that the RD (excluding ambiguous regions) evolves more slowly than cytochrome *b* as a whole. As for the single gene and combined analyses, rates of substitution appeared relatively constant across taxa, as the molecular clock hypothesis could not be rejected with heterogeneous substitution models ($-2 \ln\Lambda = 43.91$, d.f. = 44, $P = 0.476$). Although cytochrome *b* and the RD have fairly similar base composition overall, tests of the homogeneity of base composition parameters (π_i) significantly favoured heterogeneity for all comparisons: homogeneity of the substitution matrix *R* could not be rejected if base composition was allowed to vary. Equality of the Γ-distribution shape parameter α for the two regions was rejected for all comparisons. These results suggested that the least complex model that could be used for inference of relationships among species of *Campylorhynchus* should allow for heterogeneity of base composition and pattern of among-site rate variation, but enforce uniform base substitution rates, branch length equality and the molecular clock. This corresponds approximately to model 3 + 2Γ of Barker (2004), although this model allows substitution rate to vary between partitions because *baseml* does not allow both heterogeneous substitution models and equal branch lengths.

ML and Bayesian analyses

In agreement with parsimony analyses, separate ML and Bayesian analyses of the cytochrome *b* and RD data yielded similar trees, without strongly supported conflicts. Using the test of Shimodaira & Hasegawa (1999), the cytochrome *b* data rejected the three trees obtained in analysis of the RD data ($\delta = 34.4$, $P < 0.01$; 10 000 RELL replicates), whereas the RD data failed to reject the cytochrome *b* tree ($\delta = 11.0$, $P = 0.12$). Consequently, the hypothesis of shared history cannot

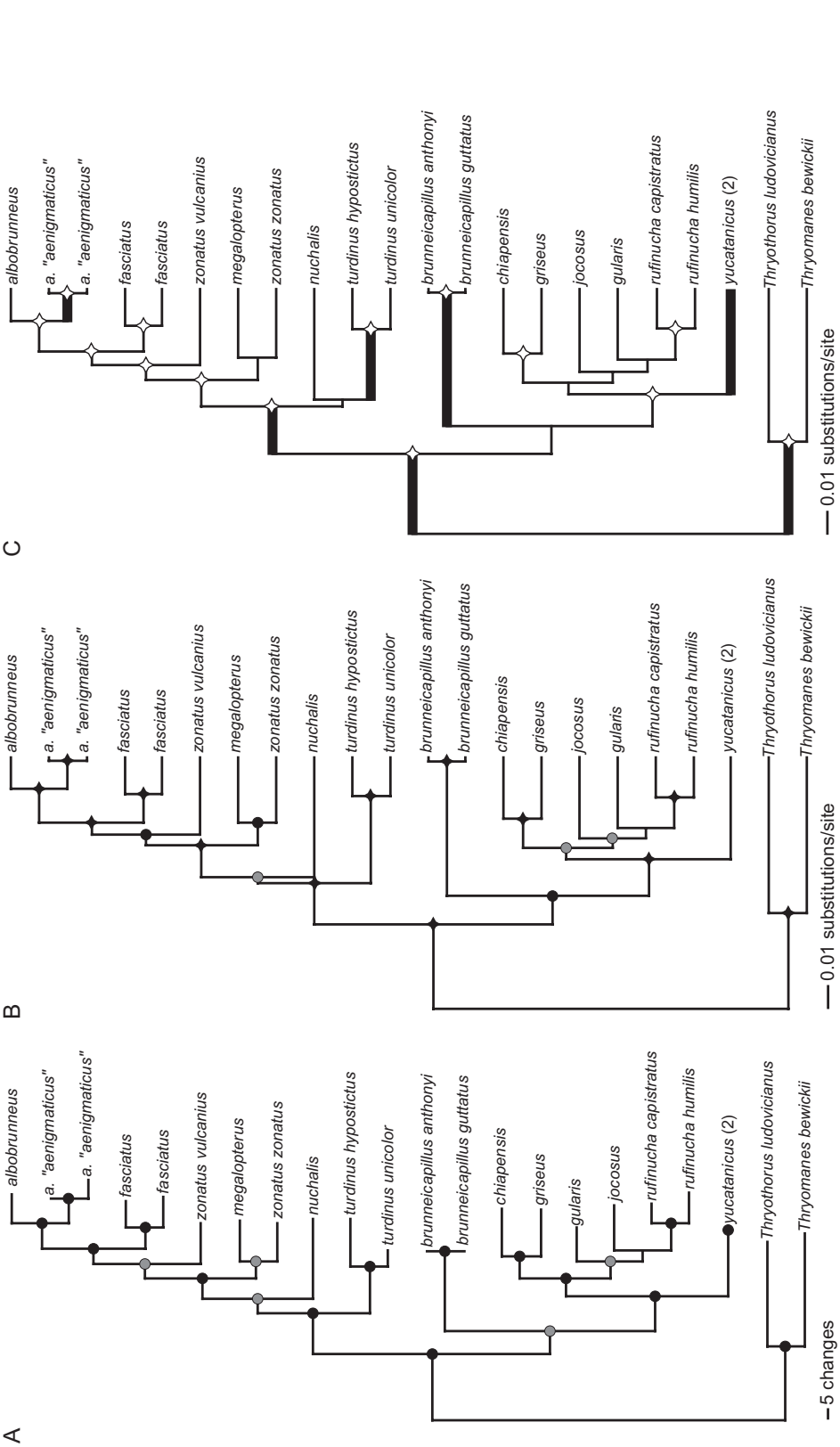

Figure 1. A. one of four equally parsimonious trees found with equally weighted parsimony analysis of cytochrome *b* and right domain (RD), excluding ambiguous regions of the alignment (L = 1079, CI = 0.435, RI = 0.600). This is the tree preferred after reweighting of characters by their rescaled consistency indices (RC, rescaled length = 379.3). Branch lengths are proportional to the inferred number of changes, subsequent to reweighting by RC. B, single tree recovered by a heuristic maximum likelihood search using a uniform model of substitution for cytochrome *b* and RD ($-\ln[\lambda] = 6986.0$; general time-reversible model, $r_{AC} = 5.65$, $r_{AG} = 55.79$, $r_{AT} = 2.87$, $r_{CG} = 1.81$, $r_{CT} = 38.64$; empirical base frequencies; proportion of invariant sites $P_{iv} = 0.516$; discrete approximation to Γ-distributed rates with $\alpha = 1.038$; molecular clock enforced). Branch lengths are proportional to the number of inferred substitutions per site. C, single tree preferred by comparison of heterogeneous-model likelihoods of near-most-parsimonious trees using PAML, under model 3 + 2Γ (see text). Branches that did not vary among the evaluated trees are thickened: branch lengths are shown proportional to the inferred number of substitutions per site. Support values for this tree (see symbol definitions below) are from a partitioned Bayesian analysis without a molecular clock enforced. Nodal support is shown by symbols: stars indicate estimated Bayesian posterior probabilities = 0.95, and circles posteriors < 0.95 (B, C), or unestimated (parsimony, A). Gray fill, bootstrap percentages between 50 and 75; black fill, bootstrap greater than 75; white fill, no bootstrap performed.

be rejected for these data. Although significant process heterogeneity was indicated by the model evaluation, an estimate of the joint likelihood tree was obtained using a full heuristic search with the data under a single substitution model (GTR + I + Γ, molecular clock enforced). This search yielded a single most likely tree (Fig. 1B), which was identical to one of the four most-parsimonious trees for these data, alough a different one from that preferred under iterative reweighting. Bootstrap proportions were moderate to high for most nodes in the tree (Fig. 1), except for relationships among the (*rufinucha*, *gularis*, *jocosus*, *chiapensis/griseus*) group, and the grouping of *C. nuchalis* with its sister-group (Fig. 1B). Bayesian analysis of the data under the same model differed from the ML tree only in the placement of *C. nuchalis* as sister to *C. turdinus* (Fig. 1C); these conflicting nodes received no appreciable support in either analysis (estimated Bayesian posterior $P = 0.48$ vs. $P = 0.47$, placing *nuchalis* as in the ML tree). Generally, the estimated Bayesian posteriors were remarkably isometric with ML bootstrap values (not shown).

The joint maximum likelihood tree, allowing for substitution model heterogeneity, was very similar to that found using homogeneous-model maximum likelihood, differing only in the arrangement of *C. nuchalis* (compare Fig. 1B, C). All trees within 1% of the length of the shortest parsimony trees were obtained using PAUP*, yielding 2441 trees = 1089 steps. The variation in topology among these trees is indicated in Figure 1C by thickened branches corresponding to nodes that did not vary among them. Bayesian analysis of the data was performed under the same model used in the heterogeneous-model ML evaluation, excepting enforcement of the molecular clock (MrBayes, version 3.0 β3 does not allow partitioned clock analysis). The majority rule consensus of trees obtained in that analysis was identical to the ML tree (Fig. 1C), except that *C. brunneicapillus* was placed as sister to all other *Campylorhynchus* species in 63% of the sampled trees (and in the ML position 26% of sampled trees; not shown), suggesting that placement of this species is sensitive to the molecular clock assumption. This conclusion is reinforced by comparison of homogeneous ML support values for placement of *C. brunneicapillus* with other Heleodytes group species (Table 1) derived from clock (95%) vs. unconstrained analyses (46%).

Biogeographical and molecular clock analyses

Dispersal-vicariance analysis of *Campylorhynchus* species was performed using both homogeneous and partitioned ML tree estimates (Fig. 1B, C). Both trees required a total of six dispersal events, with one parsimonious solution for the first tree, and three equally parsimonious solutions for the second (Fig. 2). On both topologies, the common ancestor of *Campylorhynchus* and its sister taxon (*Thryomanes* plus *T. ludovicianus*) was reconstructed as unambiguously North American (Fig. 2). The common ancestor of *Campylorhynchus* was reconstructed, depending on the topology used, as either widespread through North and South America (homogeneous ML), or equivocally northern/widespread (partitioned ML; Fig. 2). All reconstructed ancestors within the clade containing the Heleodytes group of species (Table 1) were northern (Fig. 2), with the exception of the *chiapensis/griseus* ancestor, which was inferred to have dispersed from north to south. The remaining dispersal events were reconstructed at the base of and within the clade containing the Campylorhynchus group species (Table 1; reconstructions in Fig. 2).

A likelihood ratio test under the GTR + I + Γ model failed to reject the hypothesis of rate homogeneity between wrens and Hawaiian honeycreepers ($\chi^2 = 46.2$, d.f. = 33, $P = 0.06$; including only the 790 sites shared between the two data sets), suggesting application of honeycreeper rates to wrens is not unreasonable. Parametric bootstrap error estimates of reconstructed divergence times placed only three divergences within *Campylorhynchus* at older than 3.5 Mya, the earliest estimated date for closure of the Panamanian isthmus used here (Fig. 2). Of those three, only two were reconstructed as involving dispersal between North and South America (asterisks in Fig. 2). Inferred dispersal at the basal node was unequivocal on the homogeneous ML tree, but was only required by one of the three equally parsimonious reconstructions on the partitioned ML tree. The confidence interval for that node's age excluded 3.5 Mya, suggesting that if dispersal occurred, it would have involved a water crossing. The latest initial north–south dispersal within *Campylorhynchus* in any reconstruction involved the second divergence within the clade containing the Campylorhynchus group species (Table 1; Fig. 2). The confidence interval on this latter nodal age did not exclude 3.5 Mya; incorporation of calibration error and ancestral polymorphism levels would likely render this comparison even less significant.

DISCUSSION

Phylogenetic relationships within *Campylorhynchus*

The hypothesis of relationships among species of *Campylorhynchus* proposed by Selander (1964) is the only previous attempt to define patterns of relationship within the genus, other than linear classifications. Selander's hypothesis (Fig. 3) was based upon

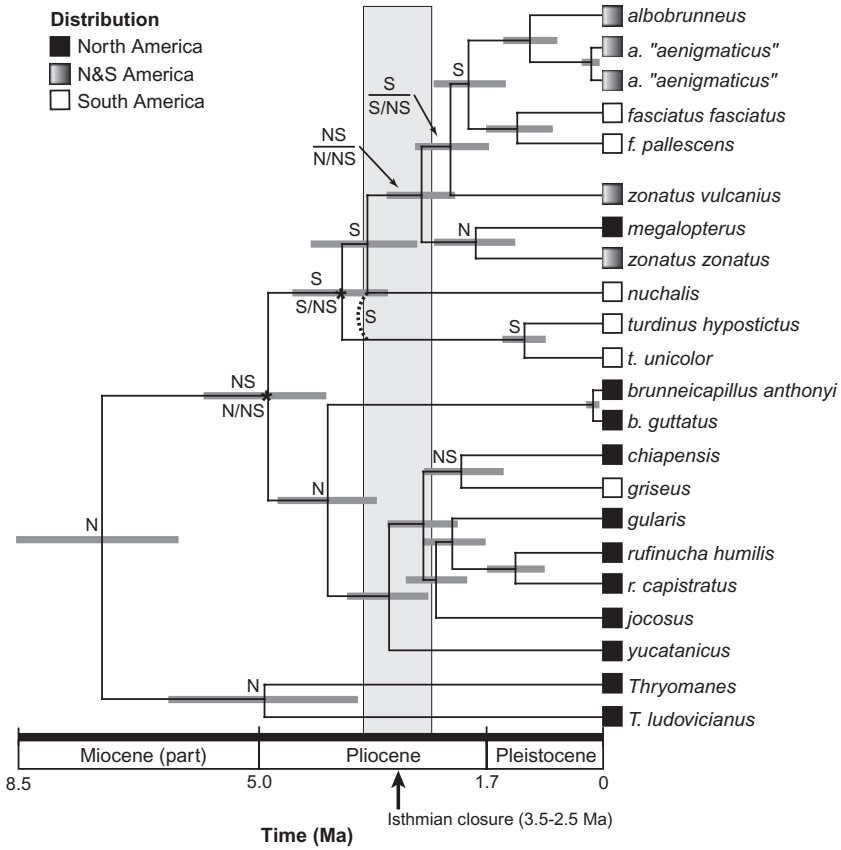

Figure 2. Biogeographical reconstructions and molecular clock calibration for divergences in *Campylorhynchus*. DIVA reconstructions for trees from Figure 1B, C are shown, respectively, above and below each branch. The single topological difference between these trees is indicated by the curved dashed line connecting *Campylorhynchus nuchalis* and *C. turdinus*: the S associated with this line indicates the optimal reconstruction when this grouping is assumed. Branches with only one reconstruction were optimized identically for both trees, and those with no reconstruction depicted share the same distribution as their immediate ancestor. Error bars are on nodal divergences are based on parametric bootstrapping of cytochrome *b* data under the assumption of a molecular clock (see Material and methods). The two nodes marked with asterisks require pre-Isthmian dispersals in at least some reconstructions.

his own morphometric studies of *Campylorhynchus* wrens, as well as his knowledge of their behaviour and ecology. Selander recognized two major 'groups' or subgenera within *Campylorhynchus*: one containing relatively large-bodied and short-winged and -tailed species tending to occupy drier habitats (Heleodytes group, Fig. 3), and the other containing relatively small-bodied, and long-winged and -tailed species preferring more forested habitats (Campylorhynchus group, Fig. 3). The existence of two major groupings within the genus, as originally defined by ecology and morphology, is largely supported by the molecular data (Figs 1, 3). The only ambiguity is caused by the relatively weak support for monophyly of the Heleodytes group in some analyses (Fig. 1), attributable to the large genetic distance of *C. brunneicapillus* from the other members of the subgenus. Other than this agreement on the basal split in the genus, few details of relationship among species corroborate Selander's hypothesis (Fig. 3).

Within these two groups, Selander recognized three superspecies complexes. The first of these, the superspecies *brunneicapillus*, comprises the species *brunneicapillus*, *jocosus*, and *yucatanicus* of the Heleodytes group. He erected this group on the basis of ecology (i.e. all three species are specialists in xeric habitats) as well as on morphology and behaviour. He considered *C. jocosus* and *C. yucatanicus* to be closely allied based on his morphometric data, as well as by plumage pattern (e.g. striping on the flanks), and vocal behaviour (individuals of both species participate in vocal duets). On the other hand, he considered tonal characteristics of the song (harsh, rhythmic, repetitive phrases) to indicate a similarity between *jocosus* and *brunneicapillus*. Notably, *C. yucatanicus* had previously been recognized as a subspecies of *brunneicapil-*

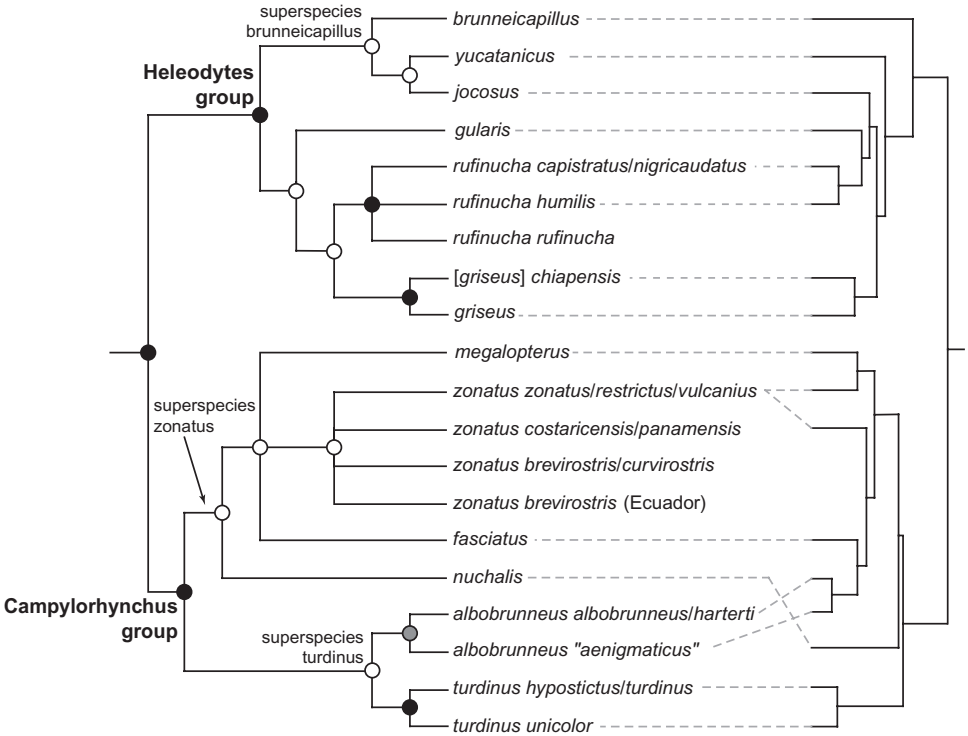

Figure 3. Hypothesis of relationships in the genus *Campylorhynchus* proposed by Selander (1964) (left), compared to a maximum likelihood estimate from molecular data (right; from Fig. 1B). Dashed lines connect comparable termini in the two trees, and nodes in Selander's hypothesis are marked with closed circles where the two trees agree, and with open circles where the two conflict (the grey circle subtending *Campylorhynchus albobrunneus* reflects the probable hybrid nature of the *Campylorhynchus albobrunneus aenigmaticus* samples; see text). The two subgenera or 'groups' are indicated, along with the three superspecies: allopatric clusters of subspecies or populations within *Campylorhynchus rufinucha*, *Campylorhynchus zonatus*, *Campylorhynchus turdinus*, and *Campylorhynchus albobrunneus* are represented as multiple unresolved lineages.

lus (Hellmayr, 1934). Although the molecular data support the splitting of *yucatanicus* from *brunneicapillus*, the three members of this superspecies are not sister taxa in any analysis, and *brunneicapillus* at least is strongly supported as distinct from the other two (Fig. 1). Although he did not recognize them as a superspecies, Selander also hypothesized a close relationship between *C. griseus* (including both *griseus* and *chiapensis*) and *rufinucha*, due mostly to clear similarities in plumage between the former and C. *rufinucha nigricaudatus* (white unmarked underparts, plain rufous back and a dark pileum), as well as some vocal similarities (use of a repeated triplet pattern in some songs). This relationship was not supported by any of the molecular analyses, although support for the optimal relationships among *rufinucha*, *griseus*, *jocosus*, and *gularis* was not strong, and varied among analyses.

The remaining two superspecies, *zonatus* and *turdinus*, are in the Campylorhynchus group. The large superspecies *zonatus* (comprising *zonatus*, *megalopterus*, *fasciatus*, and *nuchalis*) is a group of species with very similar plumage patterns, exhibiting prominently barred or striped backs, barred remiges, rectrices, and flanks, and spotted underparts. Selander placed the remaining two species in the Campylorhynchus group, *C. albobrunneus* and C. *turdinus*, together in superspecies *turdinus* on the basis of their shared simplified plumage pattern, both species showing complete (*albobrunneus*) or near-complete (*turdinus*) elimination of barring and striping on the upperparts. Additionally, *C. albobrunneus* and C. *turdinus unicolor* share immaculately unspotted underparts. The relationships inferred from molecular data for the Campylorhynchus group largely fail to support Selander's hypotheses (Figs 1, 3). A major reason for conflict between the molecular data and Selander's hypothesis is the placement of *C. albobrunneus*. Although Selander hypothesized *C. albobrunneus* to be the sister-group to C. *turdinus* based upon their shared pattern of plumage simplification (and although some taxonomies have placed these taxa in a single species; Paynter & Vaurie, 1960), the molecular data clearly support *C. albobrunneus* as a close

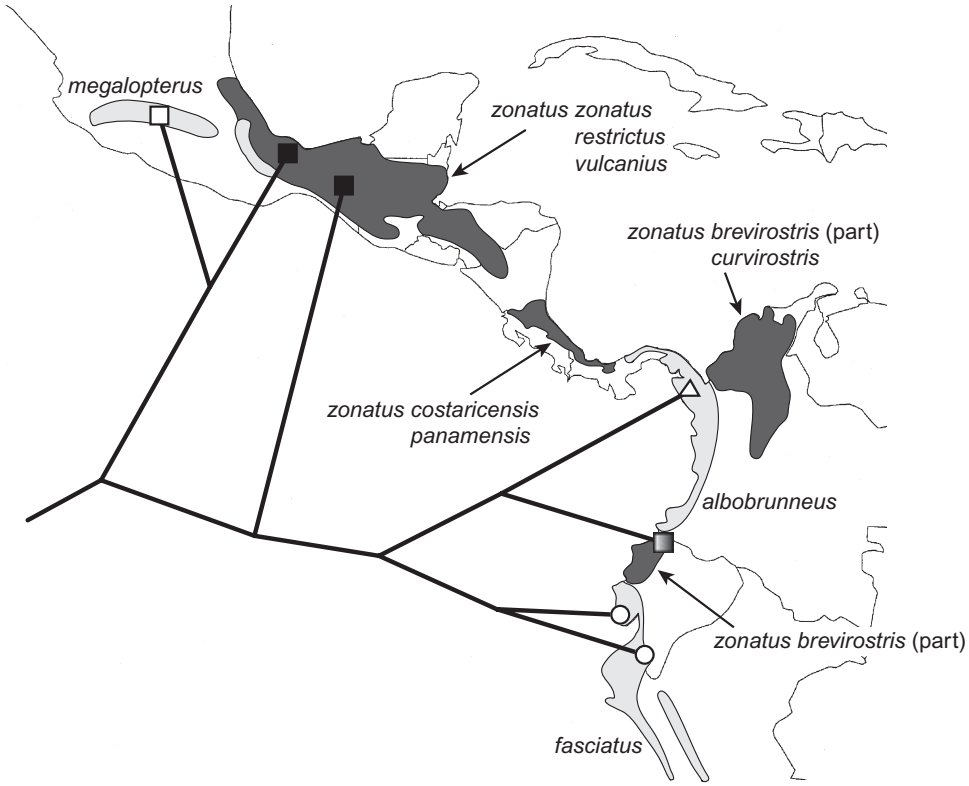

Figure 4. Relationships among samples from superspecies *zonatus*. Black squares indicate samples belonging to *zonatus*: subspecies disributed in each region are listed. Proceeding clockwise from the upper left: white square, *megalopterus*; white triangle, *albobrunneus*; white circles, *fasciatus*. The shaded square indicates the locality for samples of *albobrunneus* '*aenigmaticus*', probable hybrids between *albobrunneus* and Ecuadorian *zonatus*.

relative of *C. fasciatus*, violating monophyly of both superspecies. Additionally, the partitioned likelihood analysis recovered *C. nuchalis* as sister to C. *turdinus*, suggesting that this species is at best distantly related to other members of the *zonatus* superspecies (Fig. 1C).

Species boundaries within the Campylorhynchus group are of note. In particular, all analyses of the molecular data suggested paraphyly of the species *C. zonatus*. The sequence of *C. zonatus zonatus* from Veracruz was found to be sister to the sequence of *C. megalopterus*, whereas the sequence of *C. zonatus vulcanius* from Chiapas was found to be sister to a clade containing *fasciatus* and *albobrunneus*. Although the bootstrap support for this arrangement was moderate (Fig. 1), application of the test of Shimodaira and Hasegawa (Shimodaira & Hasegawa, 1999) failed to yield a significant difference between the homogeneous ML tree and trees where the monophyly of *C. zonatus* was constrained ($\delta = 8.12$, $P = 0.18$; 10 000 RELL replicates). Additionally, the '*aenigmaticus*' sequences, which might represent *C. zonatus brevirostris* from Ecuador, depending upon to which hybrid parent they are referable, were strongly supported as sisters to *albobrunneus*, although substantially differentiated (2.9% sequence divergence). Although this pattern of relationships was unexpected, it is remarkably congruent with spatial relationships among the populations involved (Fig. 4). Relationships in this group are consistent with the fragmentation of a single, widely-distributed ancestor by multiple vicariant events, with little or no crossed dispersal of putative vicariant derivatives. Unfortunately, interpretation of this pattern is weakened by the absence from the present study of samples of *C. costaricensis/panamensis* from Central America and of *C. zonatus brevirostris/curvirostris* from Colombia, and by the uncertain specific designation of the '*aenigmaticus*' samples from Ecuador. More extensive sampling of this group should provide additional insights.

HISTORICAL BIOGEOGRAPHY OF *CAMPYLORHYNCHUS*

It has been suggested that the subfamily Troglodytinae (the wrens) had its origin in North America (Mayr, 1946, 1964). However, no formal analysis of the biogeography of the group has yet been made, and both the

directionality and timing of wren dispersals remain in question. Dispersal-vicariance analysis of the distributions of *Campylorhynchus* and its sister taxa (*T. bewickii* and *T. ludovicianus*), based on a best-fit hypotheses of relationship (Fig. 1B, C) provides the first quantitative evidence that the entire assemblage had its origin in North America (Fig. 2). The novel grouping of exclusively North American species which forms the sister group to *Campylorhynchus* (Barker, 2004) could contain some South American forms of *Thryothorus*, potentially equivocating the northern ancestry of this group; however, nearly complete species sampling has not identified any such members (Mann *et al.*, 2006). This reconstruction reinforces the notion that wrens as a whole had their origin in North America, though a definitive analysis awaits additional data on the phylogeny of wren genera (Barker, 2004). The ancestral area for the genus *Campylorhynchus per se* is equivocal (Fig. 2). Namely, the Heleodytes group, whose only South American member is *C. griseus*, is unequivocally North American in origin, whereas the ancestral area for the Campylorhynchus group is reconstructed as either widespread through North and South America (homogeneous ML tree) or exclusively southern. At the root node of the genus, either a widespread or northern distribution is reconstructed, depending upon the topology preferred (Fig. 2). This suggests that, although the group may have had a northern origin, dispersal into South America likely occurred early in its history. Distributional data alone cannot address the timing of the dispersals which occurred, and thus the potential importance of terrestrial corridors. However, a combination of biogeographical analysis of *Campylorhynchus* phylogeny with clock analyses of the molecular data allows a critical test of the terrestrial dispersal hypothesis.

In Figure 2, the preferred maximum likelihood trees (i.e. those obtained from homogeneous- and partitioned-model ML analyses) have been plotted with branch lengths based on the cytochrome *b* data, optimized under the constraint of the molecular clock. The associated date estimates are presented for each node, along with an estimate of stochastic error. Interpretation of these values must be approached with caution because the dates may be biased by failing to incorporate estimates of ancestral polymorphism, and the associated errors are conservative because they do not include error associated with the calibration. The estimated divergence times in Figure 2 have interesting implications for the history of diversification in the genus. They indicate that the genus *Campylorhynchus* originated in the Late Miocene (~8 Mya), whereas diversification of the extant lineages appears to have commenced in the latest Miocene (~5 Mya). These estimates significantly predate even the earliest estimates of the completion of the Panamanian isthmus 3.5–2.5 Mya (Coates & Obando, 1996), suggesting that the formation of the genus and its two major morphological ecotypes (the Heleodytes and Campylorhynchus groups) occurred prior to the presence of a terrestrial corridor between the continents (see above). Formation of these two morphotypes may have been associated with early dispersal of the Campylorhynchus group into South America (Fig. 2), but this inference is dependent upon which of two statistically indistinguishable topologies is taken as optimal. At the earliest, one or more Campylorhynchus group lineages appear to have dispersed into South America some 4.7 ± 0.9 Mya [95% confidence interval (CI)]. Sometime subsequent to the earliest inferred dispersals, additional *Campylorhynchus* lineages invaded South America. However, these later dispersals probably did not require crossing of a water barrier. Within the Campylorhynchus group, optimization of continental distributions by dispersal-vicariance analysis yields multiple equally parsimonious scenarios of north–south dispersal, but detailed interpretation of these patterns is not warranted given the uncertain status of relationships in superspecies *zonatus*. Within the Heleodytes group, only *C. griseus* represents a dispersal into South America. Molecular dating of the split between *C. chiapensis* and C. *griseus* is consistent with dispersal of their common ancestor across the Isthmus after the estimated time of final closure (Fig. 2; $2.7 \geq 2.1 \geq 1.5$ Mya, 95% CI), with a subsequent split into the extant lineages.

The history of diversification of *Campylorhynchus* wrens parallels that inferred in studies of some northern mammal groups, that have indicated differentiation in North and Middle America prior to the invasion of South America, including dogs (Canidae; Wayne *et al.*, 1997), and possibly cricetid rodents (Smith & Patton, 1993; Engel *et al*., 1998). Surprisingly, given the high degree of vagility assumed, inferred, or known for many avian species (Voelker, 1999), it is difficult to demonstrate that more than a single dispersal event within *Campylorhynchus* occurred prior to closure of the Panamanian isthmus. However, the available biogeographical data, in combination with molecular clock estimates, suggest that one lineage of *Campylorhynchus* invaded South America before the availability of a terrestrial corridor, as has been suggested for some mammal groups based on fossil dates (Procyonidae; Webb, 1985), degree of morphological disparity (cricetid rodents; Hershkovitz, 1969), and molecular divergences (cricetid rodents; Engel *et al*., 1998). More importantly, this genus joins the growing list of avian taxa bearing evidence of the history of dispersal between North and South America.

As discussed above, phylogenetic studies of bird groups distributed on either side of the Panamanian

Isthmus are of particular interest because they have the potential to yield insights into the continental origins and dispersal histories of the Neotropical avifauna. The phylogeny of *Campylorhynchus* presented here adds to the tally of genera with complete (or nearly so) species-level molecular phylogenetic hypotheses. This list includes the genera *Ramphocelus* (Hackett, 1996), *Piranga* (Burns, 1998), *Icterus* (Omland, Lanyon & Fritz, 1999), *Carduelis* (Arnaiz-Villena *et al.*, 1998), *Anthus* (Voelker, 1999), *Cinclus* (Voelker, 2002), *Myiarchus* (Joseph *et al.*, 2004), *Zenaida* (Johnson & Clayton, 2000), *Cyanocompsa* (Klicka *et al*. 2001), *Catharus* (Outlaw *et al*. 2003), *Buteo* (Riesing *et al*., 2003), and *Tangara* (Burns & Naoki, 2004). Many of these previous studies have not examined the question of isthmian interchange, focusing instead on other aspects of Neotropical biogeography (e.g. *Ramphocelus*, *Tangara*), evolution of organismal characteristics (e.g. *Piranga*, *Icterus*), or phylogeny *per se* (e.g. *Buteo*). Others have explicitly examined both biogeography and dating, but yielded ambiguous results due to complex distribution patterns (*Catharus*; Outlaw *et al.*, 2003), or evolutionary rate heterogeneity (*Myiarchus*; Joseph *et al.*, 2004). Studies of *Cinclus* (Voelker, 2002) and *Zenaida* (Johnson & Clayton, 2000) failed to exclude overland dispersal (estimated dispersal times of 3.5–2.5 and 2.7–2 Mya, respectively), and the directionality of dispersal was ambiguous in both. By contrast, the study of *Anthus* relationships (Voelker, 1999) explicitly dated interchange between North and South America at approximately 6 Mya, based on divergences among South American endemic species, although the directionality of dispersal was ambiguous in that case as well. However, the study did unambiguously indicate a later dispersal of the North American *Anthus spragueii* from South America prior to isthmian closure (~4.9 Mya). In addition to these genus-level studies, two 'species'-level studies focusing on taxa distributed across the isthmus have suggested dispersal times consistent with terrestrial corridors (*Glyphorhynchus spirurus*, Marks, Hackett & Capparella, 2002; *Phaeothlypis* spp., Lovette, 2004), whereas a third (*Chlorospingus opthalmicus*; García-Moreno *et al*., 2004) indicated overwater dispersal (although the latter study did not include samples from Costa Rica and Panama that might be close to the South American populations, reducing the inferred age of dispersal).

Data on the timing and direction of avian dispersal between North and South America are just beginning to accumulate, and as yet no clear trends can be discerned. However, judging from early returns, it does not seem likely that birds responded uniformly to the absence and subsequent origin of the isthmian connection. It has been suggested that northern oscine passerines have experienced greater evolutionary success in the south than southern suboscines have in the north due to demographic differences favoured by selection in temperate and tropical source areas (Ricklefs, 2002). Similar processes have been invoked in explaining the differential success of northern and southern mammal groups (Marshall *et al*., 1982; Webb, 1985). Establishing the timing and direction of dispersal of passerine groups is a critical step in addressing such hypotheses. Studies of many additional bird lineages (passerine and otherwise) will be necessary in order to evaluate quantitatively the extent of avian participation in isthmian interchange and the evolutionary and ecogeographical contexts of dispersal. More generally, synthesis of these studies will clarify the degree to which peculiarities of avian biology may have resulted in contrasting historical patterns in the face of a barrier so critical in mammalian history.

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