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Phylogenetic relationships of woodcreepers (Aves: Dendrocolaptinae) – incongruence between molecular and morphological data

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The woodcreepers is a highly specialized lineage within the New World suboscine radiation. Most systematic studies of higher level relationships of this group rely on morphological characters, and few studies utilizing molecular data exist. In this paper, we present a molecular phylogeny of the major lineages of woodcreepers (Aves: Dendrocolaptinae), based on nucleotide sequence data from a nuclear non-coding gene region (myoglobin intron II) and a protein-coding mitochondrial gene (cytochrome *b*). A good topological agreement between the individual gene trees suggests that the resulting phylogeny reflects the true evolutionary history of woodcreepers well. However, the DNA-based phylogeny conflicts with the results of a parsimony analysis of morphological characters. The topological differences mainly concern the basal branches of the trees. The morphological data places the genus *Drymornis* in a basal position (mainly supported by characters in the hindlimb), while our data suggests it to be derived among woodcreepers. Unlike most other woodcreepers, *Drymornis* is ground-adapted, as are the ovenbirds. The observed morphological similarities between *Drymornis* and the ovenbird outgroup may thus be explained with convergence or with reversal to an ancestral state. This observation raises the question of the use of characters associated with locomotion and feeding in phylogenetic reconstruction based on parsimony.

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The woodcreepers (Dendrocolaptinae sensu Irestedt et al. 2002) constitute a highly specialized lineage within the New World suboscine radiation that usually is regarded as the sister-group to ovenbirds (Furnariinae), excluding the genus *Sclerurus* (Irestedt et al. 2002). The members of the clade primarily forage along trunks and branches, and this lifestyle has made woodcreepers similar to woodpeckers in appearance. Due to their common lifestyle woodcreepers form a morphologically homogeneous assemblage and several features in woodcreepers are certainly adaptations to their climbing behaviour, e.g. the inwards curved, stiffened tail-tips and the partly fused forwards-directed phalanges. However, the group also exhibits great variations in size and in bill morphology, such as a short chisel-shaped bill in *Glyphorhynchus* ("wedgebill"), large, powerful bills suited for tearing bark and grasping grubs and beetles in *Xiphocolaptes*, and slender and curved bills suited for probing in deep crevices or among epiphytes in *Campylorhamphus*.

Feduccia (1973) described some woodcreeper genera (*Dendrocincla*, *Sittasomus*, *Deconychura* and *Glyphorynchus*) as being morphologically "intermediate" between presumed primitive (philydorine) ovenbirds and

the typical (''strong-billed'') woodcreepers, and he therefore postulated that woodcreepers might be nested within the ovenbird group. Later studies, based on both molecular (Sibley and Ahlquist 1990, Irestedt et al. 2002) and morphological data (Raikow 1994, Clench 1995), supported the monophyly of woodcreepers. However, the molecular evidence provided in Irestedt et al. (2002) demonstrates that the situation is indeed more complex than previously assumed, with the ovenbird genus *Sclerurus* positioned basally to other ovenbirds and woodcreepers.

Relationships amongst woodcreepers are far from clear. Feduccia's (1973) distinction between ''intermediate'' and ''strong-billed'' woodcreepers is in good agreement with behavioural data (as described in several single-species accounts by Willis 1974) and with some molecular studies (Sibley and Ahlquist 1990, Aleixo 2002, Irestedt et al. 2002), although these latter lack several genera. On the other hand, a morphological study by Raikow (1994), using hindlimb muscles and the structure of bill and nostrils, lead to a different phylogenetic hypothesis. For example, Raikow's analysis placed the ground-feeding *Drymornis* as the deepest branch within the woodcreeper radiation, while Feduccia (1973) placed it among the advanced, ''strong-billed'' woodcreepers.

In this paper we use DNA sequence data obtained from both the nuclear and mitochondrial genomes to generate a hypothesis for evolutionary relationships among all genera and species groups of woodcreepers. This phylogeny is then compared to Feduccia's and Raikow's hypotheses. We have also evaluated the evolution of certain morphological characters related to feeding and locomotory adaptations by mapping them onto the molecular tree.

Material and methods

Taxon sampling, amplification and sequencing

A total of twenty-three woodcreeper species, representing all genera (taxonomy following Ridgely and Tudor 1994), was selected for the molecular analyses. We included multiple species from the speciose genera *Xiphorhynchus* (with 11 currently accepted species) and *Lepidocolaptes* (with 6 species) whose monophyly are in doubt (Raikow 1994, García-Moreno and da Silva 1997, Aleixo 2002). The phylogenetic trees were rooted by outgroup comparison (Farris 1972, cf. Nixon and Carpenter 1993), using representatives from major ovenbirds lineages suggested by Irestedt et al. (2002). Sample identifications and GenBank accession numbers are given in Table 1. The entire myoglobin intron II (varying in length from 674 bp in *Campylorhamphus trochilirostris* to 694 bp in *Anumbius annumbi*) and 999 bp of the cytochrome *b* gene were sequenced. The myoglobin intron II correspond to the region between positions 303 (exon II) and 400 (exon III) in humans (GenBank accession number XM009949) and the sequences obtained from the cytochrome *b* gene corresponds to positions 15037 to 16035 in the chicken mitochondrial genome sequence (Desjardins and Morais 1990). No insertions or deletions, stop or nonsense codons were observed in any of the cytochrome *b* sequences. The aligned sequences include totally 1696 positions.

Extraction, amplification and sequencing procedures for myoglobin intron II and cytochrome *b* follow the descriptions in Irestedt et al. (2002). Note that the internal myoglobin primers Myoint.c and Myoint.nc do not work for woodcreepers, as the binding sites are located at a region were all woodcreepers have an insertion. For each gene and taxon, multiple sequence fragments were obtained by sequencing with different primers. These sequences were assembled to complete sequences with SeqMan II™ (DNASTAR Inc.). Positions where the nucleotide could not be determined with certainty were coded with the appropriate IUPAC code. Thanks to a low number of insertions in myoglobin intron II the combined sequences could easily be aligned by eye in MegAlign™ (DNASTAR Inc.).

From all taxa (both genes) the PCR-products were used directly in the sequencing procedure. However, as *Xiphorhynchus guttatus* and *Xiphorhynchus obsoletus* produced partly unreadable sequences for the myoglobin intron II, the PCR products from these two taxa were cloned. The cloning, amplification and sequencing were done with the TOPO TA Cloning® Kit (Invitrogen Life Technologies), using the manufacturer's primers and protocol. Myoglobin belongs to the globin gene-family, which also includes β-haemoglobin and α-haemoglobins. Although it is supposedly single-copy, all five clones in *Xiphorhynchus guttatus* and four out of five clones in *Xiphorhynchus obsoletus* differed from each other. An insertion of one base pair was observed in three of the *Xiphorhynchus guttatus* clones, while one of the *Xiphorhynchus obsoletus* clones had a deletion of two base pairs. These indels were situated at the positions where the sequences from the uncloned PCR-products became unreadable and may explain the reading problems in the latter. Although the differences between clones is likely to be due to PCR errors, the rather large (up to 1%) sequence distances observed between the clones with and without indels, respectively, does not exclude the possibility that the myoglobin gene is multi-copy or is due the occurrence of pseudogenes. The influence of the occurrence of the different clones upon the phylogenetic results was estimated by including all clones in certain analyses.

Table 1. Samples used in the study. Subfamily names follow the classification of Irestedt et al. (2002). Dendrocolaptinae, Furnariinae and Sclerurinae are all subfamilies of Furnariidae. Abbreviations: AMNH = American Museum of Natural History; FMNH = Field Museum of Natural History, Chicago; LSUMZ = Museum of Natural Science, Louisiana State University; NRM = Swedish Museum of Natural History; ZMUC = Zoological Museum of the University of Copenhagen. [a] Irestedt et al. (2002), [b] Johansson et al. (2002). [c] Ericson et al. (2002).

Species	Subfamily	Sample no.	myoglobin	cytochrome *b*
Campylorhamphus trochilirostris	Dendrocolaptinae	NRM 947183	AY442961	AY442987
Campylorhamphus pusillus	Dendrocolaptinae	ZMCU S1451	AY442962	AY442988
Dendrexetastes rufigula	Dendrocolaptinae	FMNH 389815	AY442973	AY443001
Dendrocinca fuliginosa	Dendrocolaptinae	NRM 976662	AY065770 [a]	AY065713 [a]
Dendrocincla merula	Dendrocolaptinae	ZMCU S1251	AY442960	AY442986
Dendrocincla tyrannina	Dendrocolaptinae	ZMCU S1110	AY442959	AY442985
Deconychura longicauda	Dendrocolaptinae	ZMCU S1249	AY442963	AY442989
Dendrocolaptes certhia	Dendrocolaptinae	ZMCU S1253	AY442965	AY442991
Dendrocolaptes platyrostris	Dendrocolaptinae	NRM 976714	AY442964	AY442990
Drymornis bridgesii	Dendrocolaptinae	NRM 966930	AY065768 [a]	AY065711 [a]
Glyphorynchus spirurus	Dendrocolaptinae	ZMCU S1521	AY442966	AY442992
Hylexetastes perrotii	Dendrocolaptinae	LSUMZ B 13841	AY442974	AY443002
Lepidocolaptes affinis	Dendrocolaptinae	ZMCU S1106	AY442968	AY442994
Lepidocolaptes angustirostris	Dendrocolaptinae	NRM 937184	AY065767 [a]	AY078175 [b]
Lepidocolaptes fuscus	Dendrocolaptinae	NRM 937283	AY442967	AY442993
Nasica longirostris	Dendrocolaptinae	ZMCU S1831	AY442969	AY442995
Sittasomus griseicapillus	Dendrocotaptinae	NRM 967031	AY065771 [a]	AY065714 [a]
Xiphocolaptes major	Dendrocolaptinae	NRM 966847	AY065769 [a]	AY065712 [a]
Xiphocolaptes promeropirhynchus	Dendrocolaptinae	ZMCU S38	AY442970	AY442996
Xiphorhynchus erythropygius	Dendrocolaptinae	ZMCU S1616	AY442971	AY442997
Xiphorhynchus guttatus	Dendrocolaptinae	ZMCU S1523	AY442975–AY442979	AY442998
Xiphorhynchus triangularis	Dendrocolaptinae	ZMCU S45	AY442972	AY442999
Xiphorhyncnus obsoletus	Dendrocolaptinae	ZMCU S1261	AY442980–AY442984	AY443000
Anumbius annumbi	Furnariinae	NRM 966903	AY065765 [a]	AY065709 [a]
Furnarius cristatus	Furnariinae	NRM 966772	AY064279 [c]	AY078175 [c]
Lochmias nematura	Furnariinae	ZMCU S2577	AY065755 [a]	AY065699 [a]
Philydor atricapillus	Furnariinae	NRM 937334	AY065758 [a]	AY065702 [a]
Pygarrhichas albogularis	Furnariinae	AMNH PRS1128	AY065760 [a]	AY065704 [a]
Synallaxis ruficapilla	Furnariinae	NRM 956643	AY065763 [a]	AY065707 [a]
Sclerurus scansor	Sclerurinae	NRM 937258	AY065772 [a]	AY065715 [a]

Phylogenetic analyses

Bayesian analyses, using the Markov chain Monte Carlo method, were performed with MrBayes 2.01 (Huelsenbeck and Ronquist 2001) both for the genes separately and combined. All clones from *Xiphorhynchus guttatus* and *Xiphorhynchus obsoletus* were included in the analysis of the myoglobin data set. As these clones contained similar taxon specific, phylogenetic signals (see Results), we used the consensus sequences for the combined data set. Appropriate IUPAC codes were used for conflicting positions in the clones.

Models for nucleotide substitution patterns of the genes were selected with the likelihood ratio test implemented in Modeltest 3.06 (Posada and Crandall 1998). For myoglobin the Bayesian analysis was performed with the HKY model with a discrete approximation of gamma distribution rates (HKY+G). The analyses of cytochrome *b* and the combined data set were performed with the general-time-reversible model with assumed proportion of the invariable sites estimated and a discrete approximation of gamma distribution rates at the variable sites (GTR+I+G). The Markov chain Monte Carlo process was set so that four chains ran simultaneously for 500,000 generations, with trees sampled every 10th generations (resulting in 50,000 trees). The analyses were initiated at a random starting tree. The likelihood scores were plotted against the generation number to investigate when the Markov chain Monte Carlo process had reached stationarity (i.e., when the likelihood scores no longer improve). Stationarity was reached at 3000 trees for the myoglobin data set, 4000 trees for that of cytochrome *b*, and 8000 trees for the combined data set, respectively. These trees were discarded and the posterior probability values were calculated from the remaining trees.

Parsimony analyses were also performed for the combined molecular data set, using Paup*4.0b10 (Swofford 1998). Searches for the most parsimonious trees were performed under the heuristic search option with all characters coded as unordered. To reduce the risk of finding local optima only, all analyses were performed with ten random additions of taxa. Bootstrap support values were calculated from 1000 replicates. It has been shown that the regions of myoglobin analysed herein are not saturated at the levels of sequence divergence observed in the present data set (Ericson et al. 2003). The degree of saturation in the cytochrome *b* data set was investigated by plotting the number of transition and transversion substitutions against pairwise genetic distances calculated for the myoglobin sequences. The analysis showed a non-linear correlation between the

axes for the third codon positions, indicating that the cytochrome *b* gene is saturated for the most distantly related taxa. The cytochrome *b* data set were therefore analysed with transitions at third codon positions excluded.

As the morphological tree suggested by Raikow (1994) lack comparable bootstrap support values, parsimony analyses and calculation of bootstrap support values (from 1000 replicates) were also conducted from the 36 morphological characters scored by Raikow. However, species not included in the molecular data set were pruned from the morphological data set. We believe it is justified to base our comparisons on this taxonomically pruned data set since no topological differences were observed between the strict consensus tree obtained in the parsimony analysis of these data, and the tree published by Raikow (1994) based on the full data set (see below).

Of the 36 characters used by Raikow (1994), 22 derive from the myology of the hindlimb, nine are coded from variation in bill and nostril morphology, three describe variation in the structure of the tail feathers, and one the ossification of tendons in the leg (Raikow 1994: appendix I). In order to estimate the level of homoplasy in this data set the number of steps, consistency (CI) and retention indices (RI) were calculated for the individual characters on the phylogenetic tree obtained in the Bayesian inference analysis of the combined molecular data set and Raikow′s morphological phylogeny (pruned as described above), respectively.

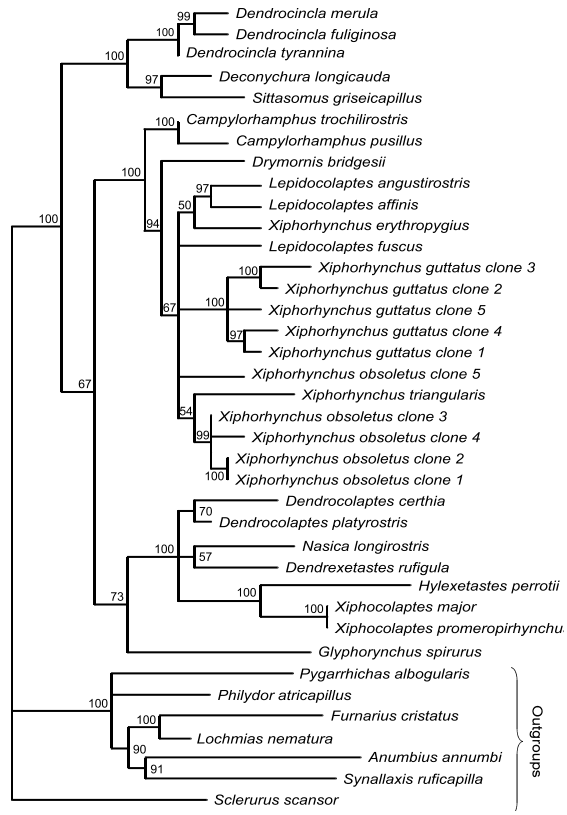

Fig. 1. Majority rule consensus tree obtained from the Bayesian analysis of myoglobin intron II. Posterior probability values from the Bayesian analysis are indicated at the nodes.

Results

A molecular phylogeny of the woodcreepers

All clones of *Xiphorhynchus guttatus* and four out of five *Xiphorhynchus obsoletus* clones grouped together in the Bayesian analyses of the myoglobin data set (Fig. 1). The fifth clone of *X. obsoletus* was placed outside a clade consisting of all other *X. obsoletus* clones and *X. triangularis*, but this arrangement received low nodal support (54%). In subsequent analyses, *X. guttatus* and *X. obsoletus* are represented by the consensus sequence based on the clones of each species.

There is a general congruence between the phylogenetic trees resulting from the Bayesian analyses of the myoglobin intron II (Fig. 1) and cytochrome *b* (Fig. 2) data sets. In both trees, the woodcreepers are recovered as monophyletic with strong support (100% posterior probability), as are three major clades of woodcreeper genera (100% each in both gene trees). The first clade includes the genera *Dendrocincla*, *Deconychura* and *Sittasomus*, the second clade *Campylorhamphus*, *Drymornis*, *Lepidocolaptes* and *Xiphorhynchus*, and the third clade *Dendrocolaptes*, *Dendrexetastes*, *Nasica*, *Hylexetastes*, and *Xiphocolaptes*. Both the myoglobin and cytochrome *b* gene tree also support a dichotomy between the first clade (*Dendrocincla*, *Deconychura* and *Sittasomus*) on one hand, and the other two clades on the other. This arrangement receives 67% and 100% posterior probability in the myoglobin and cytochrome *b* trees, respectively). The relationships within each of the three clades are also similar in the individual gene trees, and the nodes that do differ often involve short nodes that receive low posterior probabilities in at least one of the gene trees. The most obvious difference between the trees is in the position of *Glyphorynchus*. The cytochrome *b* data set strongly supports *Glyphorynchus* to be basal to all other woodcreepers (97% posterior probability), while the myoglobin intron II places *Glyphorynchus* two nodes up from this basal position, as the sister taxon to the third clade (consisting of *Dendrocolaptes*, *Dendrexetastes*, *Nasica*, *Hylexetastes* and *Xiphocolaptes*). The two nodes receive posterior probability values of 67% and 73%, respectively.

The phylogenetic tree that resulted from a Bayesian analysis of the combined data set is generally well supported with high posterior probability values (Fig. 3). The basal position of *Glyporhynchus* is now strongly supported (91%), as are each of the three major clades of

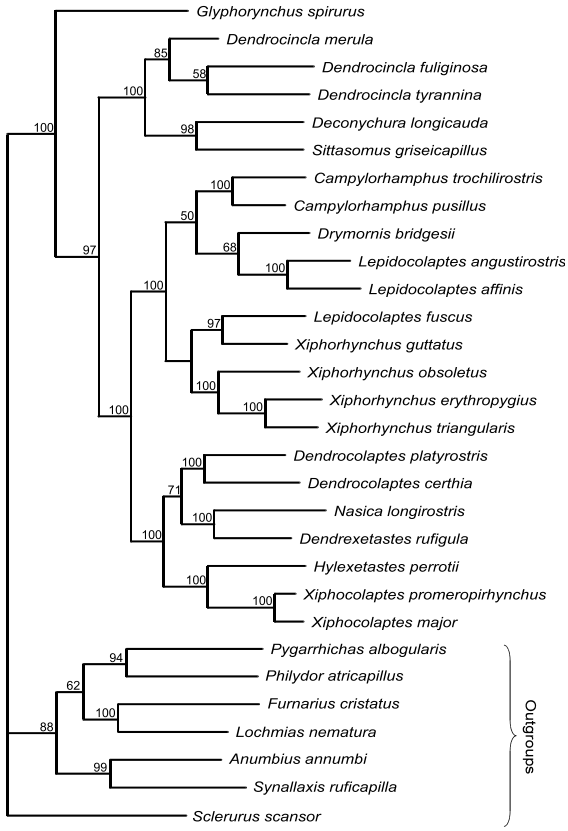

Fig. 2. Majority rule consensus tree obtained from the Bayesian analysis of cytochrome *b*. Posterior probability values from the Bayesian analysis are indicated at the nodes.

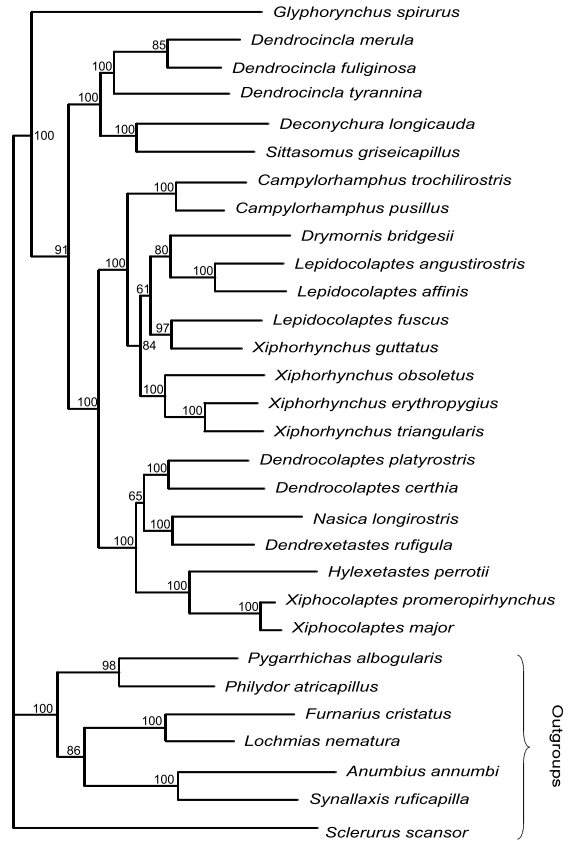

Fig. 3. Majority rule consensus tree obtained from the Bayesian analysis of the combined data set (myoglobin intron II and cytochrome *b*). Posterior probability values from the Bayesian analysis are indicated at the nodes.

woodcreeper genera suggested in the individual gene trees. Also the clade with species traditionally placed in the genera *Campylorhamphus*, *Drymornis*, *Lepidocolaptes* and *Xiphorhynchus* is well resolved. Neither *Lepidocolaptes* nor *Xiphorhynchus* were recovered as monophyletic. For example, a sister group relationship between *Lepidocolaptes fuscus* and *Xiphorhynchus guttatus* was strongly supported (97%) in the Bayesian analysis.

The phylogenetic tree obtained from the parsimony analyses of the combined data set with transitions at third codon positions excluded in cytochrome *b* (tree not shown) is topologically very similar to the Bayesian tree from the combined molecular data set. Minor topological conflicts occur (*Drymornis* being placed with the two *Campylorhamphus* species and *Dendrocincla merula* being basal in its genus), but the major structure is the same. Most relationships are also well supported by high bootstrap values. For example, the monophyly of the woodcreeper clade, the basal position of *Glyphorynchus*; the division other woodcreeper genera into three major clades; and the basal position of the *Dendrocincla-Deconychura-Sittasomus* clade in relation to the other two major clades are all supported by bootstrap values above 90%. The overall high nodal support observed in the trees calculated from the combined data set, the close resemblance between the topologies of the nuclear and mitochondrial gene trees, and the general topological agreement between our molecular phylogenies and most other studies of phylogenetic relationships of woodcreepers, suggest that Fig. 3 is a good estimate of the true phylogenetic branching pattern among woodcreepers. The analysis of the homoplasy levels in the morphological characters for these taxa is thus based on this combined molecular tree.

Comparisons with morphological data

The analysis of the taxonomically pruned data set resulted in a strict consensus tree that is fully congruent with the tree that includes all taxa studied by Raikow (1994). The basal position of *Drymornis* in relation to other woodcreepers receives a bootstrap support value of 94%, while the position of *Nasica* as sister to the

remaining woodcreepers has a 76% bootstrap support. Several other relationships are also supported by bootstrap values exceeding 50%. Another area of interest to the present study includes the representatives of *Dendrocincla*, *Deconychura*, *Sittasomus* and *Glyphorynchus*. The branching patterns among these taxa are identical to those in Raikow (1994), but they receive no bootstrap support values above 50%.

When mapping the morphological characters used by Raikow (1994) onto the molecular tree, the total number of steps for all characters is 126 (CI = 0.3810, RI = 0.5761). The corresponding figure for the most parsimonious tree based on morphological data is 101 (CI = 0.4752, RI = 0.7120).

Of the thirty-six characters included in the morphological data set only one character (the shape of the culmen of the bill) showed a somewhat better fit to the molecular tree. Of the remaining thirty-five characters, twelve had an equal fit to both trees while the remaining twenty-three had a worse fit to the molecular tree. Divided into morphological categories, a majority of the bill and nostril characters had an equal or only marginally worse fit to the molecular tree than to the morphological tree (Table 2). In contrast, most myological and tail characters showed a worse fit to the molecular tree.

Discussion

The phylogenetic tree calculated from the DNA sequences herein is in broad agreement with previously published results based on molecular data (if taken into account that several woodcreeper genera were missing from these other analyses). For example, the observation that the current allocation of species to the genera *Lepidocolaptes* and *Xiphorynchus* does not accurately mirror their actual relationships, has previously been reported based on mtDNA (García-Moreno and da Silva 1997, Aleixo 2002). Also, the higher level relationships depicted in Fig. 4 of Aleixo (2002) closely resemble those in Fig. 3 herein, but note that no outgroup was used in the study of Aleixo (2002). In fact, no explanation to why the tree was drawn with *Glyphorhynchus* in a basal position is given, but it is in agreement with the present study.

Although our molecular tree thus agrees well with previous DNA-based hypotheses of woodcreeper relationships, it conflicts in its deepest branches with the tree presented by Raikow (1994) based on morphological data. In the morphological tree, *Drymornis* is the first taxon to branch off from the other woodcreepers (with 94% bootstrap support), followed by a branch to *Nasica* (see Fig. 4B). In all DNA-based phylogenies *Drymornis* and *Nasica* are positioned several nodes (some of which have $>95\%$ support) further up in the tree, but not closely together. There is a considerably better agreement between the molecular and morphological trees at the more terminal nodes. For example, the genera *Hylexetastes* and *Xiphocolaptes* are closely related in both trees, with *Lepidocolaptes* close to them. Interestingly, if *Drymornis* and *Nasica* are excluded, the topology of the morphological tree agrees with that of our molecular tree in that *Dendrocincla*, *Deconychura*, *Glyphorynchus* and *Sittasomus* are positioned basal to the other woodcreeper genera. The node separating these groups receives a 67% bootstrap support in the re-analysis of the taxonomically pruned, morphological data set.

The molecular tree presented herein is also in good agreement with the relationships among woodcreepers suggested by Feduccia (1973). He interpreted the morphological variation in relation to two major adaptive shifts: (1) a suite of characters associated with hitching along tree-trunks and (2) specialization of the skull for more forceful feeding by secondary enforcement of the skull to protect it from strong lateral forces to which it is subjected in prying, probing and powerful grasping. For example, the molecular analysis supports both the monophyly of a group of "strong-billed" woodcreepers defined by Feduccia (1973), and his identification of "intermediates" (*Dendrocincla*, *Sittasomus*, *Deconychura* and *Glyphorhynchus*) with somewhat ovenbird characters. One important feature of the "intermediates" is their fairly light cranium, which functionally is intermediate towards the pseudo-schizorhinal ovenbird condition (with a delicate and pliable "rhynchokinetic" bill, where prokinetic movement at the fronto-nasal hinge is impaired by the extension of the interorbital septum in front of the mesethmoids, beneath the dorsal bar of the upper mandible). The "strong-billed" woodcreepers, in contrast, show a strongly ossified skull and a prokinetic and holorhinal condition, where the upper mandible can be moved forcefully about a single fronto-nasal hinge.

Contrary to Feduccia (1973) we find no support for the placement of *Glyphorhynchus* close to the "strong-billed" group (based on its broad frontal bones, strongly ossified interorbital septum, and habit of nesting in holes in trees). Instead, the basal position of *Glyphorhynchus* in the molecular phylogeny, suggests that this could be a case of convergent development were the cranial enforcement is related to the woodpecker-like habits of this species.

Although plumage variation is difficult to define as discrete character states in a way that is useful for phylogenetic analysis, the overall patterns of plumage variation is concordant with the molecular phylogenies. The "intermediate" woodcreepers are rather uniform brown (*Dentrocincla* and *Sittasomus*) or uniform with buff-spotted throat and head-sides (*Deconychura* and *Glyphorhynchus*), with broad rufous wing-bar (as in philydorine ovenbirds). The others are more conspicu-

Table 2. Measurements of fits (Cl consistency index, Rl retention index) for Raikow's (1994) morphological characters to the molecular tree (Fig. 3), compared to their fit to the most parsimonious tree calculated from these characters (the comparisons are made with the morphological tree in Fig. 4B that is taxonomically pruned to include the same taxa as in the molecular analysis).

Char no Raikow (1994)	Character	No. of states	No. of steps		Morphological tree			Molecular tree			Difference	
			Min no. of steps	Max no. of steps	No. of steps observed	Cl	Rl	No. of steps observed	Cl	Rl	Cl	Rl
20	Bill morphology	2	1	11	1	1.00	1.00	3	0.33	0.80	0.67	0.20
21	Bill morphology	2	1	8	2	0.50	0.86	2	0.50	0.86	0.00	0.00
26	Bill morphology	2	1	2	1	1.00	1.00	1	1.00	1.00	0.00	0.00
28	Bill morphology	4	3	13	7	0.43	0.60	8	0.38	0.50	0.05	0.10
29	Bill morphology	2	1	8	3	0.33	0.71	3	0.33	0.71	0.00	0.00
30	Bill morphology	3	2	9	6	0.33	0.43	6	0.33	0.43	0.00	0.00
31	Bill morphology	2	1	7	3	0.33	0.67	2	0.50	0.83	− 0.17	− 0.16
32	Bill morphology	2	1	9	3	0.33	0.75	4	0.25	0.63	0.08	0.12
36	Bill morphology	2	1	2	1	1.00	1.00	1	1.00	1.00	0.00	0.00
	Bill morphology, average					**0.58**	**0.78**		**0.51**	**0.75**	**0.07**	**0.03**
1	Myology	2	1	11	4	0.25	0.70	5	0.20	0.60	0.05	0.10
2	Myology	2	1	10	3	0.33	0.78	6	0.17	0.44	0.16	0.34
3	Myology	2	1	2	1	1.00	1.00	2	0.50	0.00	0.50	1.00
4	Myology	3	2	4	2	1.00	1.00	2	1.00	1.00	0.00	0.00
5	Myology	2	1	7	3	0.33	0.67	4	0.25	0.50	0.08	0.17
6	Myology	2	1	5	1	1.00	1.00	2	0.50	0.75	0.50	0.25
7	Myology	2	1	2	1	1.00	1.00	2	0.50	0.00	0.50	1.00
8	Myology	2	1	2	1	1.00	1.00	2	0.50	0.00	0.50	1.00
9	Myology	4	3	6	3	1.00	1.00	4	0.75	0.67	0.25	0.33
10	Myology	3	2	9	5	0.40	0.57	7	0.29	0.29	0.11	0.2B
11	Myology	2	1	5	1	1.00	1 00	2	0.50	0.75	0.50	0.25
12	Myology	2	1	10	2	0.50	0.89	3	0.33	0.78	0.17	0.11
13	Myology	2	1	2	2	0.50	0.00	2	0.50	0.00	0.00	0.00
14	Myology	3	2	5	4	0.50	0.33	5	0.40	0.00	0.10	0.33
15	Myology	2	1	10	3	0.33	0.78	6	0.17	0.44	0.16	0.34
16	Myology	3	2	15	7	0.29	0.62	9	0.22	0.46	0.07	0.16
17	Myology	3	2	11	5	0.40	0.67	6	0.33	0.56	0.07	0.11
18	Myology	2	1	10	3	0.33	0.78	5	0.20	0.56	0.13	0.22
19	Myology	2	1	8	2	0.50	0.86	2	0.50	0.86	0.00	0.00
23	Myology	2	1	2	1	1.00	1.00	2	0.50	0.00	0.50	1.00
24	Myology	2	1	2	2	0.50	0.00	2	0.50	0.00	0.00	0.00
25	Myology	2	1	2	1	1.00	1.00	2	0.50	0.00	0.50	1.00
	Myology, average					**0.64**	**0.76**		**0.42**	**0.39**	**0.22**	**0.36**
33	Tail morphology	2	1	3	1	1.00	1.00	1	1.00	1.00	0.00	0.00
34	Tail morphology	2	1	6	1	1.00	1.00	4	0.25	0.40	0.75	0.60
35	Tail morphology	2	1	8	2	0.50	0.86	5	0.20	0.43	0.30	0.43
	Tail morphology, average					**0.83**	**0.95**		**0.48**	**0.61**	**0.35**	**0.34**
22	Relative length of toes	3	2	2	2	1.00	0.00	2	1.00	0.00	0.00	0.00
27	Tendon ossification	3	2	4	2	1.00	1.00	2	1.00	1.00	0.00	0.00

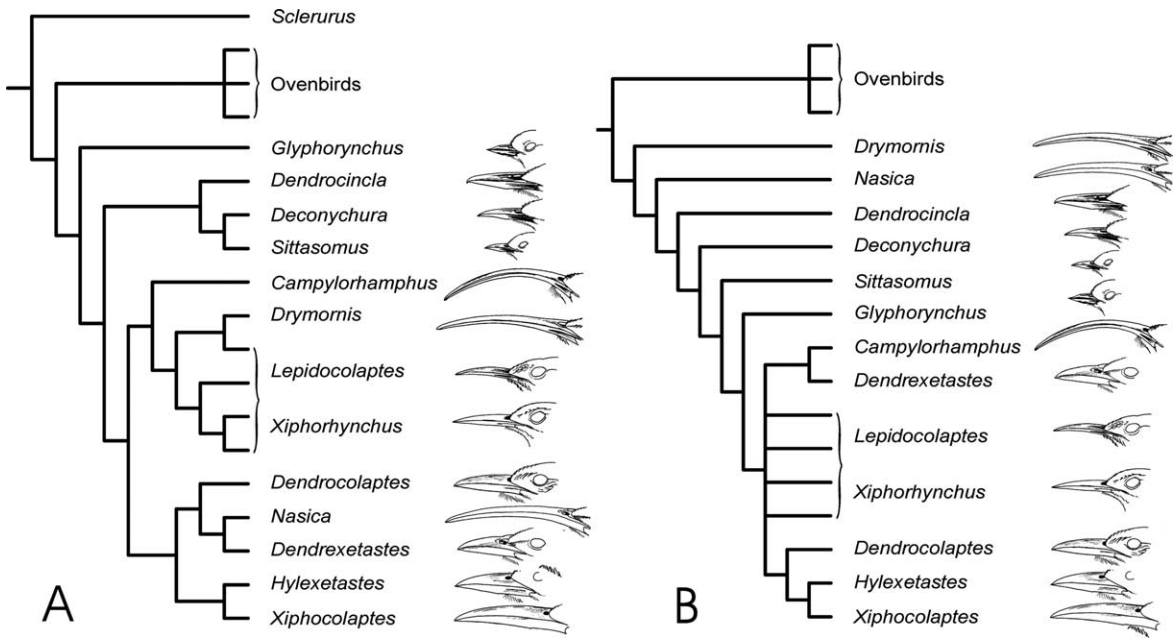

Fig. 4. Hypothetical evolution of bill morphology based on phylogenetic relationships of woodcreeper genera estimated from (A) a Bayesian analysis of the combined molecular data set (myoglobin intron II and cytochrome *b*), and (B) morphology (modified from Raikow (1994) to include the same taxa as used in the molecular study).

ously striped or spotted, with ventral barring in *Dendrocolaptes*, *Hylexetastes* (except *H. uniformis*) and *Xiphocolaptes* (but not in the *Dendrexetastes*/*Nasica* lineage of that clade). *Drymornis* share conspicuous white-striped underparts (to vent) with *Lepidocolaptes*, but has a unique facial pattern. *Lepidocolaptes fuscus* is very similar to *Xiphorhynchus guttatus*, *X. pardalotus* and *X. susurrans* in colours and pattern.

On the use of morphological characters in phylogenetic analysis

Taxonomic classifications of organisms have traditionally been based on similarities in external and internal morphology. It is thus vital to tell shared traits reflecting common ancestry (homology) apart from those reflecting functional convergence (analogy). Although no distinctions between these processes were made in the earliest classifications, systematists have been aware of this problem for a long time. The solution was to avoid all characters that were suspected to be under selective pressure (e.g., Bock 1967). However, the usual cladistic point of view is to not reject any character a priori but instead to evaluate the usefulness of characters after the phylogenetic analysis had been conducted. This approach led to the inclusion of numerous characters of doubtful phylogenetic value, which certainly increased the "noise" (homoplasy) in the data sets. It was assumed that the noise would be randomly distributed, and therefore would not obscure the phylogenetic signal. However, one might fear that nested patterns of shared adaptive characters could lead to strong support for a wrong phylogenetic hypothesis.

It is widely assumed that characters directly involved in locomotion and foraging are particularly susceptible to convergence, and the avian bill is a well-known example. Several groups of closely related species groups showed remarkable adaptive radiation in bill morphology in response to the development of various feeding strategies, e.g., Hawaiian honeycreepers, Darwin's finches, and Madagascan songbirds (Lack 1984, Grant and Grant 1995, Cibois et al. 2001). By mapping Raikow's (1994) morphological characters onto the phylogenetic trees derived from the analyses of the molecular and the morphological phylogenies, respectively (Fig. 4), it can be observed that characters that describe bill morphology shows a low fit (low CI and RI) to both trees (Table 2). In both phylogenies long and slender bills are suggested to have evolved more than once. However, given the close affinity between woodcreepers and the rather thin-billed ovenbirds (Furnariinae), the basal position of the "transitory" woodcreepers in the molecular tree (Fig. 4A) seems more plausible than inferred from the morphological tree (Fig. 4B). The generally low fit of bill morphology to the phylogenies indicates a high level of plasticity. Thus, general resemblance in bill morphology may be a poor indicator of higher-level relationships in birds.

The deeper branch incongruence between the morphological phylogeny (Raikow 1994) and our molecular phylogeny of woodcreepers also highlights a special

problem intrinsic to parsimony analyses of adaptive characters. *Drymornis* has a non-scansorial feeding behaviour, like the outgroup. It feeds running and hopping on the ground and climbs trees only for refuge. It is thus noticeable that six out of seven character states that are uniquely shared between *Drymornis* and the outgroup are obtained from the morphology of the hindlimb, and that eighteen out of twenty-two myological characters from the hindlimb showed a less fit (and the remaining an equal fit) to the molecular tree than to the morphological tree. Considering the robustness of the molecular tree, the morphological similarities between *Drymornis* and the ovenbird outgroup in Raikow′s data set is best explained by convergent evolution due to their common lifestyle (or reversals to the ancestral state in these characters).

It should be noted that also *Xiphocolaptes major* feeds much (or mostly) on the ground, but this may be a very recent specialisation, as it scores identical with *Xiphocolaptes promeropirhynchus* for Raikow's characters (1994, Table 1, see also the short branch-lengths in Fig. 3). *Drymornis bridgesii* and *Xiphocolaptes major* both inhabit a region (Chaco) of deciduous scrub and woodland, where arthropods may be very difficult to find in the trees during the driest part of the year.

The low degree of climbing specialisation is thus an important explanation to the basal position of *Drymornis* in the morphology tree. Consequently, the true phylogenetic position of a taxon that has secondarily acquired an anatomy similar to that of the outgroup, cannot be found in a parsimony analysis of characters that are under adaptive selection. A similar conflict between hindlimb characters and a molecular tree have also been reported in stifftail diving ducks (McCracken et al. 1999).

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References

Aleixo, A. 2002. Molecular systematics and the role of the "várzea"-"terra firme" ecotone in the diversification of *Xiphorhynchus* woodcreepers (Aves: Dendrocolaptidae). – Auk 119: 621–640.

Bock, W. J. 1967. The use of adaptive characters in avian classification. – Proc. XIV Int. Ornithol. Congr.: 61–74.

Cibois, A., Slikas, B., Schulenberg, T. S. and Pasquet, E. 2001. An endemic radiation of Malagasy songbirds is revealed by mitochondrial DNA sequence data. – Evolution 55: 1198–1206.

Clench, M. H. 1995. Body pterylosis of woodcreepers and ovenbirds (Dendrocolaptidae and Furnariidae). – Auk 112: 800–804.

Desjardins, P. and Morais, R. 1990. Sequence and gene organization of the chicken mitochondrial genome: A novel gene order in higher vertebrates. – J. Mol. Biol. 212: 599–634.

Ericson, P. G. P., Christidis, L., Irestedt, M. and Norman, J. A. 2002. Systematic affinities of the lyrebirds (Passeriformes: *Menura*), with a novel classification of the major groups of passerine birds. – Mol. Phyl. Evol. 25: 53–62.

Ericson, P. G. P., Envall, I., Irestedt, M. and Norman, J. A. 2003. Inter-familial relationships of the shorebirds (Aves: Charadriiformes) based on nuclear DNA sequence data. – BMC Evolutionary Biology 3: 16.

Farris, J. S. 1972. Inferring phylogenetic trees from distance matrices. – Am. Nat. 106: 645–668.

Feduccia, A. 1973. Evolutionary trends in the Neotropical ovenbirds and woodhewers. – Ornithol. Monogr. 13: 1–69.

García-Moreno, J. and da Silva, M. M. C. 1997. An interplay between forest and non-forest South American avifaunas suggested by a phylogeny of *Lepidocolaptes* woodcreepers (Dendrocolaptinae). – Studies in Neotropical Fauna & Environment 32: 164–173.

Grant, P. R. and Grant, B. R. 1995. Predicting microevolutionary responses to directional selection on heritable variation. – Evolution 49: 241–251.

Huelsenbeck, J. P. and Ronquist, F. 2001. MrBAYES: Bayesian inference of phylogenetic trees. – Bioinformatics 17: 754–755.

Irestedt, M., Fjeldså, J., Johansson, U. S. and Ericson, P. G. P. 2002. Systematic relationships and biogeography of the tracheophone suboscines (Aves: Passeriformes). – Mol. Phyl. Evol. 23: 499–512.

Johansson, U. S., Irestedt, M., Parsons, T. J. and Ericson, P. G. P. 2002. Basal phylogeny of the Tyrannoidea based on comparisons of cytochrome *b* and exons of nuclear c-*myc* and RAG-1 genes. – Auk 119: 984–995.

Lack, D. 1984. Darwin's finches, 2nd ed. – Cambridge University Press.

McCracken, K. G., Harshman, J., McClellan, D. A. and Afton, A. D. 1999. Data set incongruence and correlated character evolution: an Example of functional convergence in the hind-limbs of stifftail diving ducks. – Syst. Biol. 48: 683–714.

Nixon, K. C. and Carpenter, J. M. 1993. On outgroups. – Cladistics 9: 413–426.

Posada, D. and Crandall, K. A. 1998. MODELTEST: testing the model of DNA substitution. – Bioinformatics 14: 817–818.

Raikow, R. J. 1994. A phylogeny of the woodcreepers (Dendrocolaptinae). – Auk 111: 104–114.

Ridgely, R. S. and Tudor, G. 1994. The birds of South America. Vol. II. – Univ. of Texas Press, Austin.

Sibley, C. G. and Ahlquist, J. E. 1990. Phylogeny and classification of the birds of the world. – Yale Univ. Press, New Haven.

Swofford, D. L. 1998. PAUP*: Phylogenetic analysis using parsimony (* and other methods), ver. 4.0. – Sinauer, Sunderland, MA.

Willis, E. O. 1974. Review of evolutionary trends in the Neotropical ovenbirds and woodhewers, by Alan Feduccia. – Wilson Bull. 86: 487–489.

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