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Diversification in the Andes: the *Atlapetes* brush-finches

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With nearly 30 species, the *Atlapetes* brush-finches are one of the most species-rich genera in the New World sparrows (Passerellidae). *Atlapetes* is mainly distributed in highland forests from Mexico to north-western Argentina, with a few taxa in the foothills (<1000 m). Species diversity is highest in South America, because of high rates of local replacement but few cases of local co-occurrence, creating a mosaic of forms with different plumage colours. With the purpose of understanding phylogenetic and environmental determinants of trait evolution, we reconstructed a molecular phylogeny based on full ND2 mtDNA sequences, with focus on the core group of Andean *Atlapetes* species and related outgroups. Phylogenetic relationships revealed that most Andean species are closely related to a geographical neighbour, although these neighbours may often be phenotypically quite different, with yellow-plumaged species sister to grey-plumaged taxa. Biogeographic analyses suggest Mesoamerica as the area of origin for *Atlapetes*, which dispersed through the Panama Isthmus to the eastern Andean slope, then the western Andes and finally the southern Andes. Diversification in the genus was apparently influenced by the glacial cycles that affected the distribution of montane forests in the Neotropics, particularly during the last million years. Phenotypic plumage changes may be associated with ecological conditions, with black dorsum in the wettest areas and a pale dorsum in a group of closely related species in dry environments on the Pacific slope. Local sympatry and ecological segregation presumably does not lead to increased divergence in plumage signals or song.

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Introduction

Nearly 30 species of brush-finches in the genus *Atlapetes* (Emberizidae) are distributed in Neotropical montane forests from Mexico to north-western Argentina (Paynter 1972, 1978). Thus, the genus is highly diversified in montane forests of the northern and central Andes; a few species reach temperate latitudes and only two are distributed in forests of foothill zones below 1000 m. This genus belongs in a clade of Passerellidae (with *Pyrgisoma*, *Pipilo*, *Pezopetes*, *Torreornis* and *Melozone*), which has been rooted on Mesoamerican montane forests but later expanded to colonize the Andes (DaCosta *et al.* 2009; Barker *et al.* 2013). Up to 25 species are located along the Andes in South America, where they have long intrigued ornithologists because of the complex ways in which taxa with strikingly different colours replace each other on different mountain slopes or at different altitudes on the same slopes (Paynter 1972, 1978; Remsen & White Solomon Graves

1995). Among South American birds, the sharpness and complexity of geographical replacements is second only to that of the suboscine genus *Scytalopus* (Rhinocryptidae), where taxa differ mainly in vocalizations, but very little in morphology (Krabbe & Schulenberg 1997).

Paynter (1972, 1978) thought that most grey-breasted and yellow-breasted forms of *Atlapetes* represented distinctive monophyletic groups and explained the mosaic-like distribution of taxa in these groups as a result of competitive exclusion between them. Remsen & White Solomon Graves (1995) suggested instead that different-coloured taxa inhabiting adjacent slopes could be representatives of the same species, just with different pigment saturations. A partial molecular phylogeny of *Atlapetes* from humid cloud forests of Ecuador, Peru and Bolivia (García-Moreno & Fjeldså 1999) confirmed within-clade mosaics of grey- and yellow-breasted taxa, thereby rejecting Paynter's classification, but also demonstrating that some of these geographical neighbours were as divergent as some parapatric and competing species. The study by García-Moreno & Fjeldså (1999) compared only taxa inhabiting the cloud forests of the humid eastern Andean slopes and adjacent east-draining montane basins in Ecuador, Peru and Bolivia and did not include pale and more or less leucistic taxa found in the drier cloud forests on the Pacific slope. We therefore provide here a broader molecular analysis that includes all the grey taxa (the '*Atlapetes schistaceus* species-group' of Paynter 1972), species ranging in temperate areas in Mexico and Argentina, as well as taxa from drier cloud forests in the Peruvian Pacific slope.

Genus limits have been contentious: taxa in Central American genera *Pselliophorus* and *Pezopetes* have been sometimes included in the genus *Atlapetes* (Webster & Webster 1999), as well as taxa in the genera *Arremon* and *Buarremon*; however, recent phylogenetic studies have demonstrated that the latter are not close relatives to *Atlapetes* (Cadena et al. 2007; Navarro-Sigüenza et al. 2008; Flórez-Rodríguez et al. 2011; Barker et al. 2013; Klicka et al. 2014). Our phylogenetic hypothesis for *Atlapetes* and some closely related taxa allows for a better understanding of the evolutionary relationships in this genus. Our analysis aims, first of all, to understand the origin and evolution of phenotypes, whether plumage changes happen randomly or follow the phylogeny, or may be responses to specific environmental conditions or to the presence of congeners. For this, we focused on the region south of the equator comprising Ecuador, Peru, Bolivia and Argentina (Fig. 1), for which two of us (JF and NK) have the most detailed field experience, with detailed knowledge about ecology, vocalizations and interaction. For this region, we also try to relate the chronology of speciation events to changing environmental conditions during the evolutionary history

of this group. Finally, our genetic data set also provided insight on the species limits for some taxa, in which polytypic species may obscure real patterns of species diversity in this genus.

Materials and methods

Taxon sampling and laboratory procedures

The relationships among the New World 'sparrows' have been confusing, as recent molecular studies have demonstrated a significant mismatch between phylogenies and the traditional morphology-based classification, prompting significant taxonomic rearrangement. Many genera traditionally placed among New World sparrows are now recognized as members of other families in the large assemblage of nine-primaried oscine birds (see Klicka et al. 2000, 2007; García-Moreno et al. 2001; Yuri & Mindell 2002; Burns et al. 2003; Carson & Spicer 2003; DaCosta et al. 2009). Although the limits of these larger groups have now been clarified (Barker et al. 2013), the phylogenetic relationships among them are still surrounded with uncertainties. Nevertheless, considering the well-resolved parts of the phylogeny of the New World nine-primaried oscines, we can assume that *Atlapetes*, except for the '*Atlapetes torquatus* species-group' (of Paynter 1978; see Cadena et al. 2007; Navarro-Sigüenza et al. 2008; Flórez-Rodríguez et al. 2011) represents a monophyletic assemblage. In any case, we included a broad range of genera of New World 'sparrows' in our study (*Junco*, *Incaspiza*, *Calamospiza*, *Arremon*, *Aimophila*, *Chlorospingus*, *Pipilo*, *Melozone*, *Pezopetes* and *Pselliophorus*).

The samples used are listed in Table S1. The majority of samples were obtained during our own collecting and were complemented with loans from other institutions and sequences from GenBank (Table S1). We did not have access to samples of Colombian and Venezuelan taxa, some of which (*A. latinuchus phelpsii*, *A. melanocephalus*, *A. albofrenatus* and *A. blancae*) may be closely related to our focal group, which includes the species found south of the equator (see Klicka et al. 2014). We also miss a local form *A. terborghi* from central Peru, and we had no success extracting DNA from toe pads of museum specimens of *A. t. tricolor* and *A. canigenis*. The addition of any or all of these missing species to the phylogeny could result in changes in the position of some species, but the main structure of the tree may not be affected: our results here are very consistent with an earlier study using far fewer taxa and characters (García-Moreno & Fjeldså 1999). We extracted DNA from tissue and blood samples using the Qiagen DNeasy extraction kit, following the protocols provided by the manufacturer. We amplified and sequenced the NADH dehydrogenase subunit 2 gene (ND2) in its full length, for a total of 1041 bp. This gene has been used
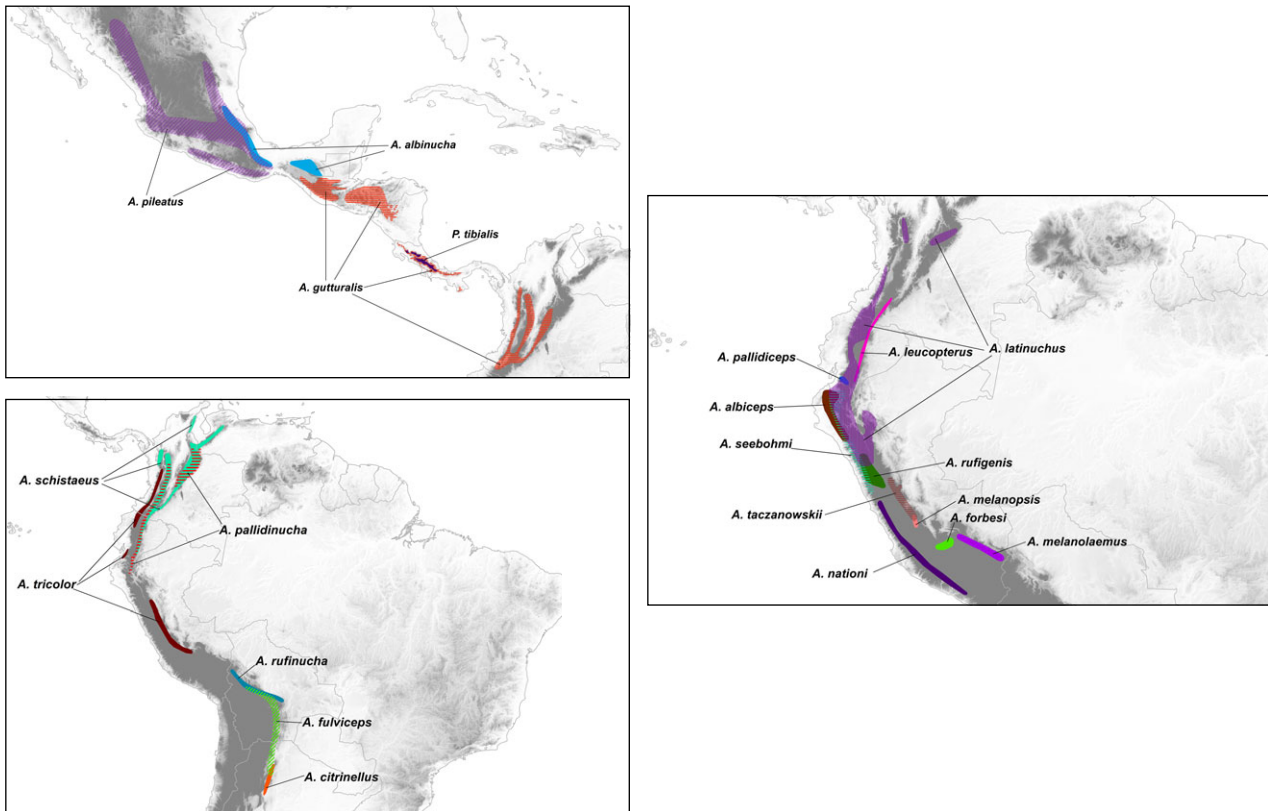
Fig. 1 Distribution of Atlapetes species included in this study.

successfully to assess phylogenetic relationships in many recent avian studies (e.g. Filardi & Moyle 2005; DaCosta & Klicka 2008; DaCosta *et al.* 2009; Barker *et al.* 2013; Klicka *et al.* 2014). Although it represents a single matrilineal locus whose history may not necessarily be fully congruent with the species tree (Huang *et al.* 2014), mitochondrial genes are believed to have a high probability of correctly tracking branching patterns of recent events of the species trees (Moore 1995; Avise 2009). The gene was amplified in two overlapping sections, by means of PCR, using the primers described in Table S2. The amplified products were cleaned by gel filtration using Sephadex G50 columns (Sigma-Aldrich, St Louis, MO, USA) and sequenced using dye-labelled terminators (BigDye v. 3.1, Applied Biosystems, Carlsbad, CA, USA). Sequencing reactions were precipitated with ethanol, and the products were resolved on an ABI377 semi-automated sequencer. All sequences have been deposited in Genbank under accession numbers KM360486-KM360525. Sequences were read using 4Peaks v. 1.6 (Griekspoor & Groothuis 2005), then aligned and proofread using SeAl v. 2.0a11 (Rambaut 2002). We corroborated the mitochondrial origin of our sequences by combining at least two of the following for

each sequence: amplifying overlapping gene segments, amplifying and sequencing the same fragment with different primer sets, sequencing both DNA strands and, whenever possible, using multiple individuals from each taxon. We found no evidence of numt contamination at any stage (Arctander 1995; Zhang & Hewitt 1996; Sorenson & Quinn 1998; Bensasson *et al.* 2001), and all sequences present a single stop codon at the expected terminal position.

Phylogenetic analyses

We conducted phylogenetic analyses of the mtDNA sequences under maximum likelihood (ML) as implemented in PAUP* (Swofford 2002). The appropriate substitution model that best fitted our data for each partition was estimated via the Akaike information criterion (AIC) in MrModeltest (Nylander 2004). The best fitting model was GRT+I+G (rate matrix: A–C = 3.22, A–G = 200.99, A–T = 3.10, C–G = 8.07, C–T = 81.85, G–T = 1; gamma distribution shape parameter = 2.13, proportion of invariable sites = 0.55; estimated base frequencies $pA = 0.32$, $pC = 0.38$, $pG = 0.09$ and $pT = 0.21$). We also performed a Bayesian inference analysis (BI) using MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2002). Three partitions of

characters were defined corresponding to first, second and third codon positions; these were allowed to vary with different evolutionary rates. We performed two simultaneous runs, each one with four Markov chains for 10^6 generations and sampling the tree space every 1000th generation. Stationarity and convergence of the Bayesian inference were inspected in Tracer v1.5 (Rambaut & Drummond 2007). After inspection, the first 2500 trees were discarded as burn-in, and consensus trees, as well as posterior probabilities for each node, were estimated based on the remaining 7500 trees (Huelsenbeck *et al.* 2002).

An ultrametric tree with divergence time estimates using the model GTR+I+G, an uncorrelated relaxed clock and the Yule speciation process as priors was obtained through BEAST 1.7 (Drummond *et al.* 2012). Selection of the clock model was performed through Bayes factors log likelihood values applying a stepping-stone method (Xie *et al.* 2011), as implemented in Mr.Bayes 3.2.2 (Ronquist *et al.* 2012), where an uncorrelated relaxed clock (log likelihood: −6221.15) was preferred over a strict clock model (log likelihood: −6278.57), following Kass & Raftery (1995). Analyses were run for 50×10^6 generations, sampling every 1000 generations. Convergence of chains was inspected in Tracer v1.4 (Rambaut & Drummond 2007). Given that appropriate fossils for calibration are not available, we relied in secondary calibrations obtained for the genus *Arremon* (Cadena *et al.* 2007), for which estimates for a most recent common ancestor have been placed about 7.6 million years ago (mya) and for the divergence between *Atlapetes* and *Pselliophorus*, estimated to have been occurred approximately 3.7 mya (Smith & Klicka 2010), using both calibration dates simultaneously. We selected default priors, except for the relative parameter *mu, where we selected a gamma distribution (bounds 0, +inf).

Biogeography

It is well established that New World nine-primaried oscines originated in the north and dispersed to South America (e.g. Smith & Klicka 2010; Barker *et al.* 2013), but the wide latitudinal distribution of *Atlapetes* makes its ancestral area and mode progress of diversification equivocal. For biogeographic reconstruction, we relied in a Bayesian method in RASP v2.1. beta (Reconstruct Ancestral States in Phylogenies, Yu *et al.* 2012a). Using of a Bayesian method is justified by the geographical context in which complexity and mosaic habitats together with a multitude of potential dispersal barriers (generally deep valleys with local rain-shadow climate; Brown & Kappelle 1995; Webster 1995; Sánchez-González *et al.* 2008; Sánchez-González & Navarro-Sigüenza 2009) give the montane forest habitat an archipelago-like structure. This insular-like distribution may render the use of Dispersal–Vicariance analysis inade-

quate in these geographical settings (see Lamm & Redelings 2009; Kodandaramaiah 2010). RASP uses a modified source code from MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and implements two models of character state evolution assuming equal rates of change with either fixed or estimated state frequencies (JC and F81 models, respectively). In biogeographic analyses, these character states are equivalent to regions, thus enabling to take characters as binary – with gamma-distributed rate variation between regions. For calculations, we used the maximum clade credibility tree as obtained from BEAST 1.7 (Drummond *et al.* 2012); we ran two independent runs of 10 chains (temperature 0.1) for 5×10^6 generations sampled every 1000 generations, using a F81+ gamma model (allowing exchange variation in dispersal between regions). Convergence between independent runs was assessed confirming a value less than 0.01 (actual value 0.0003, following Yu *et al.* 2012b). State frequencies were calculated after 25% of the total generations were discarded. Based on the results of the phylogenetic analysis, the maximum number of areas included was limited to three within ancestral distributions. For comparison purposes, we have included results obtained in S-Diva (Yu *et al.* 2010) for relevant nodes in our phylogeny (Table 1), as implemented in RASP v.2.1.beta (Yu *et al.* 2012a). Dispersal and Vicariance (DIVA) is a parsimony-based method in which dispersal is assigned a cost, thus assuming vicariance as the main mode of diversification. Dispersal and Extinction Cladogenesis (DEC) analysis is also implemented in RASP v.2.1.beta (Yu *et al.* 2012a), which is a parametric method that models range evolution as a function of time (Ree & Smith 2008); however, criticisms around the possibility of overestimation of the frequency of processes like extinction and the inference of areas not considered as part of the distributional range of the taxon under study may prevent an adequate application (Buerki *et al.* 2011). This may be especially complex in study systems like this, where recognized species may show widespread or highly localized distributional ranges, and events of extinction are unknown. Areas for reconstruction analysis follow Sánchez-González *et al.* (2008) for montane forests, based on WWF ecoregions (Olson *et al.* 2001), and Stotz *et al.* (1996) for the lowland Tumbes region (Fig. S1).

Ancestral-state reconstructions

We assessed the possible contingency of changes between populations with different pigmentations through their optimization in a time-calibrated phylogenetic framework, which allowed us to draw more solid conclusions about the mode and tempo of change of plumage in the genus. Over the years, JF and NK have reviewed the majority of museum specimens of *Atlapetes* in various museums (see Fjeldså & Krabbe 1990) and have gathered comprehensive

Table 1 Ancestral-state reconstructions and divergence dates for various nodes in *Atlapetes* data set

Node	Estimated divergence date (range)	Most probable ancestral range BBM/S-Diva	Plumage characters		Habitat	CntAb
			Upperparts (upp)	Underparts (und)		
4.A. *Atlapetes*	5.12 (3.80–6.58)	Mesoamerica: 83.71/1.00	Slaty grey: 0.86	Yellow: 0.28 White: 0.20	MEe: 0.27 ME: 0.17	MM: 0.34 UM: 0.18 UT:0.17
5.B. Mesoamerican *Atlapetes*	1.73 (1.00–2.64)	Mesoamerica: 98.43/1.00	Slaty grey: 0.73 Dull olive Green: 0.20	White: 0.85	MEe: 0.39 POF: 0.25	UT: 0.34 MM: 0.34
18.C. Central American and Andean *Atlapetes* (including *Pselliophorus*)	3.68 (2.80–4.69)	Costa Rica-Panama: 42.97/ Equivocal Eastern Andean slope: 21.19/Equivocal Mesoamerica: 19.62/Equivocal	Slaty grey: 0.94	Yellow: 0.36	MEe: 0.30 ME: 0.22	MM: 0.40
20.D. Andean *Atlapetes*	2.95 (2.20–3.73)	Eastern Andean slope: 93.49/1.00	Slaty grey: 0.99	Yellow:0.54 Creamy: 0.21	MEe: 0.41 ME: 0.23	MM: 0.51
21.E. Northern Andes *Atlapetes*	2.57 (1.87–3–32)	Eastern Andean slope: 95.91/1.00	Slaty grey: 0.99	Yellow:0.39 Creamy: 0.31	MEe: 0.40 ME: 0.20	MM: 0.46
71.F. Central Andes *Atlapetes*	1.95 (1.35–2.63)	Eastern Andean slope: 81.41/1.00	Slaty grey: 0.96	Yellow: 0.85	ME: 0.44 MEe: 0.23	MM:0.61
78.G. Southern Andes *Atlapetes*	1.53 (1.02–2.14)	Eastern Andean slope: 93.06/1.00	Slaty grey: 0.87	Yellow: 0.94	MEe: 0.80	MM: 0.63
32. H. Andes Ecuador and Peru (excluding western Andes in Ecuador)	0.97 (0.64–1.34)	Eastern Andean slope: 94.22/1.00	Slaty grey: 0.99	Creamy: 0.53 Yellow: 0.33	MEe: 0.99	MM: 0.83
1. 36. Eastern Andean slope (Ecuador and Peru) and Western slope Andes	0.75 (0.50–1.04)	Eastern Andean slope: 93.25/ Eastern Andean slope + Central Andes wet Puna: 1.00	Slaty grey: 0.99	Creamy: 0.54 Yellow: 0.32	MEe: 0.99	MM: 0.84
J.37. Eastern slope of Ecuadorian Andes and Western slope Andes	0.60 (0.40–0.85)	Eastern Andean slope: 27.04/1.00 Central Andes wet Puna: 21.30 Tumbes, Piura: 17.55	Slaty grey: 0.99	Creamy: 060 Yellow: 0.32	MEe: 0.97	MM: 0.90
45. K. Western slope Andes Taxa	0.53 (0.33–0.78)	Tumbes-Piura: 57.34 Central Andes wet Puna-Tumbes, Piura: 21.70/1.00	Slaty grey: 0.99	Creamy: 092	MEe: 0.68	MM: 0.90

Values for ancestral areas are in posterior probabilities; values for ancestral reconstructions of plumage characters; Habitat and Centre of Abundance (CntAb, both based on Stotz *et al.* 1996) are presented in proportional likelihoods. Habitat: ME, montane evergreen; MEe, montane evergreen edge. CntAb: UT, upper tropical, 900–1600 metres above sea level (masl); middle montane, 1600–2600 masl; UM, upper montane, >2600 masl.

field experience with most taxa. The matrix of 16 character states for ancestral-state reconstruction (Table 1) was based on specific examination of specimens in the Zoological Museum, University of Copenhagen and US National Museum in Washington, D.C. As colour codification may be confused due to the different terminology in published literature, colour codification was unified following the terminology of Rising (2011). This was performed also for species included in the outgroups (*Junco*, *Arremon*, *Pipilo*, *Pezopetes*, *Melozone* and *Pselliophorus*).

Plumage change in *Atlapetes* has been characterized as a leapfrog pattern, where a distinctly coloured population is inserted between phenotypically more similar populations (Remsen 1984). Although several explanations have been proposed, leapfrog variation may be the result of either stochastic or environmental factors. We explored these possible relationships by reconstructing ancestral states for the elevational zone in which a species is thought to be more common (Center of abundance, CntAb; Stotz *et al.* 1996; see legend in Table 1), which is a variable proposed as specially informative for montane species (Stotz *et al.* 1996). We also recorded the habitat, following Stotz *et al.* (1996); most *Atlapetes* species are humid montane forest species, but species representing early-diverged (northern) lineages inhabit montane pine-oak forest, and others inhabit the patches of mist vegetation of dry regions.

Plumage trait and elevational zonation evolution were analysed on a time-calibrated phylogeny (obtained in BEAST v1.7.5) using maximum likelihood and the Markov k-state one-parameter model (Mk1), which assumes equal rates of change between any of the character states, as implemented in Mesquite version 2.74 (Maddison & Maddison 2010). Rates of change in plumage characters were estimated in Mesquite v. 2.74 (Maddison & Maddison 2010), based on the input data, and results are presented as proportional likelihoods.

Vocalizations

We examined and compared 2796 dawn songs from 264 individuals of 27 species (38 taxa) of *Atlapetes* and *Pselliophorus* (examples shown in Figure S2, supplementary material), treating *taczanowskii* as a separate species from *schistaceus* and *crassus* as distinct from *tricolor*. Dawn song was defined as the varied phrases given at regular intervals by the male alone, mainly or only at dawn, and was distinguished from the male part of the duets given by pairs throughout day, a vocalization that has been confused with dawn song in some species (e.g. Rising 2011; Jaramillo *et al.* 2011). This was fairly straightforward for most species, but three species, *albincha* (including *gutturalis*), *albofrenatus*, and *Pselliophorus tibialis*, differed substantially. Due to variations between phrases, only some fundamental properties of phrases were com-

pared. Recordings of dawn songs in the public libraries XenoCanto, Macaulay Library of Natural Sounds, and Banco de Sonidos Animales, and on published media (Mayer 2000; Schulenberg 2000; Krabbe & Nilsson 2003; Boesman 2007; Moore *et al.* 2013) were available for all species except for *P. luteoviridis*, *A. nationi*, *A. rufigenis* and *A. terborghi*. Only calls and duets were available for *P. luteoviridis*. No records were available for the possibly extinct *A. blancae* and for the geographically isolated nominate form of *A. pallidinucha*. Using CoolEditPro (Syntrillium Software), each recording was measured for maximum length of a phrase, maximum number of notes in a phrase and total number of different notes of all phrases as judged from visual comparison, as well as shortest interval length between phrases in undisturbed song. For every species, mean and standard deviation was calculated for each of these variables (Table 2).

Results

Sequence characteristics and phylogenetic reconstruction

We successfully amplified the complete 1041 base pairs of the ND2 gene for a set of *Atlapetes* brush-finches and their chosen outgroups, with the exception of *Incaspiza* (only 593 bp). Among *Atlapetes*, there were 206 parsimony informative characters, distributed according to the typical pattern seen in coding sections: 153 (74%) third codon positions, 40 (19%) first positions and only 13 (6%) second codon positions. The majority of changes were transitions. Using MacClade (Maddison & Maddison 2010) and the best scoring ML/MP tree, we counted 205/214 A-G and 292/312 C-T transitions; 34/34 A–C, 16/16 A–T, 12/16 G–C; and a single/1 G–T transversion.

Our analyses placed *Incaspiza*, *Calamospiza*, *Aimophila* and *Chlorospingus* quite distant from *Atlapetes*. We therefore present here only analyses with *Junco*, *Arremon*, *Pezopetes*, *Melozone* and *Pipilo* as outgroups (Fig. 1). Our phylogenetic data reconfirms the paraphyly in *Melozone* and *Pipilo* (DaCosta *et al.* 2009) and the monophyly in *Arremon* (Cadena *et al.* 2007; Navarro-Sigüenza *et al.* 2008; Flórez-Rodríguez *et al.* 2011). Consistent with previous studies, we recovered *Pipilo chlorurus* as sister to *Atlapetes* (DaCosta *et al.* 2009; Barker *et al.* 2013). *Atlapetes* was recovered as paraphyletic, given that the Central American *Pselliphorus* was found embedded within *Atlapetes*, supporting previous suggestions based on morphological grounds (Webster & Webster 1999) and indicating that a monophyletic *Atlapetes* should include Central American *Pselliophorus* finches, a recent finding also included in Klicka *et al.* (2014).

The phylogenetic tree for *Atlapetes* showed two well-supported clades (Fig. 2). A first clade includes Mesoamerican taxa (*A. pileatus*, *A. albinucha* and *A. gutturalis*); the second clade includes *Pselliophorus* and all of the South American *Atlapetes*. Within this second clade, two further groups

Table 2 List of song recordings examined and properties of dawn songs of 27 species of *Atlapetes* and *Pselliophorus* (mean ± standard deviation and range)

Taxon	No. of cuts	No. of songs	Duration of longest phrase (s)	Max. no. of notes in a phrase	No. of different notes per cut	Min. length of pauses between undisturbed songs (s)
Pselliophorus tibialis	5	92	1.4 ± 0.4 (0.9–1.9)	10.8 ± 2.6 (7–14)	2.8 ± 2.0 (1–6)	5.0 ± 2.0 (2–7)
Atlapetes pileatus dilutus	9	41	1.0 ± 0.2 (0.8–1.3)	5.9 ± 2.3 (4–10)	4.8 ± 2.0 (2–8)	3.3 ± 0.8 (2.3–4.7)
A. albinucha[1]	16	423	2.0 ± 1.5 (0.3–4.1)	3.4 ± 1.9 (1–7)	6.5 ± 2.2 (2–10)	1.5 ± 0.6 (0.6–3.0)
A. albofrenatus[2]	12	69	2.7 ± 0.9 (1.2–4.1)	20 ± 8 (5–30)	2.7 ± 1.5 (1–7)	2.1 ± 1.2 (1.0–5.0)
A. flaviceps	6	57	1.5 ± 0.1 (1.5–1.6)	7.3 ± 1.5 (6–10)	7.8 ± 1.9 (5–10)	3.1 ± 1.0 (1.6–4.4)
A. fuscoolivaceus	4	237	1.3 ± 0.1 (1.2–1.3)	6.5 ± 0.6 (6–7)	11.5 ± 3.7 (8–16)	3.8 ± 0.7 (2.8–4.2)
A. semirufus denisei	16	144	1.3 ± 0.3 (0.8–1.8)	5.6 ± 1.4 (4–8)	4.4 ± 1.5 (2–7)	5.4 ± 1.3 (3.0–7.7)
A. p. personatus	5	36	1.2 ± 0.3 (0.9–1.6)	6.4 ± 1.1 (5–8)	3.4 ± 0.5 (3–4)	4.6 ± 1.2 (3.6–6.4)
A. leucopis	15	157	3.5 ± 0.7 (2.5–5.5)	9.5 ± 2.1 (6–15)	10.1 ± 2.6 (6–16)	4.1 ± 0.9 (2.6–5.6)
A. s. schistaceus	16	162	1.2 ± 0.2 (0.9–1.6)	2.9 ± 0.8 (2–5)	2.7 ± 1.1 (2–6)	4.9 ± 1.4 (2.8–7.8)
A. melanocephalus	7	142	1.1 ± 0.3 (0.8–1.4)	2.6 ± 0.5 (2–3)	2.6 ± 0.5 (2–3)	2.1 ± 0.3 (1.8–2.8)
A. latinuchus[3]	37	259	1.3 ± 0.3 (0.8–2.0)	5.8 ± 2.1 (3–10)	5.0 ± 2.0 (2–9)	5.1 ± 1.8 (2.6–12.0)
A. pallidinucha papallactae	5	40	1.1 ± 0.4 (0.7–1.6)	4.2 ± 2.2 (2–7)	6.4 ± 4.0 (2–10)	4.1 ± 1.3 (3.0–0.2)
A. pallidiceps	5	19	1.2 ± 0.2 (0.8–1.4)	10.0 ± 3.2 (6–14)	5.4 ± 1.8 (3–8)	9.6 ± 3.6 (7.3–5.0)
A. leucopterus[4]	10	34	1.5 ± 0.4 (1.0–2.6)	9.1 ± 3.0 (4–14)	4.2 ± 1.5 (2–6)	8.5 ± 2.2 (6.2–13.1)
A. seebohmi simonsi	6	26	1.3 ± 0.3 (0.9–1.7)	10.2 ± 2.6 (6–13)	5.7 ± 0.5 (5–6)	4.6 ± 1.3 (3.3–6.6)
A. (schistaceus) taczanowskii	7	113	1.1 ± 0.3 (0.8–1.6)	4.9 ± 1.8 (2–7)	6.1 ± 1.7 (3–8)	4.3 ± 0.9 (2.7–5.5)
A. albiceps	2	8	1.4 ± 0.4 (1.1–1.6)	8 ± 1.4 (7–9)	7.5 ± 0.7 (7–8)	5.0 ± 0.0
A. melanopsis	3	13	1.1 ± 0.6 (0.7–1.8)	3.0 ± 1.0 (2–4)	3.3 ± 0.6 (3–4)	3.1 ± 0.4 (2.8–3.5)
A. (tricolor) crassus	21	93	2.4 ± 0.5 (1.3–3.3)	12.0 ± 4.5 (4–21)	4.8 ± 1.7 (2–8)	3.9 ± 1.3 (1.9–7.8)
A. (t.) tricolor	4	67	1.4 ± 0.4 (1.1–1.9)	7.3 ± 4.3 (3–11)	8.5 ± 4.2 (4–14)	3.7 ± 1.0 (2.7–5.0)
A. canigenis	10	147	0.9 ± 0.1 (0.7–1.0)	2.1 ± 0.3 (2–3)	2.3 ± 0.7 (2–4)	5.5 ± 3.2 (2.9–14.5)
A. melanolaemus	5	28	0.9 ± 0.2 (0.7–1.3)	3.6 ± 2.1 (2–7)	2.8 ± 0.8 (2–4)	5.1 ± 0.6 (4.4–5.7)
A. forbesi	1	13	1.7	5.0	6.0	4.0
A. rufinucha[5]	15	87	1.0 ± 0.3 (0.7–1.8)	4.9 ± 3.5 (2–15)	3.6 ± 1.8 (2–8)	5.4 ± 3.4 (2.4–16.6)
A. fulviceps	9	40	0.9 ± 0.3 (0.6–1.7)	5.7 ± 1.7 (4–10)	3.3 ± 0.9 (2–5)	5.3 ± 2.3 (2.2–9)
A. citrinellus	13	249	1.1 ± 0.2 (0.8–1.4)	7.8 ± 2.4 (5–12)	4.4 ± 2.0 (2–7)	6.6 ± 4.6 (3.0–19.0)

Taxa with more subspecies included and no. recordings: [1]*albinucha 4, gutturalis 12.* [2]*albofrenatus 11, meridae 1.* [3]*yariguerum 5, elaeoprorus 2, caucae 2, spodionotus 6, comptus 3, latinuchus 15, chugurensis 4.* [4]*leucopterus 3, dresseri 5, paynteri 2.* [5]*carrikeri 1, rufinucha 14.*

were recovered: a first group including *Atlapetes [Pselliophorus] tibialis* from montane forests of Costa Rica and western Panama; and a second group including all Andean taxa. Among our Andean species, the southernmost forms, *A. rufinucha*, *A. fulviceps* and *A. citrinellus*, formed a clade with strong node support. The species distributed in central and southern Peru, *A. melanolaemus* and *A. forbesi*, were also paired in a clade with good node support (however, we did not include the forms *A. terborghi* and *A. canigenis*, which have very restricted distributions between these two). A third clade, also with high BI support, engulfed all the remaining taxa mainly from the northern Andes. This clade was characterized by very short internodes, with *melanopsis*, *leucopis* and *albiceps* being basal, followed by *rufigenis*. The remaining species are all rather similar in general appearance, except for the variation in colour of underparts; only *pallidiceps*, and to some extent *pallidinucha*, showed distinctive head patterns.

Two relatively widely distributed taxa were recovered as paraphyletic according to our phylogenetic tree. The grey-plumaged species *A. schistaceus* was partitioned in two well-supported separate groups: *A. s. taczanowskii* and *A. s. schistaceus*, from central Peru and the northern Andes in Colombia and Ecuador, respectively, whereas the yellow-plumaged *A. latinuchus* was also recovered in two well-supported and separated clades, one including *A. l. spodionotus* and *A. l. chugurensis* of north-western Ecuador and north-western Peru, and the other with *A. l. comptus* from south-western Ecuador. The latter intergrade with *A. l. spodionotus* in west-central Ecuador (Chapman 1926) and was also found to intergrade with nominate *A. l. latinuchus* of the east Andean slope during the present study. The form *chugurensis* itself appears to be variously intermediate in plumage between *latinuchus* and *baroni* of the Río Marañón drainage and locally on the Pacific slope, suggesting that all these forms intergrade. These data suggest that widely distributed taxa may include more species than previously recognized and that more than one mitochondrial type may occur in the same population (Paynter 1972, 1978; Remsen & White Solomon
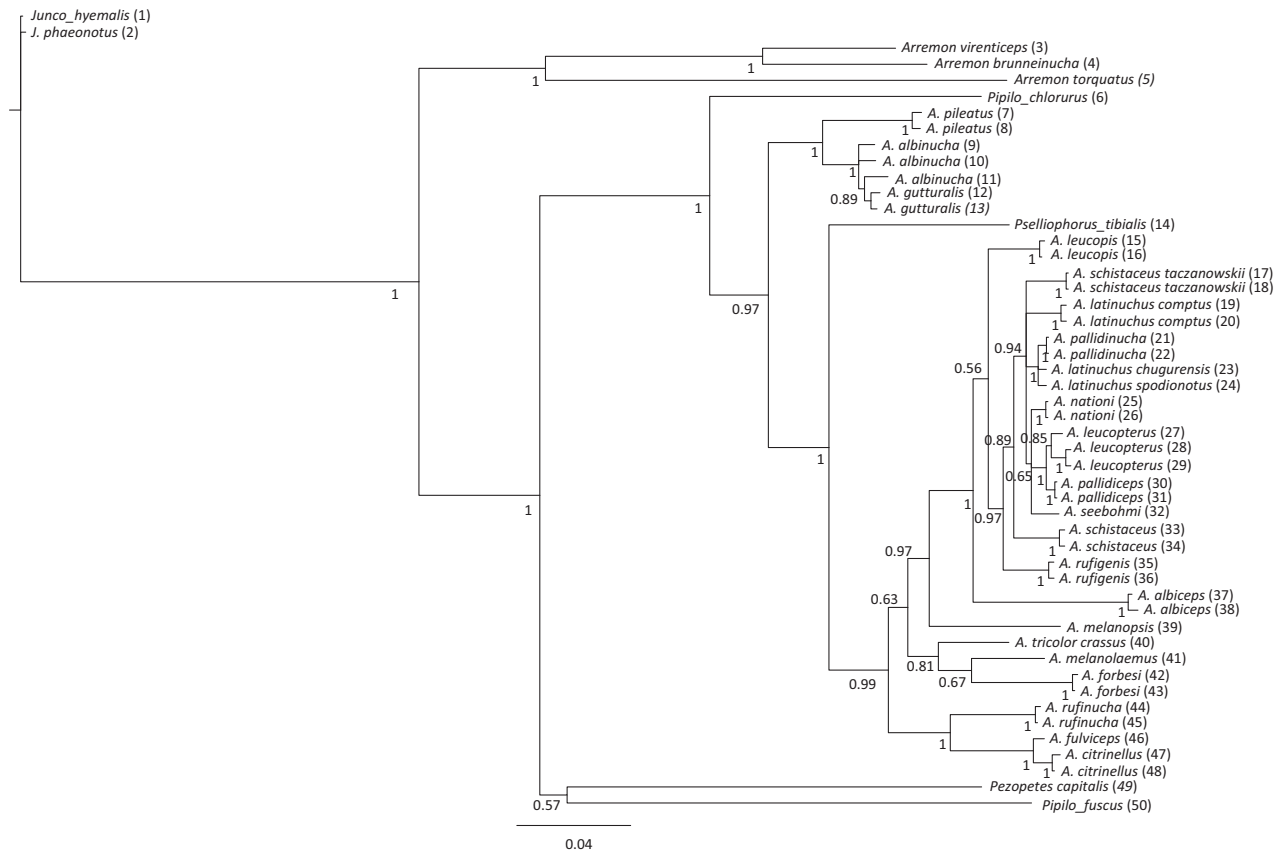
Fig. 2 Bayesian inference estimate of the phylogeny for *Atlapetes*. Numbers in the branches refer to posterior probabilities. Numbers in parenthesis refer to samples used in this study (Table S1).

Graves 1995; Hogner *et al.* 2012), calling for a denser geographical sampling and genetic comparisons involving both nuclear and mitochondrial sequences to establish degrees of intergradation or hybridization.

Biogeography and geographical replacements

Ancestral area reconstructions suggest that the origin of *Atlapetes* most probably occurred in the Mesoamerican mountains (Figs. 3 and 4). This is consistent with other studies, suggesting an expansion of New World 'sparrows' from North America through Mesoamerica, with later diversification in South America (Smith & Klicka 2010). According to our divergence dated phylogeny and RASP Bayesian Binary Method (BBM) reconstruction, *Atlapetes* (including *Pselliophorus*) originated in Mesoamerica and later dispersed into the Costa Rica–Panama highlands in the late Miocene–early Pliocene (HPD range 3.8–6.6 mya, Table 1, Fig. 3); after origin, the two main clades in the genus (Clades B and C) diverged at about 4 mya (HPD range 3.2–5.2 mya) in the mid-Pliocene.

After the initial divergence within the genus, *Atlapetes* taxa dispersed from the Costa Rica–Panama highlands to the eastern Andean slope about 3 mya (HPD range 2.8–4.6 mya), where they began intensive speciation (Clade C). Differentiation in the Andes apparently preceded differentiation of Mesoamerican taxa, which started about 1.7 mya (HPD range 1–2.6 mya). Most speciation events in *Atlapetes* seem to have occurred within the last two-three million years along the eastern Andean slope, from where, according to RASP reconstructions, it dispersed to the western Andes (*A. tricolor*) and to the southern Andes within the last one million years, as Andean taxa in clades H to K suggest. These times of divergence correspond to the largest amplitudes of climatic and vegetation cyclical changes, mostly corresponding to Pleistocene glacial cycles (Hooghiemstra *et al.* 1993). Plumage colour change in the underparts is especially notorious again in the north Andean clade (Clade E), where a higher amount of changes between grey- and yellow-plumaged species, and a change of habitats between species regarding other taxa is very conspicuous.
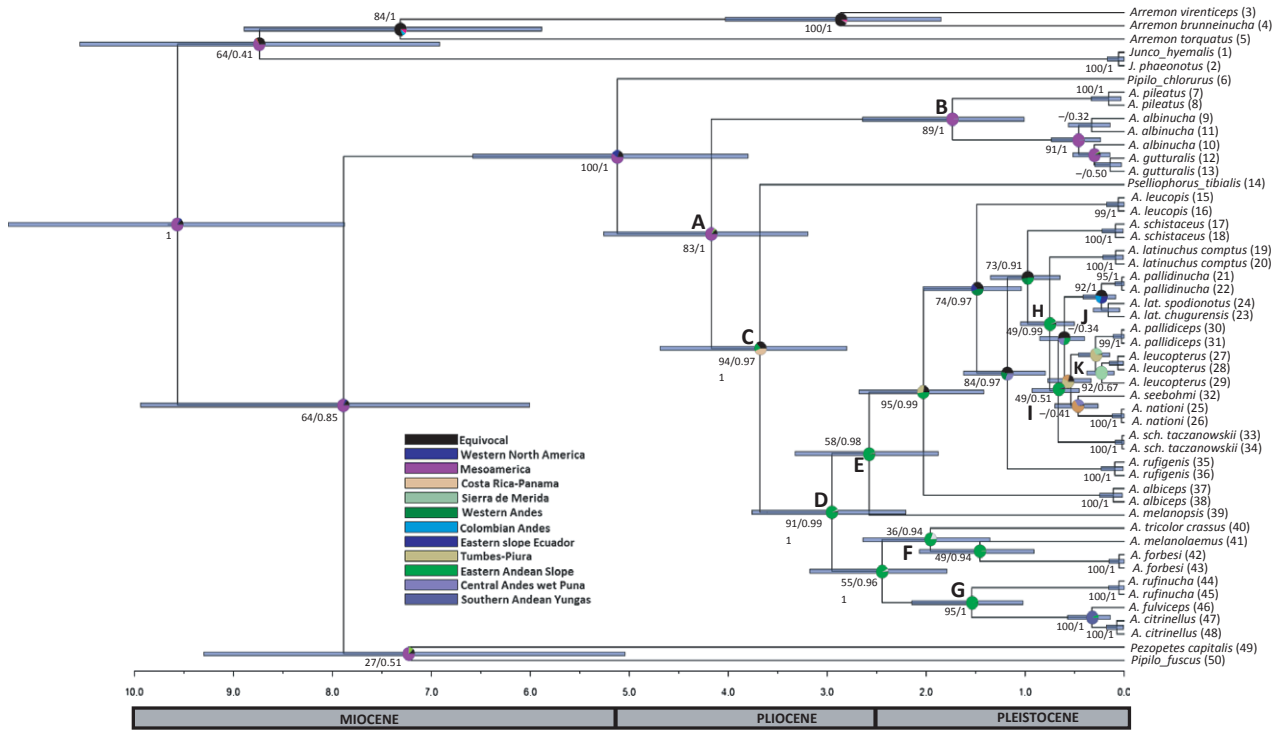
Fig. 3 Maximum clade credibility tree and ancestral areas reconstruction for *Atlapetes*. Coloured bars represent 95% high posterior density (HPD) intervals. Numbers in the branches refer to bootstrap support for the ML (before slash) and posterior probabilities from the BI (after slash). Legend refers to areas of endemism used in this study (based on Sánchez-González *et al.* (2008). Capitalized letters in nodes refer to A, *Atlapetes*; B, Mesoamerican *Atlapetes*; C, Central American and Andean *Atlapetes* (including *Pselliophorus*); D, Andean *Atlapetes*; E, northern Andes *Atlapetes*; F, central Andes *Atlapetes*; G, southern Andes *Atlapetes*; H, Andes of Ecuador and Peru (excluding western Andes in Ecuador); I, eastern Andean slope (Ecuador and Peru) and western slope of the Andes; J, eastern slope of Ecuadorian Andes and western slope Andes; K, western slope Andes taxa.

Basal taxa in the north Andean Clade are grey-plumaged and occupy higher elevation habitats, with the exception of *A. albiceps*, which is found in tropical deciduous forests in the Tumbes–Piura region, and which diverged from other taxa within this clade about 2 mya. The basal taxon in Clade H is the yellow-plumaged *A. latinuchus comptus* of the cloud forests and semi-humid shrubbery of south-western Ecuador, possibly with nominate *latinuchus* of the wet forest on the east slope in south-eastern Ecuador and adjacent northern Peru, which may have diverged 1 mya; whereas basal taxa in Clade I is grey-plumaged (*A. schistaceus* (?) *taczanowskii*), both of these are sister to a clade with yellow-plumaged species *A. latinuchus* and *A. pallidinucha*. Finally, clade K includes grey-plumaged species, which occur in mid-elevation dry cloud forests of the western slope of the Andes. Species in the central (Clade F) and southern Andes clade (Clade G) appear to have diverged also within the last two million years. Clade F includes only one grey-plumaged species (*A. forbesi*) in our data set (and potentially the missing, dark grey *A. canigenis*), which is nested within yellow-plumaged species; species in the

Argentinean Andes are both closely related and diverged relatively recently (1.5 mya).

Elevational replacements are scattered along the phylogeny (Fig. 4). According to reconstructed ancestral elevation, the most recent common ancestor of *Atlapetes* was a middle-montane (MM) to upper-tropical (UT) species of the Mesoamerican mountains. MM forests appear as the most probable ancestral habitat throughout the internal nodes in our phylogeny, suggesting that other habitats in different elevations may be the result of independent colonization events. In the Mesoamerican group (Clade B), *Atlapetes* underwent differentiation in HT (high tropical) habitat, going back to MM elevations south of the Isthmus of Tehuantepec during the Last Interglacial. In the Andes, the pattern of habitat shifts is more complex. For the central and southern Andes clades, a single colonization event of upper montane (UM) from MM is suggested in *A. forbesi* (Clade F); for Clade G, a single shift to lower montane forest is suggested in the southernmost *Atlapetes* species: *A. citrinellus*. For the northern Andes clade (Clade E), at least five independent colonizations of UM habitats
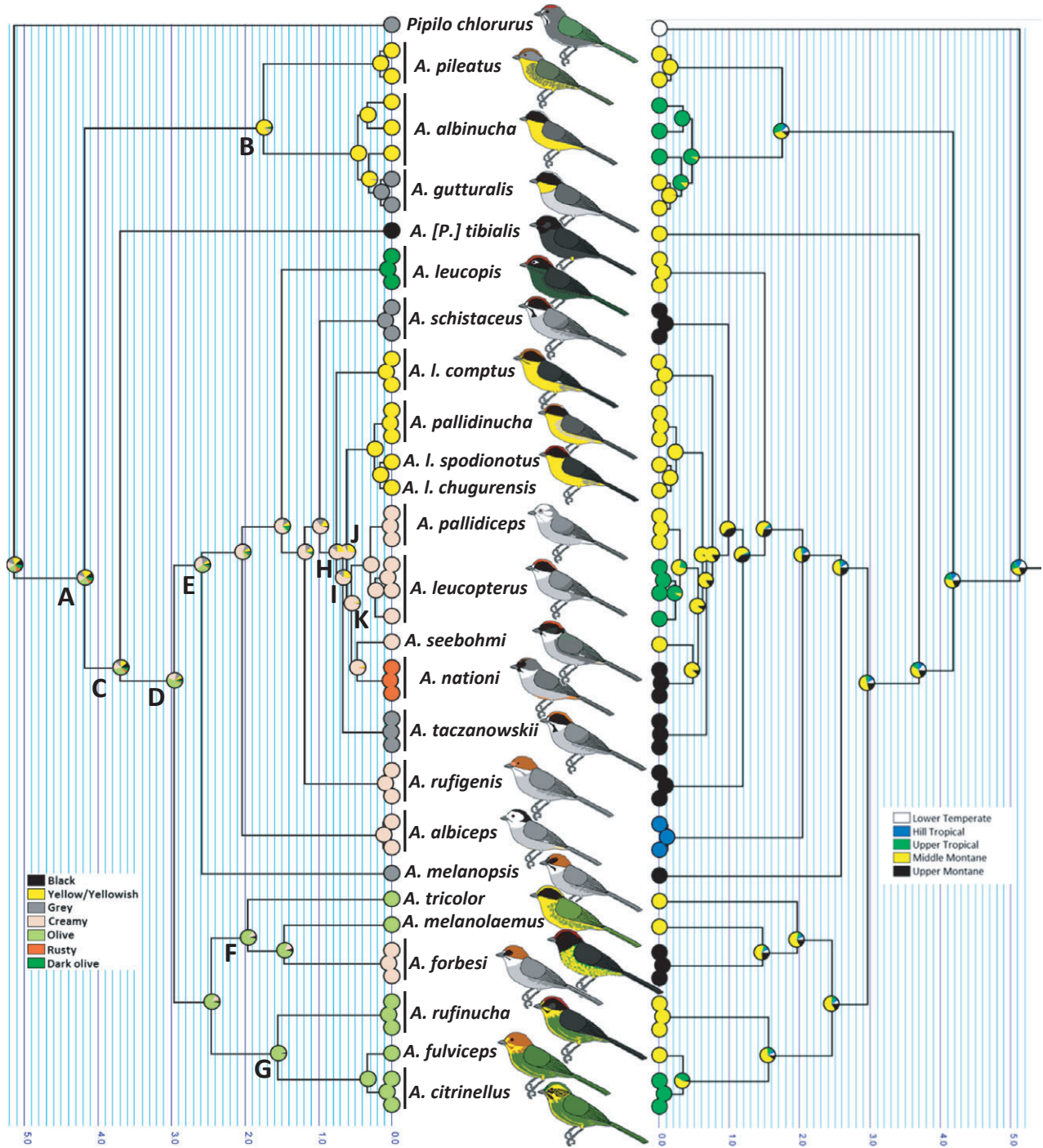
Fig. 4 Ancestral-state reconstruction for colour of the underparts (left) and CntAb (following Stotz *et al.* 1996; see Table 1 for elevational limits).

are observed within the last 2.6 mya (HPD range 1.9-3-3 mya), whereas a single event of colonization of UT in the western Andes slope in Peru is recorded within the last 2.5 kya and other to even lower HT elevations estimated

as occurred earlier in the differentiation of the northern Andean radiation in the Tumbes–Piura area.

Most species replacements are associated with a barrier or habitat gradient, but some sharp replacements are found

in places with no obvious barrier. On the East Andean slope of Ecuador, *A. schistaceus* occurs to the north and *A. latinuchus* to the south, and are sympatric along 25–50 km of the Andes just south of Río Paute in Azuay and Morona-Santiago, where they can be seen in the same mixed-species flocks, although *schistaceus* then moves more rapidly and higher in the vegetation than *latinuchus* (obs. by NK). In Peru, *A. latinuchus* and *taczanowskii*, and *A. canigenis* and *melanolaemus* meet but are not known to overlap.

Atlapetes leucopis has reached complete compatibility with other forms in this clade (*A. latinuchus* and *A. schistaceus*). *A. albiceps*, even more basal in this clade, has been reported to frequently occur in the same flocks as *A. leucopterus dresseri* in the 450 m wide altitudinal suture zone between them (Fitzpatrick 1980), suggesting that they have reached a near-complete ecological separation. The elevational overlap between *A. pallidinucha* and *A. l. latinuchus* in south-eastern Ecuador is remarkably broad (700 m), but within this zone, males of the two species countersing and apparently do not share territories (obs. by NK). The suture zone between *A. pallidiceps* (which is now restricted to a single locality) and *A. latinuchus comptus* is remarkably sharp, the latter species found highest on the slope and in the contact zone confined to the tallest and most humid vegetation; elsewhere *A. latinuchus comptus* also occurs in habitat similar to that of *A. pallidiceps* (Krabbe 2004). Elsewhere in south-western Ecuador and adjacent Peru, the distributions of *A. leucopterus*, *seebohmi* and *albiceps* resemble a draughtboard, with some level of local exclusion.

Plumage colour evolution

Given that changes in plumage colours are most prominent on the underparts, we show reconstructions for only that character (Fig. 4). As suggested by Remsen & White Solomon Graves (1995) and later confirmed by García-Moreno & Fjeldså (1999), change from grey- to yellow-underparts, and *vice versa*, is found within single clades (yellow plumaged/grey plumages): *A. albinucha/A. gutturalis* in Clade B, species in Clade H and *A. melanolaemus/A. forbesi* (and potentially *terborghi* and *canigenis*) in Clade G; our expanded data set suggest that such changes have occurred over the entire phylogeny, although they have been more frequent in the northern Andes clade (Node E, Fig. 4). According to the plumage reconstruction model, the *Atlapetes* ancestor most likely had neutral-grey upperparts and yellow-to-white underparts (Node A, Table 1), suggesting that plumage change probably progressed from these basic plumage colours to the others present in the genus (Fig. 4). Regarding the underparts, there is a tendency for the grey-plumaged taxa to inhabit higher elevations and drier habitats, as in clades B and F, where

grey-plumaged taxa *A. gutturalis* and *A. forbesi*, respectively, inhabit higher elevations than their respective sister species. This pattern of change in underparts colour seems to be more complicated in Clade E (north and central Andean taxa), where changes between grey-plumage and yellow-plumage seem to follow a random pattern as grey-plumaged species are basally located, whereas yellow-plumaged species appear to be more derived (Clades H and J). Two grey-plumaged species *A. melanopsis* (Clade E) and *A. forbesi* (Clade F) inhabit adjacent areas in the intermontane basins of central Peru. These species are phenotypically so similar that they could be regarded as subtle variants of the same species, yet they belong in different clades. This underscores that grey-plumage may be the ancestral phenotype in the core group in this study.

Elevation seems to have played a central role of plumage evolution in this genus. Most yellow-plumaged taxa inhabit middle-montane forests, whereas grey-plumaged species are found in higher elevation habitats, suggesting that a dark plumage colour may be advantageous at higher altitudes (Fig. 4). However, according to our results, dry environments may also play a selective role in mostly dry habitats as taxa grouped in Clade K (*A. pallidiceps*, *A. leucopterus*, *A. seebohmi* and *A. nationi*) are all grey-plumaged.

Vocalizations

All species gave dawn songs of short phrases at regular intervals, but *albinucha* often gave nearly continuous song of a few different single notes at short intervals. Songs of most species were mainly composed of rather sharp notes of various types, but songs of *melanopsis*, *tricolor*, *forbesi*, *schistaceus*, *taczanowskii*, *canigenis* and *leucopis* consistently included loud drawn-out notes, those of *latinuchus* and *melanocephalus* often so (Table 2). For each species, the phrases usually varied both in terms of themes and number of notes, but still, the songs of most species could be told apart either by ear or by one or another of the variables measured (Table 2), except *pallidiceps* songs, which could not be distinguished with certainty from those of the closely related *leucoptera*.

The general structure of the commonest phrases was fairly similar in many species belonging to different clades (Fig. S2): one, or more commonly two, loud, drawn-out notes followed by one or two series of repeated notes usually at slightly lower volume, not unlike song of *Zonotrichia capensis*. Song of *Pselliophorus tibialis* differed by lacking drawn-out notes, by intervals between phrases being very short and by parts of each phrase being often composed of repeated alternating notes of different pitches. In many species, each phrase was initiated by a variable number of *tick* or *chip* notes, but in *albofrenatus*, such notes were an integral part of the phrases, which instead lacked the loud

drawn-out notes. In *leucopis*, phrases were very long and slow-paced, highly varied and contained many loud drawn-out notes.

Different subspecies were generally found to give similar songs. Thus, we could neither detect any constant difference between songs of seven subspecies of *latinuchus* except that one (*yariguerum*) was perhaps generally impoverished in numbers of notes and different notes, nor could we find consistent differences between three subspecies of *leucopterus*, and two subspecies each of *albinucha*, *albofrenatus* and *rufinucha*. Songs of *A. tricolor crassus* and *A. t. tricolor* and songs of *A. s. schistaceus* and *A. schistaceus taczanowskii*, on the other hand, differed as much from each other as did many species (Table 2). The longest phrases of *crassus* were longer and with more notes than in *tricolor*, but songs were composed of fewer different notes. Phrases of *schistaceus* were always very simple, composed of just 1–3 notes, each individual giving on average 2.7 ± 1.1 ($n = 16$) different notes during a bout of songs, whereas those of *taczanowskii* were considerably more varied and complicated, composed on average of 6.1 ± 1.7 ($n = 7$) different notes in a bout of songs.

Discussion

Phylogenetic relationships

Phylogenetic relationships of the Passerellidae (including the genus *Atlapetes*) have been recently addressed using a multigene framework (Klicka *et al.* 2014). The phylogenetic tree obtained for the genus *Atlapetes* in the work by Klicka *et al.* (2014) shows strong similarities in the general structure to the tree obtained in our work, in which *Atlapetes* is divided in two main clades, with one clade including all of the Mesoamerican taxa, and the other clade including all of the South American taxa plus the Central American *Pselliophrus* finches deeply embedded. These similarities suggest that our single-gene phylogeny may be a reliable hypothesis. Additionally, the study by Klicka *et al.* (2014) recovered the earliest speciation bursts in South America (node D in our phylogeny) as poorly resolved, with red-headed species, *A. personatus* in the Tepuis are and *A. semirufus* in Colombia Venezuela, as deep branches, with the latter grouped with *A. albofrenatus* and *A. melanocephalus* in the same area, and all of them with yellow breast.

Main differences between our hypothesis and that in Klicka *et al.* (2014) are in our Northern Andes Clade E and central Andes Clade F. These results suggest that a comprehensively sampled study is critical to resolve the clustering order at the tips, as both works include taxa excluded from the other study's sampling, making direct comparison difficult. For example, the placement of a couple of species, *A. melanopsis* and *A. forbesi*, seems unclear as they belong in the same clade in Klicka *et al.* (2014) but in

different clades in our work, although with low support. Although with differences, both phylogenies contain information that may be complementary. For instance, in Klicka *et al.* (2014), the yellowish and dark olive Colombian endemics *A. fuscoolivaceus* and *flaviceps* group with *A. leucopis* (with high Bayesian probability).

In general, our molecular phylogeny of the genus *Atlapetes* provides an increased taxon sampling of populations distributed south of the equator (Fig. 1). Our phylogenetic framework allowed us to test and corroborate some taxonomic and biogeographic hypotheses regarding the evolutionary history of the genus. For example, a seminal paper on the phylogenetic relationships of *Atlapetes* brush-finches using molecular characters rejected the past hypothesis of separate groups of yellow and grey forms (García-Moreno & Fjeldså 1999). The inclusion of *Pselliophorus* in *Atlapetes* was previously suggested on anatomical grounds (Webster & Webster 1999); however, phenotypic differentiation from *Atlapetes* taxa prevents their inclusion in this genus. With the inclusion of *Pselliophorus* in *Atlapetes*, taxonomic arrangement within this latter genus changes, and the two *Pselliophorus* species recognized should be renamed as *A. luteoviridis* and *A. tibialis* (Klicka *et al.* 2014).

Phylogenetic clustering of the major clades seems to correspond to geography. We recovered a monophyletic *Atlapetes*, in which two different clades are clearly distinguished: a Mesoamerican species clade (Clade B) and a clade that includes *Atlapetes* species from Costa Rica–Panama (formerly included in *Pselliophorus*) and the Andes. The Andean clade is further subdivided, and clades associated with geography are also recognizable, with most species in each clade grouped in northern, central and southern clades.

Biogeography

Some previous studies on New World nine-primaried oscines have suggested that taxa included in this family may have had North America as their ancestral area and have speciated extensively in South America after dispersal (Smith & Klicka 2010; Barker *et al.* 2013). This has been suggested for *Buarremon* (Cadena *et al.* 2007; Navarro-Sigüenza *et al.* 2008), and for some species in the paraphyletic genus *Pipilo* (e.g. *Pipilo chlorurus*), some of which are closely related to *Atlapetes* (DaCosta *et al.* 2009; Barker *et al.* 2013). The same pattern is found in *Atlapetes*, in which the middle elevation forests of the Mesoamerican mountains show the highest probabilities as the ancestral area of the group.

Diversification in the genus *Atlapetes* is apparently correlated with glacial cycles that modified the elevation of the montane forests, which may have been the engine for the speciation of this genus and other montane forest birds,

especially in the Andes (García-Moreno & Fjeldså 2000; Weir 2006). After a rapid dispersal to Central and South American montane forests about 3.8 and 3 mya, respectively, *Atlapetes* underwent an intensive species radiation. The correspondence between clades and geography may be attributed to the key role that geographical barriers have played for the differentiation and diversification of montane avifaunas in the Neotropics (Vuilleumier 1969; Sánchez-González *et al.* 2008; Weir 2009). These intensive speciation events were apparently followed by isolation of *A. melanopsis* and dispersal to the southern Andes clade (clades F+G) in the central Peruvian valley system; a topographically complex area thought to have been ecologically resilient throughout the climatic fluctuations that characterized the Pleistocene (Fjeldså 1995; Fjeldså *et al.* 1999, 2012). The southern Andes clade may have undertaken further expansion, reaching the more seasonal climatic zone in north-western Argentina by 1.6 mya. The northern group (Clade E) experienced a burst of differentiation, likely associated to Pleistocene glacial cycles that promoted climatic change along the Andes. Within the last one million years, glacial cycles appear to have been more frequent and severe (Hooghiemstra *et al.* 1993); this intense period of climatic change produced elevational shifts in the distribution of montane forests which at times increased their extension as a consequence of an altitudinal shift downwards of approximately 1000 m (Veer & Hooghiemstra 2000; Hooghiemstra & van der Hammen 2004; Weng *et al.* 2007). *Atlapetes* taxa in Clade H seem to fit closely to this pattern: intermingling of species with yellow and grey underparts seem to describe a period of diversification promoting the speciation of UM taxa in high glacial cycles (*A. melanopsis, A. albiceps, A. rufigenis, A. taczanowskii* and *A. schistaceus*); whereas in high humidity periods (interglacial), differentiation of yellow-plumaged taxa in lower middle-montane elevations may have been possible (*A. comptus, A. pallidinucha* and *A. latinuchus*). It should also be born in mind that some Central Peruvian species are missing from our data set, and an expanded data set could result in a different pattern from the one shown here. In fact, the position of some interesting species from Peru that are missing in our phylogeny could be inferred from the results obtained by Klicka *et al.* (2014). This study included two local endemics from Cordilleras Vilcanota and Vilcabamba (Cuzco, Peru), with the very dark grey (almost patternless) *A. canigenis* inserted between the yellow-breasted and strongly pigmented *A. terborghi* and *A. melanopsis*, and all of them as part of a strongly supported clade from central Peru. An expanded molecular data set, as suggested above, may therefore change the patterns showed here. Late Pleistocene changes seem also responsible for differentiation in the Mesoamerican group (Clade B) and in the

southern Andes clade (Clade G). At the regional scale, differentiation may have proceeded at a slower pace when compared to the north Andean clade probably due to a correlation between regional elevation and a low number of geographical barriers promoting vicariance. However, at the local scale, patchiness in the cloud forests at higher elevations in south-western Ecuador and western Peru may have played a role in the differentiations process (Best & Kessler 1995), as suggested by the yellow-breasted *A. latinuchus* (?) *comptus* in the wettest cloud forests and grey birds with more or less white heads in drier vegetation types in the area. We lack tissue samples of nominate *latinuchus* from the central Andes ridge of southern Ecuador, which would be necessary to explore horizontal gene transfers among these populations.

Habitat shifting is likely associated to Pleistocene glacial cycles. In the geographical extremes of the *Atlapetes* range, a single habitat shift is detected in the Mesoamerican clade, where one species differentiated in UT habitats and then went back to MM elevations; the opposite pattern occurred in the southern Andes clade (Clade G), where the southernmost species in the genus colonized UT elevations. As mentioned earlier, the pattern of habitat shifting is more complicated in the northern Andes where lineages underwent at least five different colonization events of UM habitats, some of which early in the diversification of the genus; one species in this clade even invaded the lowest elevations recorded for species in the genus, reaching the HT forests of the Tumbes–Piura region. This suggests that the ancestor of Clade E may have been an ecologically tolerant species. It also suggests that glacial climatic cycles and the complicated geography of this part of the Andes may have been fundamental for the rich radiation of this genus in the area, as suggested in other bird groups (Weir 2006, 2009).

Plumage evolution
Atlapetes brush-finches have long been recognized for the change in plumage that different species show along the Andes and have been cited as one of the most pervasive examples of the leap-frog pattern (Remsen 1984; Remsen & White Solomon Graves 1995; García-Moreno & Fjeldså 1999). Most plumage changes in this study (detected mainly in the colour of the underparts) seem to have occurred within the last 2.5 mya, with a burst of colour-plumage change in the last one mya. As mentioned earlier, dark grey species are mostly associated with high elevation habitats (and with dry habitats on the Pacific slope; see below), whereas yellow-plumaged species are associated with MM habitats, suggesting a possible adaptive role of the colour of the plumage in this genus. Some yellow- or grey-plumaged species seem to inhabit elevational gradients

that are wider than the elevation as predicted from their underparts colour plumages; this has been suggested as an effect of interspecific competition (Remsen & White Solomon Graves 1995).

Whereas all *Atlapetes* species seem to conform to a relatively simple pattern of plumage with species mostly grey- or yellow-underparts and a variously ornamented head, *A. luteoviridis* and *A. tibialis* (hitherto *Pselliphorus*) from the Costa Rica–Panama highlands present a completely different plumage pattern: bright-yellow thighs and an unicoloured (*A. tibialis*) or bicoloured (*A. luteoviridis*) plumage (with some phenotypic resemblance to three Colombian species, *A. melanocephalus*, *flaviceps* and *fuscoolivaceus*). Both species diverged from other *Atlapetes* species around 3.7 mya and inhabit MM cloud forests as other typical *Atlapetes* species. This suggests that long isolation (as suggested by the long branch of *A. tibialis*) in a highly isolated area may be responsible for their high phenotypic differentiations. The only other atypical *Atlapetes* species in this study, *A. leucopis*, is found locally in wet montane forest in Ecuador and southern Colombia (*A. personatus* and *A. semirufus* are also atypical, but not included in this study). Basal species in the clade (Clade E) are inhabitants of UM or HT dry environments of the western Andes in Ecuador and Peru and are characterized by light plumages and in some cases more or less whitish heads, suggesting that UM *A. leucopis* may have developed a highly saturated plumage (dark green overall) as a result of colonization of a comparatively high-humid environment.

Most plumage colour changes have occurred in a relatively short time frame corresponding to the most severe glacial cycles in the Andes and that have been suggested to have occurred in the last million years (Hooghiemstra *et al.* 1993; van der Hammen & Hooghiemstra 1997). This suggests that changes in the underparts plumage colour may be associated to corresponding climatic changes in this period. Other explanations may also account in the explanation of this pattern: where there is only one species of *Atlapetes*, it may occupy the entire elevational range, conversely, where more than one species occurs, the elevational gradient is partitioned. This in turn suggests that competition may constrain the distribution of species (Remsen & White Solomon Graves 1995; but see Cadena & Loiselle 2007).

Non-adaptive vs. adaptive morphological variation

All species of *Atlapetes* species occur in dense brush or undergrowth, and many tolerate a wide humidity range and variation in floral compositions. Their distributional ranges seem to be widely governed by competition as the species replace each other sharply in different geographical areas, with apparently little niche segregation, but with some sharp replacements in places with no obvious physical barrier or environmental gradient, suggesting that these replacements are secondary contact zones between distinct (and incompatible) species. Considering that sympatric species are generally farther apart in the phylogeny (García-Moreno & Fjeldså 2000), there is therefore no evidence to suggest that morphological divergence is driven by selection for isolating mechanisms.

The most xeric habitats of the Pacific-slope harbour the two palest species, *A. albiceps* and *A. pallidiceps*, which have partially white heads, and more or less white heads are also seen in some populations of *leucopterus* and *nationi*. Similarly, species entirely restricted to the lower strata of humid forest may be very dark throughout, as *A. leucopis*, and it also appears to be significant that all the black-backed and yellow underparts species are primarily associated with closed humid forest. In *A. latinuchus*, the western Andean subspecies, which often frequent more open habitats, are all grey-backed, whereas nominate *latinuchus*, confined to humid forest on the east Andean slope, is black-backed (like other eastern slope taxa, *A. melanolaemus* and *rufinucha*). Most taxa inhabiting the arid western Peru are pale-backed and have grey underparts. Thus, the darkness of the upper parts and the distinctive colour of the underparts may show some correlation with climate.

Vocalizations

Consistent vocal differences were found between most *Atlapetes* species and the taxa *schistaceus* and *taczanowskii*. The species with the most different songs were *Atlapetes albinucha* and *Atlapetes tibialis* (and presumably *A. luteoviridis* for which no dawn songs were available, although duets are very similar to those of *tibialis*). Other species with fairly different songs were *fuscoolivaceus*, *albofrenatus* and *leucopis*, also all northern Andean forms. The theme in many phrases of *pileatus*, a species found to be closest related to *albinucha*, was somewhat similar to a theme common to many Andean forms, evidently a plesiomorphic trait.

As expected (Patten *et al.* 2004) loud, drawn-out and relatively low-pitched notes were most prominent in *leucopis*, the most forest based of all the species. Not all forest-based species gave loud, drawn-out and relatively low-pitched notes and at least one species of more open habitats (*forbesi*) did, but the fact that most of the species that did were forest based and belonged to widely different clades suggests that such notes may be environmental adaptations, and not evidence of phylogenetic relationship. Similarly, poor phrases of only 1–3 notes characterized several species of different clades, perhaps suggesting independent loss of repertoire. Interestingly, *pallidiceps* maintained a rather varied repertoire despite having been through a recent population bottleneck of less than 40 pairs (Krabbe 2004).

The explanation might be that these 40 pairs were all in close proximity to each other, while several other Andean species could have been through similar bottlenecks in their very patchy habitats. It remains difficult, however, to explain why the widespread and forest-based *schistaceus* has such a poor repertoire. It might suggest that short (or slow, as in *leucopis*) phrases of loud, drawn-out and relatively low-pitched notes may be an adaptation to a forested environment. Contra the expected (Patten *et al.* 2004), the two species with consistently long pauses between songs (*leucopterus*, *pallidiceps*) were birds of fairly open habitats.

Geographical speciation and secondary contact

Over all, the gene tree coincides well with geographical structure, as in other humid montane forests taxa as *Chlorospingus flavopectus* (García-Moreno *et al.* 2004; Bonaccorso *et al.* 2008; Weir *et al.* 2008), or *Buarremon* (Cadena *et al.* 2007; Navarro-Sigüenza *et al.* 2008) and *Myioborus* (Pérez-Emán 2005), more than with morphology. A few species, representing rather deep branches in the Andean radiation (*A. melanopsis*, *A. albiceps*, *A. leucopis* and possibly *A. tricolor* (?) *crassus*), may represent relictual taxa with small and widely separate distributions. The placing in Klicka *et al.* (2014) of the yellowish and dark olive Colombian endemics *A. fuscoolivaceus* and *A. flaviceps* with *A. leucopis* makes geographical sense.

Only in few cases does geographical contact between closely related species seem to have led to ecological segregation for the species inhabiting south-western Ecuador and adjacent Peru, as described above for *A. schistaceus* vs. *latinuchus*, and for *A. leucopis* vs. *schistaceus* and *latinuchus*. Despite the apparent recent speciation in the genus, hybridization between accepted species must be rare and restricted. Hybrids appear to be very rare among *Atlapetes* species. When examining hundreds of museum specimens, we found a single bird that may be a hybrid between *A. seebohmi* and *A. rufigenis* (The Natural History Museum, Tring, 1900–10.2.95); a single intermediate individual between *A. melanolaemus* and *A. rufinucha* is also known (García-Moreno & Fjeldså 1999). A specimen claimed by Chapman (1926) to be intermediate between *A. latinuchus spodionotus* and *A. latinuchus comptus* was re-examined by NK and found to represent a misinterpretation of an artefact of the skinning method. However, other intermediates between *latinuchus* and *comptus* were collected (ZMUC) and observed during the present study at sites where the two forms meet, leaving little doubt that these two forms intergrade.

The southern population of *A. leucopterus* (*dresseri*) was identical in its ND2 sequence to the most northern (nominate) form, suggesting that *pallidiceps* evolved in isolation for some time and that *leucopterus* only recently colonized the Tumbesian region by dispersal (pp = 0.98) past the range of *pallidiceps*, a small jump across Río Jubones in the humid zone downstream from the Yunguilla Valley. The small population sizes resulting from such a colonization in small habitat patches squeezed between populations of *A. seebohmi* and *A. latinuchus comptus* might account for the great site-to-site variability and widespread leucism in *dresseri* and the Huancabamba Valley population *paynteri*.

Overall, most morphological differences between *Atlapetes* species are probably results of population fragmentation and evolutionary stasis (Ricklefs & Latham 1992), as populations which persist in fairly constant environments diverge slowly, the rate depending on population size and generation time, in arbitrary directions. This non-adaptive divergence results in differences in pigments and patterns in populations inhabiting different slopes. However, some adaptations to local environments seem to take place, as the palest forms occur in the driest, most open habitats, and the black-backed forms in the most humid forest. Additionally, there is a tendency (with some exceptions) for strong pigment saturation in humid zones and loss of pigmentation, with a predominance of grey colours, in populations that live in rather dry cloud forest. Under favourable climatic conditions, such as the present interglacial period, the populations expand and establish secondary contact zones, generally without introgression, and in a few cases full compatibility has evolved. The Pacific-slope populations would represent a particularly interesting case for studying the detailed historical population structure.

Taxonomic implications

The phylogenetic hypothesis presented supports, in general, the classification suggested by García-Moreno & Fjeldså (1999), including the Central American *Pselliophorus* ground finches (Klicka *et al.* 2014; this study). Although formerly suggested based on morphological grounds (Webster & Webster 1999), Central American *Pselliophorus* ground finches were maintained as a genus, given its marked plumage differences from typical *Atlapetes* species.

In addition, our phylogenetic hypothesis shows that *A. schistaceus* forms a sister clade to the rest of the northern Andean group. The taxon *taczanowskii*, isolated 700 km away from nominate *schistaceus*, appears to be closer to the various taxa referred to *A. latinuchus*, thus representing yet another example of independent shift of pigmentation in the genus. Geographically, it is isolated from its nearest relatives, and morphologically it differs as much from these forms, as do some species living in partial sympatry, such as *A. l. latinuchus* and *A. s. schistaceus*. Additionally, it differs distinctly from *schistaceus* vocally. We therefore suggest that it is best treated as a full species: Taczanowski's Brush-finch *Atlapetes taczanowskii*.

In the taxa investigated, the subspecies were found to differ little from each other vocally, but one exception is *A. t. crassus* and *tricolor* (Table 1), which are distributed in western Colombia and Ecuador, and 900 km away in central Peru. They also differ in elevational distributions 300–2000 m against 1750–3050 m) and morphologically (*crassus* with bill much larger, crown, back and underparts differently coloured; Fjeldså & Krabbe 1990; Schulenberg *et al.* 2007). We suggest that the northern form is best treated as a full species: Chocó Brush-Finch *Atlapetes crassus*. In fact, the study by Klicka *et al.* (2014) included samples of *A. t. tricolor* from Peru and recovered it as sister to our clade H, which includes *Atlapetes* from a clade including mainly eastern Andean slope forms, widely separated from our Ecuadorian *A. t. crassus* sample, thus confirming the taxonomic differentiation in these two taxa.

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References

Arctander, P. (1995). Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. *Proceedings of the Royal Society of London Series B*, 262, 13–19.

Avise, J. C. (2009). Phylogeography: retrospect and prospect. *Journal of Biogeography*, 36, 3–15.

Barker, F. K., Burns, K. J., Klicka, J., Lanyon, S. M. & Lovette, I. J. (2013). Going to extremes: contrasting rates of diversification in a recent radiation of new world passerine birds. *Systematic Biology*, 62, 298–320.

Bensasson, D., Zhang, D.-X., Hartl, D. & Hewitt, G. M. (2001). Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology and Evolution*, 16, 314–321.

Best, B. J. & Kessler, M. (1995). *Biodiversity and Conservation of Tumbesian Ecuador and Peru*. Cambridge, UK: BirdLife.

Boesman, P. (2007). *Birds of Venezuela*. [DVD-ROM]. The Netherlands: Birdsounds.nl.

Bonaccorso, E., Navarro-Sigüenza, A. G., Sánchez-González, L. A., Peterson, A. T. & García-Moreno, J. (2008). Genetic differentiation of the *Chlorospingus ophthalmicus* complex in Mexico and Central America. *Journal of Avian Biology*, 39, 311–321.

Brown, A. D. & Kappelle, M., 1995. Introducción a los bosques nublados del Neotrópico: una síntesis regional. In M. Kappelle & A. D. Brown (Eds) *Bosques nublados del Neotrópico* (pp. 25–40). Costa Rica: InBio.

Buerki, S., Forest, F., Alvarez, N., Nylander, J. A., Arrigo, N. & Sanmartín, I. (2011). An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. *Journal of Biogeography*, 38, 531–550.

Burns, K. J., Hackett, S. J. & Klein, N. K. (2003). Phylogenetic relationships of Neotropical honeycreepers and the evolution of feeding morphology. *Journal of Avian Biology*, 34, 360–370.

Cadena, C. D. & Loiselle, B. A. (2007). Limits to elevational distribution in two species of emberizine finches: disentangling the role of interspecific competition, autoecology, and geographic variation in the environment. *Ecography*, 30, 491–504.

Cadena, C. D., Klicka, J. & Ricklefs, R. E. (2007). Evolutionary differentiation in the Neotropical Montane Region: Molecular Phylogenetics and Phylogeography of *Buarremon* brush-finches (Aves, Emberizinae). *Molecular Phylogenetics and Evolution*, 44, 993–1016.

Carson, R. J. & Spicer, G. S. (2003). A phylogenetic analysis of the emberizid sparrows based on three mitochondrial genes. *Molecular Phylogenetics and Evolution*, 29, 43–57.

Chapman, F. M. (1926). *The Distribution of Bird-life in Ecuador: a Contribution to a Study of the Origin of Andean Bird-Life*. Bulletin of the American Museum of Natural History, 55 (p. 784). New York.

DaCosta, J. M. & Klicka, J. (2008). The Great American Interchange in birds: a phylogenetic perspective with the genus Trogon. *Molecular Ecology*, 17, 1328–1343.

DaCosta, J. M., Spellman, G. M., Escalante, P. & Klicka, J. (2009). A molecular systematic revision of two historically problematic songbird clades: *Aimophila* and *Pipilo*. *Journal of Avian Biology*, 40, 206–216.

Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29, 1969–1973.

Filardi, C. E. & Moyle, R. G. (2005). Single origin of a pan-Pacific bird group and upstream colonization of Australasia. *Nature*, 438, 216–219.

Fitzpatrick, J. W. (1980). A new race of *Atlapetes leucopterus*, with comments on widespread albinism in *A. l. dresseri* (Taczanowski). *Auk*, 97, 883–887.

Fjeldså, J. (1995). Geographical patterns of neoendemic and older relict species of Andean forest birds: the significance of ecologically stable areas. In S. P. Churchill, H. Balslev, E. Forero & J. L. Luteyn (Eds) *Biodiversity and Conservation of Neotropical Montane Forests* (pp. 89–102). New York: New York Bot. Garden.

Fjeldså, J. & Krabbe, N. (1990). *Birds of the High Andes*. Apollo Books, Svendborg, Denmark, and Zoological Museum, University of Copenhagen.

Fjeldså, J., Lambin, E. & Mertens, B. (1999). Correlation between endemism and local ecoclimatic stability documented by comparing Andean bird distributions and remotely sensed land surface data. *Ecography*, 22, 63–78.

Fjeldså, J., Bowie, R. C. K. & Rahbek, C. (2012). The role of mountain ranges in the Diversification of birds. *Annual Review of Ecology, Evolution and Systematics*, 43, 249–265.

Flórez-Rodríguez, A., Carling, M. D. & Cadena, C. D. (2011). Reconstructing the phylogeny of "*Buarremon*" brush-finches and near relatives (Aves, Emberizidae) from individual gene trees. *Molecular Phylogenetics and Evolution*, 58, 297–303.

García-Moreno, J. & Fjeldså, J. (1999). A re-evaluation of species limits in the genus *Atlapetes* based on mtDNA sequence data. *Ibis*, *141*, 199–207.

García-Moreno, J. & Fjeldså, J. (2000). Chronology and mode of speciation in the Andean Avifauna. *Bonner Zoological Monographs*, *46*, 25–46.

García-Moreno, J., Ohlson, J. & Fjeldså, J. (2001). MtDNA sequences support monophyly of *Hemispingus* tanagers. *Molecular Phylogenetics and Evolution*, *21*, 424–435.

García-Moreno, J., Navarro-Sigüenza, A. G., Peterson, A. T. & Sánchez-González, L. A. (2004). Genetic variation coincides with geographic structure in the common bush-tanager (*Chlorospingus ophthalmicus*) complex from Mexico. *Molecular Phylogenetics and Evolution*, *33*, 186–196.

Griekspoor, A. & Groothuis, T. (2005). 4Peaks, version 1.6. Available via mekentosj.com.

van der Hammen, T. & Hooghiemstra, H. (1997). Chronostratigraphy and correlation of the Pliocene and Quaternary of Colombia. *Quaternary International*, *40*, 81–91.

Hogner, S., Laskemoen, T., Lifjeld, J. T., Porkert, J., Kleven, O., Albayrak, T., Kabasakal, B. & Johnsen, A. (2012). Deep sympatric mitochondrial divergence without reproductive isolation in the Common Redstart *Phoenicurus phoenicurus*. *Ecology and Evolution*, *2*, 2974–2988.

Hooghiemstra, H. & van der Hammen, T. (2004). Quaternary Ice-Age dynamics in the Colombian Andes: developing an understanding of our legacy. *Philosophical Transactions of the Royal Society of London B*, *359*, 173–181.

Hooghiemstra, H., Melice, J. L., Berger, A. & Shackleton, N. J. (1993). Frequency-spectra and paleoclimatic variability of the high-resolution 30-1450-Ka Funza I pollen record (Eastern Cordillera, Colombia). *Quaternary Sciences Review*, *12*, 141–156.

Huang, H., Tran, L. A. P. & Knowles, L. L. (2014). Do estimated and actual species phylogenies match? Evaluation of East African cichlid radiations *Molecular Phylogenetics and Evolution*, *78*, 56–65.

Huelsenbeck, J. P. & Ronquist, F. R. (2002). Mr Bayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*, 754–755.

Huelsenbeck, J. P., Larget, B., Miller, R. E. & Ronquist, F. (2002). Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology*, *51*, 673–688.

Jaramillo, A., Rising, J. D., Copete, J. L., Ryan, P. G. & Madge, S. C. (2011). Family Emberizidae. In J. del Hoyo, A. Elliott & D. Christie (Eds) *Handbook of the Birds of the World, Vol. 16*, (pp. 428–683). Barcelona: Lynx Edicions.

Kass, R. E. & Raftery, A. E. (1995). Bayes factors. *Journal of the American Statistical Association*, *90*, 773–795.

Klicka, J., Johnson, K. P. & Lanyon, S. M. (2000). New world nine-primaried oscine relationships: constructing a mitochondrial DNA framework. *Auk*, *117*, 321–336.

Klicka, J., Burns, K. & Spellman, G. M. (2007). Defining a monophyletic Cardinalini: a molecular perspective. *Molecular Phylogenetics and Evolution*, *33*,*45*, 1014–1032.

Klicka, J., Keith Barker, F., Burns, K. J., Lanyon, S. M., Lovette, I. J., Chaves, J. A. & Bryson, R. W., Jr (2014). A comprehensive multilocus assessment of sparrow (Aves: Passerellidae) relationships. *Molecular Phylogenetics and Evolution*, *77*, 177–182.

Kodandaramaiah, U. (2010). Use of dispersal–vicariance analysis in biogeography—a critique. *Journal of Biogeography*, *37*, 3–11.

Krabbe, N. (2004). Pale-headed brush-finch *Atlapetes pallidiceps*: notes on population size, habitat, vocalizations, feeding, interference competition and conservation. *Bird Conservation International*, *14*, 77–86.

Krabbe, N. & Nilsson, J. 2003. *Birds of Ecuazrdor*. [DVD-ROM]. The Netherlands: Bird Songs International.

Krabbe, N. & Schulenberg, T. S. (1997). Species limits and natural history of *Scytalopus* tapaculos (Rhinocryptidae), with descriptions of the Ecuadorian taxa, including three new species. *Ornithological Monographs*, *48*, 47–88.

Lamm, K. S. & Redelings, B. D. (2009). Reconstructing ancestral ranges in historical biogeography: properties and prospects. *Journal of Systematics and Evolution*, *47*, 369–382.

Maddison, W. P. & Maddison, D. R. (2010). *Mesquite: A Modular System for Evolutionary Analysis. 2010. Version 2.74*. Available at: mesquiteproject. org/mesquite/download/download. html.

Mayer, S. (2000). *Birds of Bolivia*. [CD-ROM]. The Netherlands: Bird Songs International.

Moore, W. S. (1995). Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, *49*, 718–726.

Moore, J. V., Krabbe, N. & Jahn, O. (2013). *Bird Sounds of Ecuador*. [DVD-ROM]. San José, California: John V. Moore Nature Recordings.

Navarro-Sigüenza, A. G., Peterson, A. T., Nyari, A., García-Deras, G. M. & García-Moreno, J. (2008). Phylogeography of the *Buarremon* brush-finch complex (Aves, Emberizidae) in Mesoamerica. *Molecular Phylogenetics and Evolution*, *47*, 21–35.

Nylander, J. A. A. (2004). *MrModeltest v2*. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University.

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., D'Amico, J. A., Itoua, I., Strand, H. E., Morrison, J. C., Loucks, C. J., Allnutt, T. F., Ricketts, T. H., Kura, Y., Lamoreux, J. F., Wettengel, W. W., Hedao, P. & Kassem, K. R. (2001). Terrestrial ecoregions of the world: a new map of life on Earth. *BioScience*, *51*, 933–938.

Patten, M. A., Rotenberry, J. T. & Zuk, M. (2004). Habitat selection, acoustic adaptation, and the evolution of reproductive isolation. *Evolution*, *58*, 2144–2155.

Paynter, R. A. (1972). Biology and evolution of the *Atlapetes schistaceus* species-group (Aves: Emberizinae). *Bulletin of the Museum of Comparative Zoology*, *143*, 297–320.

Paynter, R. A. (1978). Biology and evolution of the avian genus *Atlapetes* (Emberizinae). *Bulletin of the Museum of Comparative Zoology*, *148*, 323–369.

Pérez-Emán, J. L. (2005). Molecular phylogenetics and biogeography of the Neotropical redstarts (*Myioborus*; Aves, Parulinae). *Molecular Phylogenetics and Evolution*, *37*, 511–528.

Rambaut, A. (2002). SE-AL v. 2.0a11 ± sequence alignment program. Available via http://tree.bio.ed.ac.uk/software/seal/.

Rambaut, A. & Drummond, A. J. (2007). *Tracer v1.4*. Available via http://beast.bio.ed.ac.uk/Tracer.

Ree, R. H. & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, *57*, 4–14.

Remsen, J. V., Jr (1984). High incidence of "leapfrog" pattern of geographic variation in Andean birds: implications for the speciation process. *Science*, *224*, 171–173.

Remsen, J. V., Jr & White Solomon Graves, I. V. (1995). Distribution patterns and zoogeography of *Atlapetes* brush-finches (Emberizinae) of the Andes. *Auk*, *112*, 210–224.

Ricklefs, R. E. & Latham, R. E. (1992). Intercontinental correlation of geographical ranges suggests stasis in ecological traits of relict genera of temperate perennial herbs. *American Naturalist*, *139*, 1305–1321.

Rising, J. D. (2011). Family Emberizidae (Buntings and New World Sparrows). In J. Del Hoyo, A. Elliot & D. A. Christie (Eds) *Handbook of the Birds of the World. Vol. 16. Tanagers to New World Sparrows* (pp. 428–683). Barcelona: Lynx Edicions.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S. & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542.

Sánchez-González, L. A. & Navarro-Sigüenza, A. G. (2009). History meets ecology: a geographical analysis of ecological restriction in the Neotropical humid montane forests avifaunas. *Diversity and Distributions*, *15*, 1–11.

Sánchez-González, L. A., Morrone, J. J. & Navarro-Sigüenza, A. G. (2008). Distributional patterns of the Neotropical humid montane forest avifaunas. *Biological Journal of the Linnean Society*, *94*, 175–194.

Schulenberg, T. S. (2000). *Voices of Andean Birds*. Vol. 2. [Audio CD]. Ithaca, New York: Library of Natural Sounds.

Schulenberg, T. S., Stotz, D. F., Lane, D. F., O'Neill, J. P. & Parker, T. A. III (2007). *Birds of Peru*. Princeton: Princeton University Press.

Smith, T. B. & Klicka, J. (2010). The profound influence of the Late-Pliocene Panamanian uplift on the exchange, diversification, and distribution of New World Birds. *Ecography*, *33*, 333–342.

Sorenson, M. D. & Quinn, T. W. (1998). Numts: a challenge for avian systematics and population biology. *Auk*, *115*, 214–221.

Stotz, D. W., Fitzpatrick, J. W., Parker, T. A. III & Moscovits, D. K. (1996) *Neotropical Birds: Ecology and Conservation*. Chicago, Illinois: University of Chicago Press.

Swofford, D. (2002). *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4.0b10. Sunderland, Mass: Sinauer Associates.

Veer, R. V. T. & Hooghiemstra, H. (2000). Montane forest evolution during the last 650 000 years in Colombia: a multivariate approach based on pollen record Funza-I. *Journal of Quaternary Science*, *15*, 329–346.

Vuilleumier, F. (1969). Pleistocene speciation in birds living in the high Andes. *Nature*, *117*, 9–1180.

Webster, G. L. (1995). The panorama of neotropical cloud forests. In S. P. Churchill, H. Balslev, E. Forero & J. M. Luteyn (Eds), *Biodiversity and Conservation of Neotropical Montane Forests* (pp. 53–77). New York: The New York Botanical Garden.

Webster, J. D. & Webster, J. R. (1999). Skeletons and the genera of sparrows (Emberizinae). *Auk*, *116*, 1054–1074.

Weir, J. T. (2006). Divergent timing and patterns of species accumulation in lowland and highland neotropical birds. *Evolution*, *60*, 842–855.

Weir, J. T. (2009). Implications of genetic differentiation in neotropical montane forest birds. *Annals of the Missouri Botanical Garden*, *96*, 410–433.

Weir, J. T., Bermingham, E., Miller, M. J., Klicka, J. & González, M. A. (2008). Phylogeography of a morphologically diverse Neotropical montane species, the Common Bush-Tanager (*Chlorospingus ophthalmicus*). *Molecular Phylogenetics and Evolution*, *47*, 650–664.

Weng, C., Hooghiemstra, H. & Duivenvoorden, F. (2007). Response of pollen diversity to the climatic-driven altitudinal shift of the vegetation in the Colombian Andes. *Philosophical Transactions of the Royal Society B*, *274*, 919–928.

Xie, W., Lewis, P. O., Fan, Y., Kuo, L. & Chen, M. H. (2011). Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology*, *60*, 1–11.

Yu, Y., Harris, A. J. & He, X. (2010). S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution*, *56*, 848–850.

Yu, Y., Harris, A. J. & He, X.-J. (2012a). RASP (Reconstruct Ancestral State in Phylogenies) v. 2.0. Available at http://mnh.scu.edu.cn/soft/blog/RASP.

Yu, Y., Harris, A. J. & He, X. (2012b). A rough guide to RASP. Available online at http://mnh.scu.edu.cn/soft/blog/RASP.

Yuri, T. & Mindell, D. P. (2002). Molecular phylogenetic analysis of Fringillidae, "New World nine-primaried oscines" (Aves: Passeriformes). *Molecular Phylogenetics and Evolution*, *23*, 220–243.

Zhang, D.-X. & Hewitt, G. M. (1996). Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, *11*, 247–251.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of samples used in this study.

Table S2. Primer used in this study.

Fig. S1. Endemism areas used for the biogeographic reconstruction of the genus *Atlapetes*.

Fig. S2. Sonograms for different *Atlapetes* species considered in this study. S2.1. Sonograms of fairly similar song phrases in three species of *Atlapetes* belonging to three widely different clades.