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Molecular systematics of the new world screech-owls (*Megascops*: Aves, Strigidae): biogeographic and taxonomic implications ☆

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ABSTRACT

Megascops screech-owls are endemic to the New World and range from southern Canada to the southern cone of South America. The 22 currently recognized *Megascops* species occupy a wide range of habitats and elevations, from desert to humid montane forest, and from sea level to the Andean tree line. Species and subspecies diagnoses of *Megascops* are notoriously difficult due to subtle plumage differences among taxa with frequent plumage polymorphism. Using three mitochondrial and three nuclear genes we estimated a phylogeny for all but one *Megascops* species. Phylogenies were estimated with Maximum Likelihood and Bayesian Inference, and a Bayesian chronogram was reconstructed to assess the spatio-temporal context of *Megascops* diversification. *Megascops* was paraphyletic in the recovered tree topologies if the Puerto Rican endemic *M. nudipes* is included in the genus. However, the remaining taxa are monophyletic and form three major clades: (1) *M. choliba*, *M. koepckeae*, *M. albogularis*, *M. clarkii*, and *M. trichopsis*; (2) *M. petersoni*, *M. marshalli*, *M. hoyi*, *M. ingens*, and *M. colombianus*; and (3) *M. asio*, *M. kennicottii*, *M. cooperi*, *M. barbarus*, *M. sanctaecatarinae*, *M. roboratus*, *M. watsonii*, *M. atricapilla*, *M. guatemalae*, and *M. vermiculatus*. *Megascops watsonii* is paraphyletic with some individuals more closely related to *M. atricapilla* than to other members in that polytypic species. Also, allopatric populations of some other *Megascops* species were highly divergent, with levels of genetic differentiation greater than between some recognized species-pairs. Diversification within the genus is hypothesized to have taken place during the last 8 million years, with a likely origin in Central America. The genus later expanded over much of the Americas and then diversified via multiple dispersal events from the Andes into the Neotropical lowlands.

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1. Introduction

The New World screech-owl genus *Megascops*, recently split from *Otus* based on vocal and molecular evidence (van der Weyden, 1975; Marshall and King, 1988; Wink and Heidrich, 1999, 2000; Fuchs et al., 2008), currently includes 22 species divided into *ca.* 63 taxa according to Marks et al. (1999), or 21 species according to the American Ornithologists' Union (Banks et al., 2003; Remsen et al., 2015). Recognition of species limits and inferences about relationships are notoriously difficult for this genus as a result of considerable plumage similarity among taxa and polymorphism within taxa (Weske and Terborgh, 1981; Fitzpatrick and O'Neill, 1986; Sick, 1997; Wink and Heidrich, 2000). For instance, several subspecies described by Hekstra (1982) based solely on plumage are not currently recognized, being instead considered as individual variation within recognized taxa (Marks et al., 1999). In other cases, polytypic species such as *Megascops guatemalae* are variably treated either as one polytypic species or as many as four separate species with allopatric taxa distributed from Mexico to the Bolivian Andes (Marks et al., 1999).

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Previous phylogenetic hypotheses for *Megascops* (Heidrich et al., 1995; Wink and Heidrich, 2000; Proudfoot et al., 2007; Wink et al., 2009) all lacked extensive taxon sampling and only used partial DNA sequences of one or two genes. Also, authors have elevated forms of some polytypic taxa to species-level based on morphological and/or vocal evidence. Thus, sampling multiple taxa and populations in these polytypic species is important for resolving the controversial taxonomy of *Megascops* and is critical for reconstructing the evolutionary history of the group. Here, we present the most complete phylogenetic data set for *Megascops* to date based on broad taxon sampling covering all but one currently recognized species. The proposed phylogeny is used to discuss the historical diversification and taxonomy of *Megascops*.

2. Material and methods

2.1. DNA extraction, amplification and sequencing

Our analyses included tissue samples from 44 individuals of 29 *Megascops* taxa (see Supplementary Material) belonging to all but one species recognized by Marks et al. (1999) and an unnamed taxon (see results). We were unable to find available tissues for *M. seductus*. DNA was extracted using the DNeasy tissue extraction kit (Qiagen, Valencia, California) or a phenol–chloroform protocol (Sambrook and Russel, 2001). We obtained sequences of six different genes including three mitochondrial genes (Cytochrome-*b* [Cytb, 1035 bps], NADH dehydrogenase subunit 2 [ND2, 1040 bps] and Cytochrome c oxidase subunit 1 [COI, 379 bps]), one nuclear intron (ß-fibrinogen Intron 5 [Bfib5, 560 bps]) and two Z-linked introns (chromohelicase-DNA binding protein intron 18 [CHD, 349 bps] and muscle skeleton receptor tyrosine kinase intron 4 [MUSK, 605 bps]). Primers used to amplify and sequence each gene are listed in Table 1. For amplification and sequencing of ND2 for this study we designed and used two internal primers: L5758Mega (5′RRTGRGARATDGATGARAAGGC3′) and H5776Mega (5′GGNTGRATRGGCYTRAACCARAC3′). Not all genes could be sequenced for all individuals; Cytb was sequenced for all individuals; COI was sequenced for 43 individuals of all taxa; ND2 was sequenced for 43 individuals of all taxa; Bfib5 was sequenced for 39 individuals of 26 taxa; MUSK was sequenced for 39 individuals of 25 taxa, and CHD was sequenced for 34 individuals of 24 taxa (see Supplementary Material).

MtDNA fragments were PCR-amplified using standard thermal cycling conditions: denaturation at 94 °C, annealing between 46 and 56 °C, and extension at 72 °C, for 30 or 35 cycles. For the nuclear genes, we used a touch-down PCR protocol in which the annealing temperature was incrementally decreased from 58 °C for five cycles to 54 °C for five cycles and 50 °C for 30 cycles. The PCR products were run on a 1% agarose gel to verify whether amplification was successful and of sufficient quantity for sequencing. PCR products were cleaned using either Exonuclease and Shrimp Alkaline Phosphatase (ExoSap) enzymatic reactions (United States Biochemical), PEG 8000 20% NaCl 2.5 M, or GELase (Epicentre Technologies, Madison, WI). We cloned individual intron sequences exhibiting length variant heterozygosity using the TOPO TA cloning kit (Invitrogen), following the manufacturer's protocol. These cloned products were PCR amplified and then directly sequenced to verify the sequence length of the variant haplotypes. All PCR and cloned amplicons were cycle-sequenced using a BigDye 3.1 Terminator kit (BigDye, Applied Biosystems, Foster City, CA) with the same primers used for amplification. Cycle sequencing reactions were cleaned with ethanol EDTA precipitation, and resuspended in Hi–Di formamide. Sequences were then visualized through an ABI 3730 automated sequencer and aligned and reconciled using Sequencher 3.1.1 (Gene Codes Corp, Ann Arbor, MI).

2.2. Phylogenetic analyses

Uncorrected genetic distances between lineages were calculated with MEGA 5 (Tamura et al., 2011), using the concatenated mitochondrial data. We aligned sequences of all sampled individuals in Sequencher or Bioedit v. 7.1.3 (Tom Hall, Ibis Biosciences), and concatenated them into one single dataset. Saturation was evaluated with DAMBE (Xia and Xie, 2001). We used samples from the following owl taxa as outgroups: *Psiloscops* (*Otus*) *flammeolus*, *Glaucidium peruanum*, *Otus megalotis*, *Lophostrix cristata* and *Asio clamator* (Fig. 1). The phylogeny was reconstructed using Maximum Likelihood (ML) as implemented in RAxML 7.0.3 (Stamatakis, 2006), as well as Bayesian Inference (BI) as implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003).

The best-fit likelihood model of nucleotide substitution for each gene was determined using MrModeltest 2.3 (Nylander, 2004), based on Akaike's information criterion (AIC). We tested different partitioning schemes in BI: one partition (all data combined); two partitions (mitochondrial genes combined and nuclear genes combined); four partitions (mitochondrial genes separate and nuclear genes combined; mitochondrial genes combined and nuclear genes separate); and six partitions (all genes separate). The selected partitioning scheme – i.e. the one with the best Bayes factor value – had all mitochondrial genes separate and all nuclear genes combined. We also analyzed mitochondrial and nuclear datasets separately. For BI analysis we ran two parallel runs, with four Markov chains and 10 million generations each, sampling the chains every 500 generations. We discarded the first 5000 generations as burnin and used the remaining trees to create a 50% majority-rule consensus tree and to calculate Bayesian posterior probabilities (PP) as an assessment of nodal support.

2.3. Diversification timing and ancestral area reconstruction analyses

A Bayesian relaxed-clock analysis was performed in BEAST v. 1.8.0 (Drummond et al., 2012) to assess species divergence times

Table 1
Primers used in the study.

Gene	Primer	References
Cytochrome-b (Cytb, 1035 bps)	L14841	Kocher et al. (1989)
	H16065	Helm-Bychowski and Cracraft (1993)
NADH dehydrogenase subunit 2 (ND2, 1040 bps)	L5215	Hackett (1996)
	H6313	Sorenson et al. (1999)
	L5758Mega	This study
	H5776Mega	This study
Cytochrome c oxidase subunit I (COI, 379 bps)	L6625, H7005	Hafner et al. (1994)
Intron 5 of the nuclear b-fibrinogen gene (Bfib5, 560 bps)	FIB5L, FIB5H	Driskell and Christidis (2004)
Chromohelicase-DNA binding protein intron 18 (CHD, 349 bps)	CHD-18F, CHD-18R	Jacobsen et al. (2010)
Muscle skeleton receptor tyrosine kinase intron 4 (MUSK, 605 bps)	MUSK-F, MUSK-R	Kimball et al. (2009)

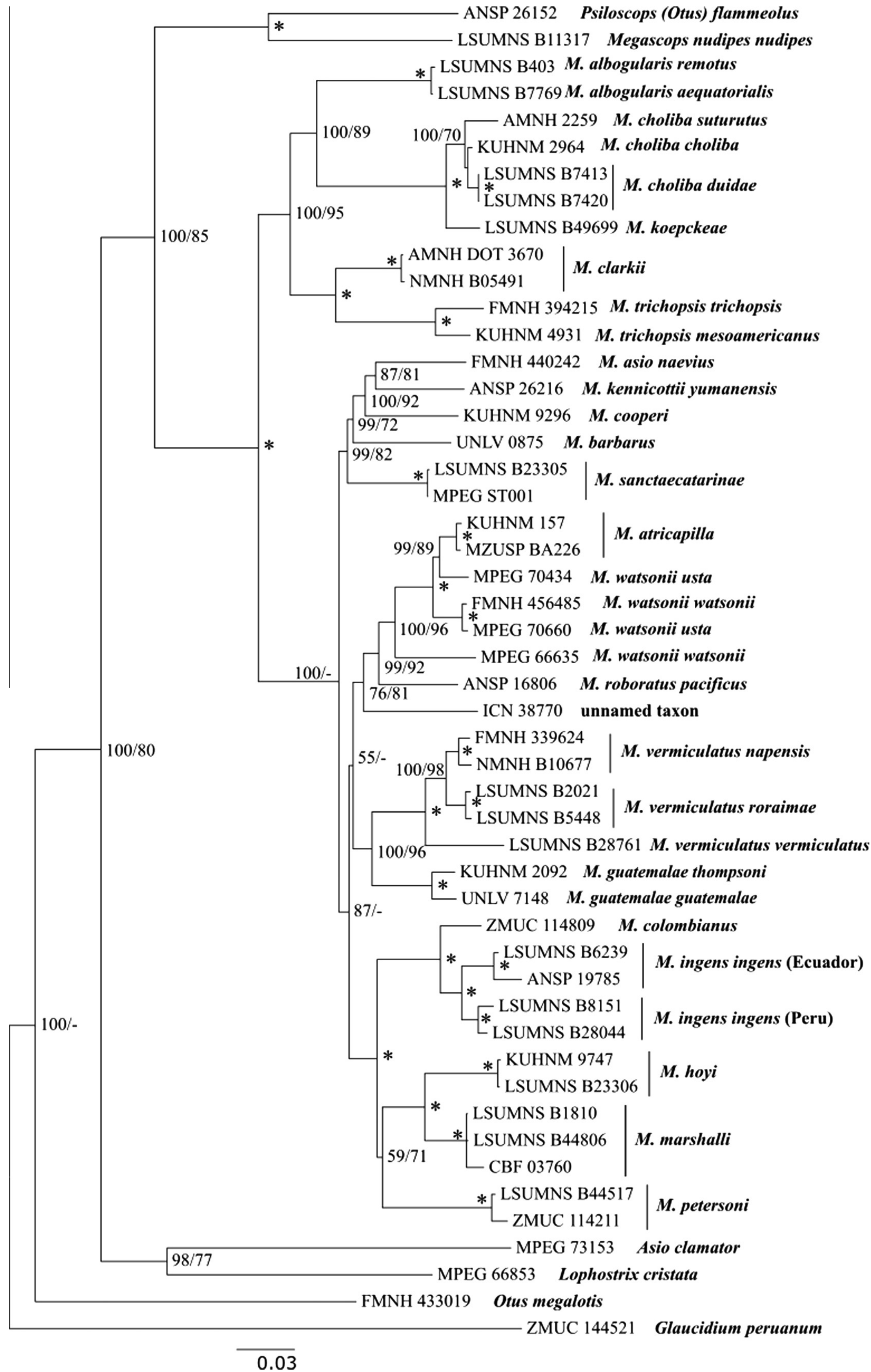

Fig. 1. Bayesian Inference phylogeny estimate based on a concatenation of all sequenced genes (Cytb, ND2, COI, BF5, CHD and MUSK). Node labels are BI/ML posterior probability and bootstrap support values, respectively. Asterisks associated with nodes indicate that both BI and ML support values are equal to or above 95%. Nodes denoted with a "–" indicate that they were not recovered by the ML analysis.

using the COI, Cytb and ND2 genes. Only one individual per taxon was used in the analysis. We linked the trees and clock (uncorrelated lognormal) models across all genes, but unlinked their substitution models (Drummond et al., 2006). The mean (parameter ucld. mean) for the clock rate was treated as an unknown parameter following a normal distribution ($\mu = 1.105\%$; $\sigma = 0.34\%$) of substitutions/site/branch/Ma. This prior was based on a widely used cytb calibration rate of 0.01105 substitutions per million years (Weir and Schluter, 2008). We used a Yule speciation process for the tree prior. MCMC chains were run for 50 million generations sampling every 5000 generations. Because ESSs parameter values associated with some initial runs were below the recommend threshold, we carried out multiple runs (up to 31) until values above 200 were obtained for these parameters, combining all runs to estimate the resulting chronogram. TRACER v. 1.5 was used to visualize the posterior distributions for every parameter.

To reconstruct the ancestral history of the genus *Megascops*, we carried out an ancestral area reconstruction analysis using BioGeoBEARS (BioGeography with Bayesian (and likelihood) Evolutionary Analysis in R Scripts; Matzke, 2013; http://cran. rproject.org/web/packages/BioGeoBEARS/index.html). This R package implements in a likelihood framework several ancestral area reconstruction models, such as LaGrange Dispersal-Extinction Cladogenesis Model (DEC) (Ree and Smith, 2008), a likelihood version of DIVA (DIVALIKE), and likelihood versions of range evolution models such as BayArea and the Bayesian Binary Model (BBM) of RASP (Yu et al., 2015). In BioGeoBEARS, founder event speciation can be added to any of the previously described models, and left as a free parameter estimated from the data, creating "DEC+J", "DIVALIKE+J", and "BAYAREA+J" models. We defined eight biogeographic areas based on the distribution of *Megascops* taxa: (1) North America; (2) Central America and trans-Andean South America; (3) Caribbean; (4) Andes; (5) Tepuis; (6) Amazonia; (7) Atlantic Forest; and (8) non-forested areas of cis-Andean South America. Our Bayesian relaxed-clock tree was used to infer the ancestral area probability, which was computed for each node and subsequently plotted on the majority-rule chronogram. Finally, we compared the six different models for statistical fit in two ways, using likelihood values and Akaike Information Criterion (AIC), both implemented in the BioGeoBEARS R package (Matzke, 2013). Only one individual of each taxon was used in the analysis (Supplementary Material).

3. Results

3.1. Sequence divergence

Plots of uncorrected transition versus transversion divergence did not indicate saturation among ingroup taxa. Uncorrected mitochondrial sequence divergence levels among all *Megascops* taxa analyzed (Supplementary Material) ranged from 2.8% (between *M. watsonii usta* and *M. atricapilla* to around 18% (between *M. nudipes* and *M. vermiculatus napensis*). Divergence levels among populations or individuals within *M. vermiculatus* (3.5–6.5%), *M. trichopsis* (4.1%, not shown in the table), *M. ingens* (3.0%) and *M. watsonii* (6.3%) were above the lowest divergence value between recognized species.

3.2. Phylogenetic relationships

A four-partition scheme was selected as the best for the BI and ML phylogenetic estimates using concatenated mitochondrial and nuclear genes. For this partitioning scheme, each mitochondrial gene had its own model applied to it and all nuclear genes were treated as a single partition. Analysis of the mitochondrial and nuclear concatenated (mit + nuc) data, and a separate analysis of mtDNA data only (not shown) provided similar results, with a few major discrepancies discussed below.

Megascops nudipes is sister to *Psiloscops flammeolus* (Fig. 1), rendering the genus *Megascops* paraphyletic. The ML and BI nodal support values for the placement of *M. nudipes* outside the main *Megascops* clade were high (Figs. 1 and 2). The remaining species of the *Megascops* were divided into two major clades, both supported with significant BI values, but with just one of them recovered and supported by the ML phylogeny (Fig. 1). One clade includes *M. choliba* and *M. koepckeae* grouped as sisters with *M. albogularis*. Members of this subclade are mainly South American

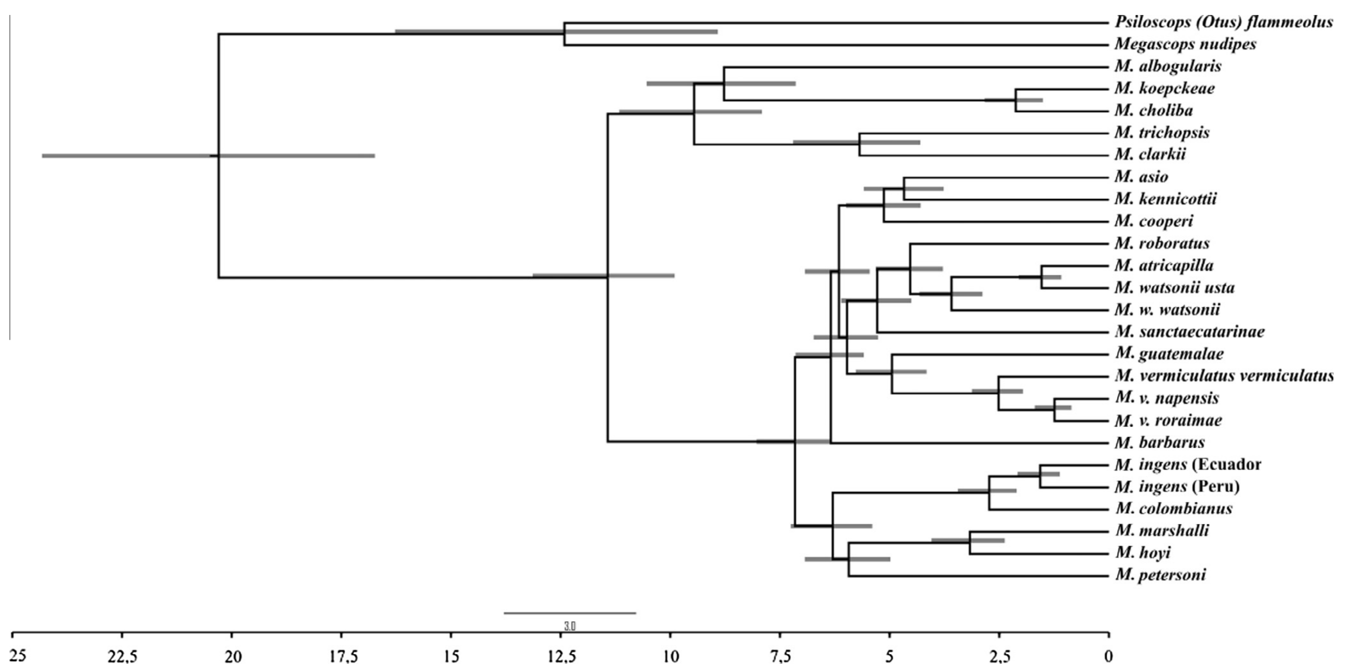

Fig. 2. Bayesian chronogram inferred from the mitochondrial genes sequenced (see text for details). Horizontal bars denote 95% posterior probability age intervals. *M.* = *Megascops*.

(SA) species (except *M. choliba*, which is distributed as far north as Costa Rica); this subclade in turn is sister to *M. trichopsis* and *M. clarkii*, two Central American (CA) taxa. *Megascops choliba duidae*, a Tepui endemic subspecies with a very different plumage from other *M. choliba* subspecies, was nested well inside this species' clade (samples LSUMZB-7413 and LSUMZB-7420 in Fig. 1). The two *M. trichopsis* subspecies (FMNH 394215: *M. t. trichopsis*, from Mexico and KUHNM 4931: *M. t. mesoamericanus*, from El Salvador) differ by a 3.7% uncorrected *p*-distance based on mitochondrial genes, surpassing the lowest level of divergence recovered herein between currently recognized species (*M. watsonii* and *M. atricapilla*; Marks et al., 1999).

The second major clade contains the remaining *Megascops* species divided into at least four subclades. The composition of these subclades differs slightly between the mtDNA only and mtDNA + nuc analyses. One subclade includes five montane SA species: *M. ingens*, *M. colombianus*, *M. petersoni*, *M. hoyi*, and *M. marshalli*. With the exception of the basal node in the *M. petersoni/hoyi/marshalli* clade, all support values in this clade were moderate to high (Fig. 1). The *M. ingens* samples were divided into two groups, an Ecuadorian and a Peruvian one (Fig. 1). Although they are considered the same subspecies (*ingens*), these samples differed genetically by 3.5% (uncorrected mitochondrial *p*-distance). The position of another well-supported group within this sub-clade, *M. guatemalae/vermiculatus*, is unresolved (Fig. 1); in contrast, internal relationships in this clade are all well supported and within-species divergences are relatively high. For example, two samples of *M. guatemalae* (*M. g. guatemalae and M. g. thompsoni*) from Mexico are 1.8% divergent from one another (uncorrected mitochondrial *p*-distance) and are monophyletic with respect to the *M. vermiculatus* group. Within *M. vermiculatus*, the Tepuian (*roraimae*) and Andean (*napensis*) populations are sisters, with the Panamanian sample (*vermiculatus*) sister to these two. Pairwise uncorrected genetic distances between *M. guatemalae* and *M. vermiculatus* samples ranged from 8.4% to 9.4%, with distances within *M. vermiculatus* ranging from 3.5% (*roraimae–napensis*) to 6.3% (*vermiculatus–napensis*).

The other groups contain the remaining SA, CA and North American (NA) species, including a well-supported South American clade with *M. roboratus*, *M. watsonii*, and *M. atricapilla* (Fig. 1), and an undescribed *Megascops* species from a population from the Santa Marta Mountains (ICN 38770) reported as *M. choliba* by Todd and Carriker (1922), but which does not fit with this or any of the recognized species in this study. We refer to this individual as "unnamed taxon". Within this clade, *M. watsonii* is paraphyletic with respect to *M. atricapilla* (Fig. 1). *Megascops roboratus*, an open habitat to deciduous forest species from western Ecuador and Peru, is the sister species to the *M. watsonii/atricapilla* clade, with the unnamed taxon from the Santa Marta mountains coming out as sister to this entire group, although with low statistical support, perhaps because only Cytb sequences were available for this particular specimen.

A clade of North and Central American species including *M. asio*, *M. kennicotti*, *M. cooperi* and *M. barbarus*, and the southern SA taxon *M. sanctaecatarinae* is strongly supported by ML (ML bootstrap = 100%) but not by BI (BI posterior probability = 85% analysis; Fig. 1). This group was not well supported by the analysis of the mtDNA only dataset (tree not shown), where neither *Megascops barbarus* nor *M. sanctaecatarinae* were placed in this clade, leaving the phylogenetic position of these species unresolved with respect to one another.

Most relationships recovered by trees obtained exclusively with the nuclear dataset (not shown) were similar to the ones based on mitochondrial and both mitochondrial and nuclear datasets, with some sister relationships receiving high support, such as *P. flammeolus/M. nudipes*, *M. ingens/M. colombianus*, and *M. choliba/M. koepckeae*. However, in the nuclear-only tree most clades, such as the *M. colombianus/ingens/hoyi/marshalli/petersoni* group, were poorly supported or unresolved. Similarly, *Megascops trichopsis* and *M. clarkii* were not closely related to any species, whereas the CA taxon *M. vermiculatus vermiculatus* fell into a CA/NA clade, that also included *M. sanctaecatarinae*.

Table 2
Models and parameters from each of the analyses conducted using BioGeoBEARS. Dispersal (*d*), Extinction (*e*), Founder (*j*), values of Log-Likelihood (ln*L*) and Akaike Information Criterion (AIC) scores from each model implemented.

Model	*d*	*e*	*j*	ln*L*	AIC
DEC	0.007	0	0	−66.05	136.1
DEC+J	0.0021	0	0.0499	−52.5	111
DIVALIKE	0.0092	0	0	−61.7	127.4
DIVALIKE+J	0.0027	0	0.0467	−52.05	110.1
BAYAREALIKE	0.0076	0.1244	0	−78.47	160.9
BAYAREALIKE+J	0.0021	0	0.0568	−54.85	115.7

3.3. Diversification timing and ancestral area reconstructions

The split between *Megascops nudipes/Psiloscops flammeolus* and the remaining *Megascops* was estimated to have occurred ca. 20 million years ago (mya) (height 95% HPD: 16.7–24.3 mya; Fig. 2); 2). The first split in *Megascops* (excluding *M. nudipes*) occurred in the Miocene, ca. 11.4 mya. Most splits in the genus occurred during the Miocene and Pliocene, with a few occurring more recently, during the Pleistocene (*M. watsonii usta* and *M. atricapilla*; *M. vermiculatus napensis* and *M. v. roraimae*). The chronogram topology was similar to those obtained in the concatenated nuclear and mitochondrial BI/ML trees, except for the position of *M. sanctaecatarinae*, which came out as sister to the *M. roboratus/watsonii/usta/atricapilla* clade according to the mitochondrial chronogram. Similarly, the chronogram recovered *M. barbarus* as sister to the *M. roboratus/watsonii/u-sta/atricapilla/sanctaecatarinae/cooperi/kennicottii/asio/guatemalae/vermiculatus* clade. Both *M. sanctaecatarinae* and *M. barbarus* were related to the *M. cooperi/kennicottii/asio* clade in the concatenated analyses.

The best ancestral area reconstruction model was DIVALIKE+J (Ln*L* = −52.05; AIC = 110.1). The most likely ancestral area reconstruction and the parameters and scores for each model (Table 2) suggested that the core members of the genus *Megascops* were in Central America during the Miocene (ca. 12.5 mya), after splitting from a common ancestor with *Megascops nudipes* and *Psiloscops flammeolus* (Fig. 2). Also in the Miocene, but after this split, there was colonization of South America including the Andes (~7.5 mya), and Atlantic Forest and non-forested South America areas. The Tepuis were colonized once from Andean ancestors and North America was colonized from Central America. The analysis suggests Amazonian lowlands were colonized from Atlantic Forest, and that at least one Andean/trans-andean species (*M. roboratus*) derived from Atlantic Forest/Amazonian ancestors.

4. Discussion

4.1. Systematics and taxonomy of the genus Megascops

Our study provides a comprehensive phylogeny for all species of the genus *Megascops*, except *M. seductus*, which is sometimes treated as a subspecies of either *M. asio* or *M. kennicottii* and is geographically limited to a small range in southwestern Mexico. The Puerto Rican endemic *M. nudipes* clearly is distantly related to the other *Megascops* and its closest living relative among the species included in this study appears to be *Psiloscops* (*Otus*) *flammeolus* (Figs. 1–3), a western North American species with at least

some populations wintering as far south as Central America (Marks et al., 1999). Unfortunately, tissue from *Margarobyas lawrencii* of Cuba, possibly a close relative of *M. nudipes* (Marshall and King, 1988, but see also Banks et al., 2003), was not available for this study. The *M. nudipes/P. flammeolus* clade was recovered as the sister group to *Megascops* with high support. Thus, we suggest that *nudipes* does not belong in the genus *Megascops* as currently defined. One possibility would be to place *nudipes* into the genus *Psilocops* or, alternatively, a more conservative approach might be to resurrect the genus *Gymnoglaux* (Cabanis, 1855; see also Olson and Suárez, 2008) for *nudipes* until data are available for *Margarobyas lawrencii*.

Although relationships among many species and clades are resolved in our reconstructions, the phylogenetic positions of some groups remain unclear. For example, the basal relationships among clades *M. guatemalae/vermiculatus*, *M. barbarus/cooperi/kennicottii/asio/sanctaecatarinae*, and *M. roboratus/watsonii/atricapilla* are poorly supported (Fig. 1). Similarly, basal relationships within the well-supported *M. petersoni/hoyi/marshalli/colombianus/ingens* clade are not well resolved (Fig. 1). The position of *M. sanctaecatarinae*, a southern SA species, is perhaps the most unexpected, because it belongs in a clade including only NA and CA species (*M. asio*, *M. kennicottii*, *M. cooperi and M. barbarus*). The mitochondrial tree (not shown) placed *M. sanctaecatarinae* within the *M. wat-sonii/atricapilla/roboratus*/unnamed taxon clade, but with low support, whereas *M. barbarus* was not included in the *M. asio/M. kennicotti/M. cooperi* clade; therefore, the phylogenetic affinities of *M. sanctaecatarinae* and *M. barbarus* reported herein (Fig. 1) should be interpreted with caution until more data become available. The relationships involving the remaining species did not change in the mitochondrial-only tree compared to the concatenated mitochondrial + nuclear analysis.

In contrast, our molecular data confirmed some hypothesized relationships based on plumage and vocalizations. For example, *M. koepckeae* and *M. choliba* are sister taxa, confirming the independent species status of *M. koepckeae* as proposed by Fjeldså et al. (2012). According to current taxonomy, the only paraphyletic species recovered by the molecular data was *M. watsonii*, with

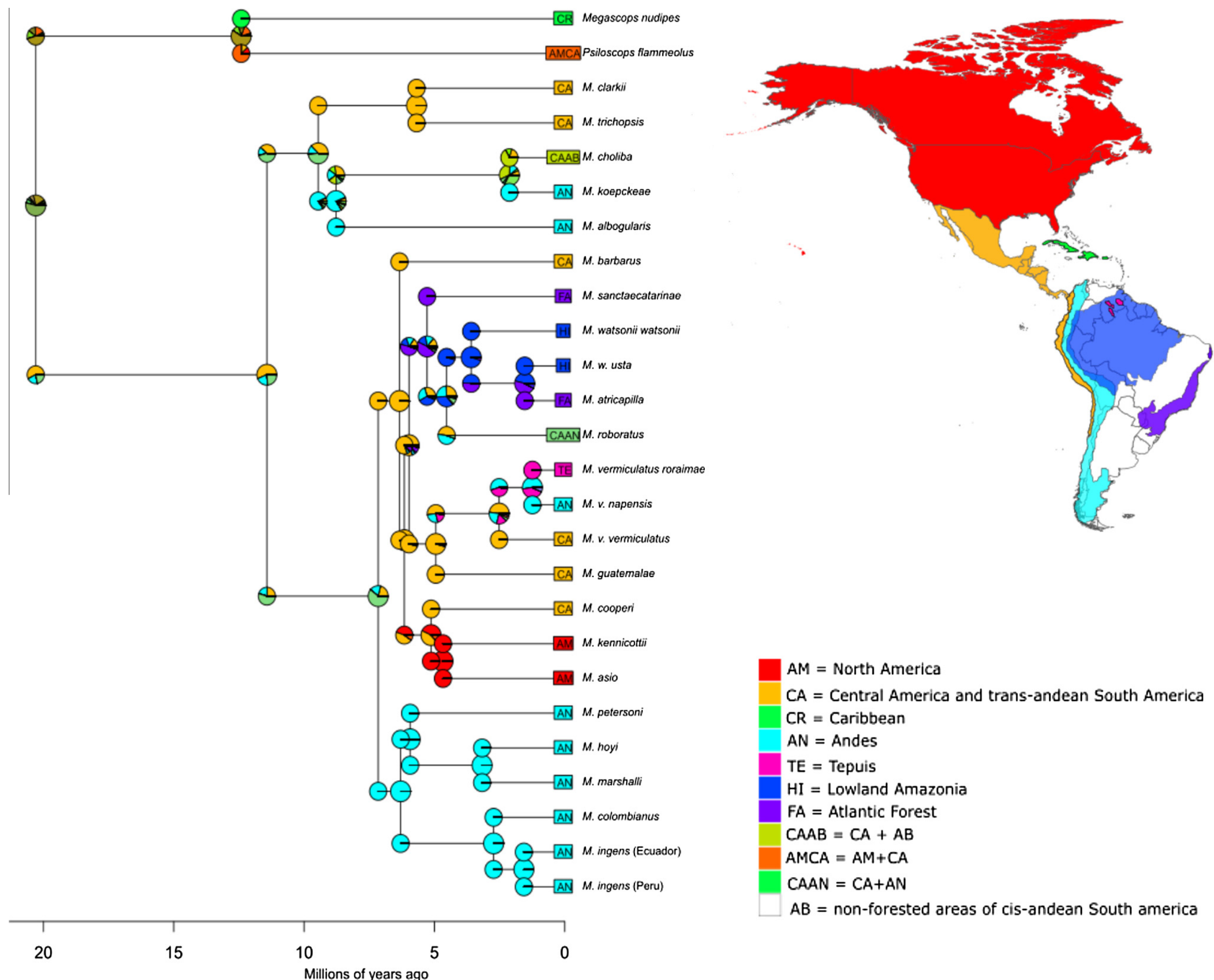

Fig. 3. Ancestral area reconstruction from BioGeoBEARS, derived from the Bayesian chronogram. The best-fit model was DIVALIKE+J. Most likely biogeographic areas are shown in the circles, and the colors in the squares indicate the current species distribution. The map shows the location of the areas used. Combinations of two areas (for example CAAN) are not shown on the map. We considered Mexico as Central America for this study.

populations from easternmost Amazonia grouping as sister to *M. atricapilla*, an Atlantic Forest endemic. More detailed analyses dealing with the phylogeography and species limits in the *M. watsonii/atricapilla* clade will be published elsewhere (Dantas et al., unpublished data). Other species, although monophyletic, showed considerable genetic variation among populations, indicating long-term isolation warranting consideration of species status. The molecular data support not only the split between *M. guatemalae* and *M. vermiculatus* (as adopted by Marks et al. (1999) but not by Banks et al. (2003)), but high genetic divergences and reciprocal monophyly among *M. vermiculatus* populations allow the recognition of three main groups, each corresponding to a named subspecies. Average uncorrected *p*-distances among these clades (6%) are substantially higher than between other currently recognized species (e.g., *M. watsonii* and *M. atricapilla*), indicating that they are probably best treated as distinct species. Although genetic distance may not be sufficient to advocate for the split of species, this case does provide "...corroborative evidence of species status" (Johnson et al., 1999). The split of *M. vermiculatus* into three (*M. vermiculatus*, *M. roraimae*, and *M. napensis*) separate species was suggested by König and Weick (2008), who based their treatment solely on morphological and vocal differences. Although tissues were not available for *M. v. pallidus* (Hekstra, 1982) from northern Venezuela, this taxon appears to be vocally distinct (18 recordings from Zulia and Aragua compared with 12 recordings of *roraimae* from Suriname, Guyana, and Sierra de Lema, Bolívar, Venezuela) and could well deserve recognition as a separate species (Krabbe, unpublished data).

Two Eastern Andean slope populations of *M. ingens* from Ecuador and Peru, regarded as belonging to the same taxon (*M. ingens ingens*), are divergent by 3.0% (uncorrected *p*-distance), and the central Mexican (*trichopsis*) and El Salvadorian (*mesoamericanus*) subspecies of *M. trichopsis* are divergent by 4.1% (uncorrected *p*-distance), which suggests that multiple species-level taxa are involved in each of these groups. Molecular studies with better geographic and population level sampling coupled with morphological and vocal characters would help to confirm whether this variation in *M. vermiculatus*, *M. ingens* and *M. trichopsis* is consistent with recognizing these subspecies as species-level taxa. In both cases, these divergences may fall across biogeographic breaks that are established for other taxa (e.g., Winger and Bates, 2015).

The unnamed taxon (ICN 38770) comes from a population of *Megascops* originally identified as *M. choliba* by Todd and Carriker (1922), although these authors noted that it was very distinct in plumage from that species, and could be an undescribed taxon. The sequenced specimen did not fall within *M. choliba* or any other *Megascops* clade, and its lowest uncorrected genetic distance to other groups in this study (6.1%, between it and *M. w. usta*) is higher than that recovered between other recognized *Megascops* species. These results support the recognition of this population as a distinctive species, which will be described elsewhere (Krabbe, unpublished data).

4.2. Historical biogeography

According to the dating analyses, the first split within *Megascops* (except *M. nudipes*) took place in the late Miocene, ca. 11 mya, separating the widespread *M. choliba/koepckeae/albogu-laris/clarkii/tri chopsis* clade from the remaining species (Fig. 2). The ancestral area reconstructions suggest a CA/Andes ancestral distribution for the ancestor of all "true" *Megascops* species (Fig. 3). The fact that the genus is more speciose in CA and the Andes is consistent with these areas being inferred as the centers of origin for *Megascops*.

According to the dating and ancestral area reconstruction analyses, the trans-Andean/NA clade *M. clarkii/trichopsis* split from the widespread SA *M. choliba/koepckeae/albogularis* clade around 10 mya, with the ancestor of all these species most likely distributed in the Andes, CA and trans-Andean SA (Fig. 3). Also, SA was colonized independently by *Megascops* at least three times, and NA was colonized independently at least twice (Fig. 3). The timing of these events span the last 9 mya, before the estimated end of the Andean uplift (Gregory-Wodzicki, 2000) and after the more recent estimates for the uplift of the Panama isthmus (Farris et al., 2011; Montes et al., 2015). Most CA *Megascops* species occupy montane habitats, and thus dispersal to the northern Andes is feasible for these lineages. Other vertebrate groups from SA have crossed into CA after the end of the uplift of the northern Andes, including capuchin monkeys (*Cebus* sp.; Alfaro et al., 2012) and *Dendrocincla* woodcreepers (Weir and Price, 2011), and thus permeability must not have been so limited in that area (see also Smith et al., 2014). Closure of the Panama isthmus has long been thought to have occurred at 3.5 mya (Coates and Obando, 1996), but it has recently been suggested that the geological closure of the isthmus likely occurred much earlier, by the middle Miocene (13 to 15 Ma; Farris et al., 2011; Montes et al., 2015). Thus, CA colonization events in many avian taxa, such as the core tanagers (Sedano and Burns, 2009), doves (Johnson and Weckstein, 2011), even theoretically low-dispersal species such as *Sclerurus*(d'Horta et al., 2013), and *Megascops* may have taken place either before or after the final closure of the isthmus, depending on the age estimated for this event according to different authors (Coates and Obando, 1996; Farris et al., 2011; Montes et al., 2015).

The reconstruction suggesting that the Amazon forest species (*M. watsonii*) and one Andean/trans-andean species (*M. roboratus*) were derived from Atlantic Forest ancestors should be interpreted with caution. The model implemented by BioGeoBEARS suggests that *M. watsonii usta* had Atlantic Forest ancestors, whereas *M. w. watsonii* had Amazonian ancestors. The tree used in the BioGeoBEARS analysis has *M. sanctaecatarinae*, a southern Atlantic Forest species, separating earliest in the *M. sanctaecatarinae/M. roboratus/M. watsonii/M. atricapilla* clade, which might have caused this result. The position of *M. sanctaecatarinae* in the phylogeny was not well resolved by this study, so using this topology for the biogeographic analysis could have biased the reconstruction.

Megascops diversification in the Andes took place during the last 9 million years, with most species originating in the Miocene/Pliocene (during the last 5–7.5 million years). Most of the species that diverged at this time inhabited the central and southern Andes, with only three species found north of Ecuador. The uplift of the Andes is believed to have had a very important role in the diversification of the Neotropical avifauna, by producing a series of isolated areas/habitats, where populations could evolve independently (Sedano and Burns, 2009; Chaves et al., 2010; Weir and Price, 2011; McGuire et al., 2014). Overall, our data are consistent with the hypothesis that the Andean uplift occurred from south to north (e.g. Garzione et al., 2008), with the ages associated with nodes of the more central and southern species being older than those of the northernmost ones, as observed in an Andean *Megascops* clade containing *M. ingens*, *M. colombianus*, *M. petersoni*, *M. clarkii*, and *M. hoyi*. Bonaccorso et al. (2011) found a similar pattern in some southern clades of *Aulacorhynchus* toucanets. However, *M. albogularis*, occurring in the central/northern Andes, has an old origin (ca. 8.7 mya), with *M. koepckeae* and *M. vermiculatus napensis*, both from the central Andes, having a relatively recent origin (ca. 2–1 mya). However, *Megascops v. napensis* is derived from a more recent colonization of the Andes by a CA group. A similar pattern is also found in other avian taxa, such as the *Drymophila caudata* antbird superspecies (Isler et al., 2012) and *Andigena* toucans (Lutz et al., 2013). Orogeny of the Andes seems to have played an important role in the diversification of *Megascops*, and this process seems to have occurred in many geographic directions and habitats.

Cis-Andean SA was colonized at least three times, probably by Andean ancestors in all instances, during the last 5 my (Fig. 3). Only one *Megascops* lineage diversified extensively in the Cis-Andean lowlands (*M. watsonii/atricapilla*). The first splitting event (dating to about 3.5 mya) isolated Guianan shield populations of *M. watsonii* from the remaining ones in the *M. watsonii/atricapilla* clade and could be related to the formation of the Amazon and Negro rivers in the Amazonian lowlands. This is consistent with the timing suggested for the origin of the modern trans-continental Amazon River (ca. 2.5 mya; Ribas et al., 2012; d'Horta et al., 2013). Populations outside the Guianan Shield later split into three clades, including western and eastern Amazonian clades and an Atlantic Forest clade. These splitting events, plus the separation of eastern Amazonian and Atlantic Forest taxa, occurred during the last 1–2 my, a similar time range estimated for the splits of eastern Amazonian populations of *Psophia* (Ribas et al., 2012), some *Sclerurus* species (d'Horta et al., 2013), and *Thamnophilus aethiops* (Thom and Aleixo, 2015). A more detailed account of the relationships and biogeographical history of these Amazonian and Atlantic Forest *Megascops* species complexes will be presented elsewhere.

4.3. Conclusions

The present study clarifies the taxonomic status of some *Megascops* taxa and species groups, such as *M. koepckeae*, *M. guatemalae*, *M. vermiculatus*, and an undescribed species from the Santa Marta Mountains in Colombia. Nonetheless, broader geographic and population level sampling of species with some degree of differentiation within them, such as *M. watsonii*, *M. ingens* and *M. trichopsis*, will clarify the taxonomy and species limits of these taxa. Additional sampling might also help reconstruct the phylogenetic position of species in the NA and CA *M. asio*, *M. kennicottii*, *M. barbarus* and *M. cooperi* species complexes.

From a biogeographical perspective, our data support a complex scenario of diversification in the Neotropics, with an origin in the highlands of Central America or the Andes and multiple invasions and recolonizations involving all major sectors and biomes of SA and CA.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.09.025.

References

Alfaro, J.W.L., Boubli, J.P., Olson, L.E., Fiori, A.D., Wilson, B., Gutiérrez-Espeleta, G.A., Chiou, K.L., Schulte, M., Neitzel, S., Ross, V., Schwochow, D., Nguyen, M.T.T., Farias, I., Janson, C.H., Alfaro, M.E., 2012. Explosive Pleistocene range expansion leads to widespread Amazonian sympatry between robust and gracile capuchin monkeys. J. Biogeogr. 39, 272–288.

Banks, R.C., Cicero, C., Dunn, J.L., Kratter, A.W., Rasmussen, P.C., Remsen Jr., J.V., Rising, J.D., Stotz, D.F., 2003. Forty-fourth supplement to the American Ornithologists' Union Check-list of North American birds. Auk 120, 923–931.

Bonaccorso, E., Guayasamin, J.M., Peterson, A.T., Navarro-Sigüenza, A.G., 2011. Molecular phylogeny and systematics of Neotropical toucanets in the genus *Aulacorhynchus* (Aves, Ramphastidae). Zoolog. Scr. 40, 336–349.

Chaves, J.C., Cuervo, A.M., Miller, M.J., Cadena, C.D., 2010. Revising species limits in a group of *Myrmeciza* antbirds reveals a cryptic species within *M. laemosticta* (Thamnophilidae). Condor 112, 718–730.

Coates, A.G., Obando, J.A., 1996. The geological evolution of the Central American isthmus. In: Jackson, J.B.C., Budd, A.F., Coates, A.G. (Eds.), Evolution and Environment in Tropical America. University of Chicago Press, Chicago, IL, pp. 21–56.

d'Horta, F.M., Cuervo, A.M., Ribas, C.C., Brumfield, R.T., Myiaki, C.Y., 2013. Phylogeny and comparative phylogeography of Sclerurus (Aves: Furnariidae) reveal constant and cryptic diversification in an old radiation of rain forest understorey specialists. J. Biogeogr. 40, 37–49.

Driskell, A.C., Christidis, L., 2004. Phylogeny and evolution of the Australo-Papuan honeyeaters (Passeriformes, Meliphagidae). Mol. Phylogenet. Evol. 31, 943–960.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Farris, D.W., Jaramillo, C., Bayona, G., Restrepo-Moreno, S., Montes, C., Cardona, A., Mora, A., Speakman, R.J., Glasscock, M.D., Reiners, P., Valencia, V., 2011. Fracturing of the Panamanian Isthmus during initial collision with South America. Geology 39, 1007–1010.

Fitzpatrick, J.W., O'Neill, J.P., 1986. *Otus petersoni*, a new screech-owl from the eastern Andes, with systematic notes on *O. colombianus* and *O. ingens*. Wilson Bull. 98, 1–14.

Fjeldså, J., Baiker, J., Engblom, G., Franke, I., Geale, D., Krabbe, N.K., Lane, D.F., Lezama, M., Schmitt, F., Williams, R.S.R., Ugarte-Núñez, J., Yábar, V., Yábar, R., 2012. Reappraisal of Koepcke's Screech Owl Megascops koepckeae and description of a new subspecies. Bull. Brit. Ornithologist́ Club 132, 180–193.

Fuchs, J., Pons, J., Goodman, S.M., Bretagnolle, V., Melo, M., Bowie, R.C.K., Currie, D., Safford, R., Virani, M.Z., Thomsett, S., Hija, A., Cruaud, C., Pasquet, E., 2008. Tracing the colonization history of the Indian Ocean scops-owls (Strigiformes: *Otus*) with further insight into the spatio-temporal origin of the Malagasy avifauna. BMC Evol. Biol. 8, 197.

Garzione, C.N., Hoke, G.D., Libarkin, J.C., Withers, S., MacFadden, B., Eiler, J., Ghosh, P., Mulch, A., 2008. Rise of the Andes. Science 320, 1304–1307.

Gregory-Wodzicki, K.M., 2000. Uplift history of the Central and Northern Andes: a review. GSA Bull. 112, 1091–1105.

Hackett, S.J., 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Mol. Phylogenet. Evol. 5, 368–382.

Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W., Nadler, S.A., 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science 265, 1087–1090.

Heidrich, P., König, C., Wink, M., 1995. Molecular phylogeny of South American screech owls of the Otus atricapilla complex (Aves: Strigidae) inferred from nucleotide sequences of the mitochondrial cytochrome b gene. Zeitschriftfür Naturforschung 50c, 294–302.

Hekstra, G.P., 1982. Description of twenty-four new subspecies of American *Otus* (Aves, Strigidae). Bull. Zool. Mus., Universiteit van Amsterdam 9, 49–63.

Helm-Bychowski, K., Cracraft, J., 1993. Recovering phylogenetic signal from DNA sequences: relationships within the Corvine assemblage (class Aves) as inferred from complete sequences of mitochondrial cytochrome-b gene. Mol. Biol. Evol. 10, 1196–1214.

Isler, M.L., Cuervo, A.M., Bravo, G.A., Brumfield, R.T., 2012. An integrative approach to species-level systematics reveals the depth of diversification in an Andean thamnophilid, the Long-tailed Antbird. Condor 114, 571–583.
Jacobsen, F., Friedman, N.R., Omland, K.E., 2010. Congruence between nuclear and mitochondrial DNA: combination of multiple nuclear introns resolves a well-supported phylogeny of New World orioles (*Icterus*). Mol. Phylogenet. Evol. 56, 419–427.
Johnson, K.P., Weckstein, J.D., 2011. The Central American land bridge as an engine of diversification in New World doves. J. Biogeogr. 38, 1069–1076.
Johnson, N.K., Remsen Jr., J.V., Cicero, C., 1999. Resolution of the debate over species concepts in ornithology: a new comprehensive biologic species concept. In: Adams, N.J., Slowtow, R.H. (Eds.), Proceedings of the 22 International Ornithological Congress, Durban. Birdlife South Africa, Johannesburg, pp. 1470–1482.
Kimball, R.T., Braun, E.L., Barker, F.K., Bowie, R.C.K., Braun, M.J., Chojnowski, J.L., Hackett, S.J., Han, K., Harshman, J., Heimer-Torres, V., Holznagel, W., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Reddy, S., Sheldon, F.H., Smith, J.V., Witt, C.C., Yuri, T., 2009. A well-tested set of primers to amplify regions spread across the avian genome. Mol. Phylogenet. Evol. 50, 654–660.
Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. 86, 6196–6200.
König, C., Weick, F. (Eds.), 2008. Owls of the World. A&C Black, p. 528.
Lutz, H.L., Weckstein, J.D., Patané, J.S.L., Bates, J.M., Aleixo, A., 2013. Biogeography and spatio-temporal diversification of *Selenidera* and *Andigena* Toucans (Aves: Ramphastidae). Mol. Phylogenet. Evol. 69, 873–883.
Marks, J.S., Cannings, R.J., Mikkola, H., 1999. Family strigidae. In: del Hoyo, J., Elliott, A., Sargatal, J. (Eds.), Handbook of the Birds of the World Vol. 5: Barn-Owls to Hummingbirds. Lynx Edicions, Barcelona.
Marshall, J.T., King, B., 1988. Genus Otus. In: Amadon, D., Bull, J. (Eds.), Hawks and Owls of the World: A Distributional and Taxonomic List. Proceedings of the Western Foundation of Vertebrate Zoology, vol. 3, pp. 296–357.
Matzke, N.J., 2013. Probabilistic historical biogeography: new models for founder event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Front. Biogeogr. 5, 242–248.
McGuire, J.A., Witt, C.C., Remsn Jr., J.V., Cori, A., Rabosky, D.L., Altshuler, D.L., Dudley, R., 2014. Molecular phylogenetics and the diversification of hummingbirds. Curr. Biol. 24, 1–7.
Montes, C., Cardona, A., Jaramillo, C., Pardo, A., Silva, J.C., Valencia, V., Ayala, C., Pérez-Angel, L.C., Rodriguez-Parra, L.A., Ramirez, V., Niño, H., 2015. Middle Miocene closure of the Central American Seaway. Science 348, 226–229.
Nylander, J.A.A., 2004. MrModeltest v2. Evolutionary Biology Centre, Uppsala University, Program distributed by the author.
Olson, S.L., Suárez, W., 2008. A new generic name for the Cuban Bare-legged Owl *Gymnoglaux lawrencii* Sclater and Salvin. Zootaxa 1960, 67–68.
Proudfoot, G.A., Gehlbargh, F.R., Honeycutt, R.L., 2007. Mitochondrial DNA variation and phylogeography of the eastern and western screech-owls. Condor 109, 617–627.
Ree, R., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.
Remsen, J.V., Jr., Cadena, C.D., Jaramillo, A., Nores, M., Pacheco, J.F., Pérez-Emán, J., Robbins, M.B., Stiles, F.G., Stotz, D.F., Zimmer, K.J. (Eds.), 2015. A Classification of the Bird Species of South America. American Ornithologists' Union, http://www.museum.lsu.edu/~Remsen/SACCBaseline.html.
Ribas, C.C., Aleixo, A., Nogueira, A.C.R., Myiaki, C.Y., Cracraft, J., 2012. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. Proc. R. Soc. B 279, 1712–1729.
Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Sambrook, J., Russel, D.W., 2001. Molecular Cloning: A Laboratory Manual, third ed. Cold Spring Harbor Laboratory Press.
Sedano, R.E., Burns, K.J., 2009. Are the Northern Andes a species pump for Neotropical birds? Phylogenetics and biogeography of a clade of Neotropical tanagers (Aves: Thraupini). J. Biogeogr. 37, 325–343.
Sick, H. (Ed.), 1997. Ornitologia Brasileira. Nova Fronteira, p. 912.
Smith, B.T., McCormack, J.E., Cuervo, A.M., Hickerson, M.J., Aleixo, A., Cadena, C.D., Pérez-Emán, J., Burney, C.W., Xie, X., Harvey, M.G., Faircloth, B.B., Glenn, T.C., Derryberry, E.P., Prejean, J., Fields, S., Brumfield, R.T., 2014. The drivers of tropical speciation. Nature 515, 406–409.
Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T., Mindell, D.P., 1999. Primers for a PCR based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol. Phylogenet. Evol. 12, 105–114.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Thom, G., Aleixo, A., 2015. Cryptic speciation in the white-shouldered antshrike (*Thamnophilus aethiops*, Aves – Thamnophilidae): the tale of a transcontinental radiation across rivers in lowland Amazonia and the Northeastern Atlantic Forest. Mol. Phylogenet. Evol. 82, 95–110.
Todd, W.E.C., Carriker, M.A., 1922. The Birds of the Santa Marta Region of Colombia: A Study in Altitudinal Distribution. Carnegie Institute, Pittsburgh.
Van der Weyden, W.J., 1975. Scops and screech owls: vocal evidence for a basic subdivision in the genus *Otus* (Strigidae). Ardea 63, 65–77.
Weir, J.T., Price, M., 2011. Andean uplift promotes lowland speciation through vicariance and dispersal in *Dendrocincla* woodcreepers. Mol. Ecol. 20, 4550–4563.
Weir, J.T., Schluter, D., 2008. Calibrating the avian molecular clock. Mol. Ecol. 17, 2321–2328.
Weske, J.S., Terborgh, J.W., 1981. *Otus marshalli*, a new species of screech-owl from Perú. Auk 98, 1–7.
Winger, B., Bates, J.M., 2015. The tempo of trait divergence in humid montane forests: avian speciation across the Marañon valley of Peru. Evolution 69, 772–787.
Wink, M., Heidrich, P., 1999. Molecular evolution and systematics of owls (Strigiformes). In: König, C., Weick, F.E., Becking, J.H. (Eds.), Owls: A Guide to the Owls of the World. Yale University Press, New Haven, pp. 39–57.
Wink, M., Heidrich, P., 2000. Molecular systematics of owls (Strigiformes) based on DNA-sequences of the mitochondrial cytochrome b gene. In: Chancelor, R.D., Meyburg, B.U. (Eds.), Raptors at Risk. WWGBP/Hancock House, pp. 819–828.
Wink, M., El-Sayed, A., Sauer-Gürth, H., Gonzalez, J., 2009. Molecular phylogeny of owls (Strigiformes) inferred from DNA sequences of the mitochondrial cytochrome b and the nuclear RAG-1 gene. Ardea 97, 581–590.
Xia, X., Xie, Z., 2001. DAMBE: data analysis in molecular biology and evolution. J. Hered. 92, 371–373.
Yu, Y., Harris, A.J., Blair, C., He, X.J., 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Mol. Phylogenet. Evol. 87, 46–49.