Zootaxa 4949 (3): 401–444
https://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4949.3.1
http://zoobank.org/urn:lsid:zoobank.org:pub:2CAB47C9-2109-45DA-8F02-50D74D593DF2

Multi-character taxonomic review, systematics, and biogeography of the Black-capped/Tawny-bellied Screech Owl (*Megascops atricapilla-M. watsonii*) complex (Aves: Strigidae)

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Abstract

Megascops is the most species-rich owl genus in the New World, with 21 species currently recognized. Phylogenetic relationships within this genus are notoriously difficult to establish due to the considerable plumage similarity among species and polymorphism within species. Previous studies have suggested that the widespread lowland Amazonian *M. watsonii* might include more than one species, and that the Atlantic Forest endemic *M. atricapilla* is closely related to the *M. watsonii* complex, but these relationships are as yet poorly understood. A recently published phylogeny of *Megascops* demonstrated that *M. watsonii* is paraphyletic with respect to *M. atricapilla* and that genetic divergences among some populations of *M. watsonii* are equal to or surpass the degree of differentiation between some *M. watsonii* and *M. atricapilla*. To shed light on the taxonomic status of these species and populations within them, we conducted a multi-character study based on molecular, morphological, and vocal characters. We sequenced three mitochondrial (cytb, CO1 and ND2) and three nuclear genes (BF5, CHD and MUSK) for 49 specimens, covering most of the geographic ranges of *M. watsonii* and *M. atricapilla*, and used these sequences to estimate phylogenies under alternative Bayesian, Maximum Likelihood, and multilocus coalescent species tree approaches. We studied 252 specimens and vocal parameters from 83 recordings belonging to 65 individuals, distributed throughout the ranges of *M. watsonii* and *M. atricapilla*. We used Discriminant Function Analysis (DFA) to analyze both morphometric and vocal data, and a pairwise diagnostic test to evaluate the significance of vocal differences between distinct genetic lineages. Phylogenetic analyses consistently recovered six statistically well-supported clades whose relationships are not entirely in agreement with currently recognized species limits in *M. watsonii* and *M. atricapilla*. Morphometric analyses did not detect significant differences among clades. High plumage variation among individuals within clades was usually associated with the presence of two or more color morphs. By contrast, vocal analyses detected significant differentiation among some clades but considerable overlap among others, with some lineages (particularly the most widespread one) exhibiting significant regional variation. The combined results allow for a redefinition of species limits in both *M. watsonii* and *M. atricapilla*, with the recognition of four additional species, two of which we describe here as new. We estimated most cladogenesis in the *Megascops atricapilla-M. watsonii* complex as having taken place during the Plio-Pleistocene, with the development of the modern Amazonian and São Francisco drainages and the expansion and retraction of forest biomes during interglacial and glacial periods as likely events accounting for this relatively recent burst of diversification.

Key words: Bioacoustics, cryptic speciation, morphometry, plumage variation, population genetics

Accepted by P. Rasmussen: 9 Feb. 2021; published: 26 Mar. 2021

Introduction

The genus *Megascops* Kaup, 1848 (Strigidae), with approximately 21 recognized species distributed throughout the Americas (Dickinson & Remsen 2013; Enriquez *et al.* 2017; Clements *et al.* 2019; Gill *et al.* 2020), is the largest exclusively Neotropical owl genus. It was long treated as a subgenus of *Otus* Pennant (AOU 1910), but more recently is treated separately because of a rather distant phylogenetic relationship, as supported by vocal and genetic evidence (van der Weyden 1975; Marshall & King 1988; Wink & Heidrich 1999; Wink & Heidrich 2000; Fuchs *et al.* 2008; Krabbe 2017; Salter *et al.* 2020). The genus *Megascops* comprises small- to medium-sized owls inhabiting a wide variety of habitats, and reaches maximum species diversity in mountainous areas, such as the Andes and Central American highlands (Marks *et al.* 1999). Species limits and phylogenetic relationships among *Megascops* have been notoriously difficult to establish due to lack of adequate geographic sampling, plumage similarity among species, and considerable polymorphism within species (Dantas *et al.* 2016; Krabbe 2017). Cryptic coloration and conserved appearance are common features in nocturnal birds such as owls and nightjars (Han *et al.* 2010), and traditional morphological taxonomy is insufficient for defining relationships and identifying taxonomic limits of this cryptic groups of birds (Salter *et al.* 2020). Complex variation in plumage coloration is also commonly exhibited by owls, further complicating character diagnoses for defining species limits (Roulin *et al.* 2011). Thus, assessments of these limits in difficult complexes within the genus *Megascops* should benefit from an appraisal of genetic variation in concert with an analysis of both morphological and vocal data to determine the taxonomic status of the various species and populations within the clade (Dantas *et al.* 2016; Enriquez *et al.* 2017).

In *Megascops*, some currently recognized species such as *M. watsonii* (Cassin, 1849) are likely complexes of species with uncertain species limits (Dantas *et al.* 2016). Some populations of *M. watsonii* are grouped under *M. w. usta* (Sclater, 1858), which has been alternatively treated as a species or subspecies (Marks *et al.* 1999; Enriquez *et al.* 2017). However, most taxonomic sources do not consider *M. w. usta* a separate species (Dickinson & Remsen 2013; Clements *et al.* 2019; Gill *et al.* 2020; Remsen *et al.* 2020), mainly because of "inadequate geographic sampling and analysis" of previous studies investigating evolutionary relationships in *Megascops* (König *et al.* 1999; Wink *et al.* 2008). The treatment of *M. w. usta* as a distinct species from *M. watsonii* has been suggested on the basis of plumage and vocal differences, with *usta* possessing more reddish (rather than grayish) overall plumage and a slower-paced song (König *et al.* 1999; Marks *et al.* 1999; König & Weick 2008). However, the high degree of plumage and vocal variation in the available sample of *M. watsonii* challenges these generalizations (SMD pers. obs.; Weick 2006). The literature is contradictory concerning body size in this group, with Marks *et al.* (1999) stating that *M. w. usta* is slightly smaller than *M. w. watsonii*, whereas König & Weick (2008) report the opposite. Vocal differences between non-oscine bird populations are usually regarded as indicative of species level divergence (Isler *et al.* 1998; Remsen 2005), but only limited information on vocal variation is available for *M. watsonii* and *M. atricapilla* (Temminck, 1822) (König *et al.* 1999; Krabbe 2017). Thus, previous studies are insufficient to provide a robust framework for diagnosing species limits among these taxa (Remsen *et al.* 2020). Recent molecular phylogenetic studies (Heidrich *et al.* 1995; Dantas *et al.* 2016) have recovered *M. watsonii* populations in Amazonia as paraphyletic with respect to *M. atricapilla* of the eastern South American Atlantic Forest, and thus are in accordance with the previous treatment of the *Megascops atricapilla-M. watsonii* complex as a single species (Hekstra 1982). Therefore, depending on the taxonomic source, between one and three different species have been recognized in the *Megascops atricapilla-M. watsonii* complex, underscoring the difficulty in establishing interspecific limits in this group (Hekstra 1982; König & Weick 2008; Enriquez *et al.* 2017; Remsen *et al.* 2020).

Roda & Pereira (2006) reported on a previously unknown disjunct population of *M. atricapilla* from the northernmost portion of the Brazilian Atlantic Forest as vocally and morphometrically distinct from the southern "core" populations of the same species, suggesting that it could possibly be a separate species. However, this has not been evaluated by any comparative study to date. If this recently discovered population of *M. atricapilla* is truly a distinct evolutionary lineage, it would be in extreme danger of extinction due to severe habitat fragmentation within its tiny range (Roda & Pereira 2006). Therefore, to assess the taxonomic status of this and other *M. watsonii* and *M. atricapilla* populations, we conducted a multi-character study to provide a biogeographic and evolutionary framework to guide taxonomic decisions concerning the ranking of these taxa.

Materials and methods

DNA extraction, amplification and sequencing. We obtained tissue samples from 56 individuals of *Megascops watsonii* and *M. atricapilla* (Fig. 1; Appendix), and three outgroup species, *M. guatemalae* (Sharpe), *M. roboratus* (Bangs & Noble) and *M. sanctaecatarinae* (Salvin), which were chosen based on previous taxonomic studies and a nearly complete molecular phylogeny of *Megascops* (Dantas *et al.* 2016). We also extracted DNA from a toepad of a lectotype of *Megascops watsonii* (ANSP 2445) from "South America" to determine what lineage corresponded to this named taxon (see Chapman 1928; Appendix). DNA was extracted using the DNeasy tissue extraction kit (Qiagen, Valencia, California) or with a phenol-chloroform protocol (Sambrook & Russel 2001). We sequenced DNA from six loci including three mitochondrial (mtDNA; Cytochrome-*b* [cytb], NADH dehydrogenase subunit 2 [ND2] and Cytochrome c oxidase subunit I [COI]), and three nuclear genes (*b*-fibrinogen Intron 5 [fib5], Chromohelicase-DNA binding protein intron 18 [CHD] and Muscle skeleton receptor tyrosine kinase intron 4 [MUSK]). The primers used for amplification and sequencing are listed in Table 1.

TABLE 1. Primer sequences used in this study.

Gene	Primers	Source
Cytochrome-b (cytb, 1035 bps)	L14841	Kocher *et al*. 1989
	H16065	Helm-Bychowski & Cracraft 1993
NADH dehydrogenase subunits 2 (ND2, 1040 bps)	L5215	Hackett 1996
	H6313	Sorenson *et al*. 1999
	L5758Mega	This study
	H5776Mega	This study
Cytochrome c oxidase subunit 1 (CO1, 379 bps)	L6625, H7005	Hafner *et al*. 1994
Intron 5 of the nuclear b-fibrinogen gene (BF5, 560 bps)	FIB5L and FIB5H	Driskell & Christidis 2004
Chromohelicase-DNA binding protein intron 18 (349 bps)	CHD-18F, CHD-18R	Jacobsen *et al*. 2010
Muscle skeleton receptor tyrosine kinase intron 4 (605 bps)	MUSK-F, MUSK-R	Kimball *et al*. 2009

Fragments were PCR amplified using standard thermocycling 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, 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 to be sequenced. Most products were cleaned using Exonuclease and Shrimp Alkaline Phosphatase (ExoSap) enzymatic reactions (United States Biochemical, Cleveland, OH, USA), and some were purified using PEG 8000 20% NaCl 2,5 M or GELase (Epicentre Technologies, Madison, WI, USA). The products of heterogeneous (length variant) nuclear genes were cloned using a TOPO TA cloning kit (invitrogen), following the manufacter´s protocol, and the PCR product was recovered by direct amplification. Amplifications were cycle-sequenced using a BigDye 3.1 Terminator kit (BigDye, Applied Biosystems, Foster City, CA, USA) and the same primers were used for amplification. Cycle sequencing reactions were cleaned with ethanol EDTA precipitation, and resuspended in Hi-Di formamide. Sequences were then read by an ABI 3730 automated sequencer and aligned and reconciled using the computer program Sequencher 3.1.1 (Gene Codes Corp., Ann Arbor, MI, USA). To obtain the gametic phase of the nuclear genes for the coalescent analyses, we used the algorithm PHASE 2.1.1 (Stephens *et al.* 2001; Stephens & Donnelly 2003), implemented in DnaSP 6.10.01 (Librado & Rozas 2009). We assumed a 70% probability limit as an acceptable value for phase definition; lower values were considered ambiguous and the respective sites were coded as missing data.

The toepad sample from the *Megascops watsonii* lectotype (ANSP 2445) was processed in a laboratory in Drexel University where no modern bird tissues have been handled, following an extraction technique developed by Andres Cuervo and colleagues (pers. comm.). The toepad was first soaked in absolute ethanol followed by buffer AE from a QIAamp DNA Micro kit and then minced and incubated (at 56°C while rotating) for two overnights in a mixture of ATL, Proteinase K, and 20 ul of 1M DTT. After incubation, we largely followed the DNeasy Blood and Tissue Kit protocol for tissue samples with a few exceptions as follows. First, we added 1µl of Carrier RNA during step 1. In step 2, we added cold absolute ethanol and incubated the ethanol plus sample mixture for one hour at 4°C.

In step 5, we used spin columns from a QIAquick PCR purification kit as the toepad was over 50 years old. After the sample was eluted, we concentrated the resulting extract to 100µl in a SpeedVac and quantified dsDNA concentration using a real-time qPCR fluorescent detection method (Blotta *et al.* 2005) with the Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen, Eugene, OR, USA).

The toepad extract contained less than 1000 µg of DNA and thus the entire extract was submitted to RAPiD Genomics (Gainesville, FL, USA) for library preparation and sequencing of ultraconserved elements using the standard 2.5k probe set (Faircloth *et al.* 2012) with additional avian-specific UCE probes (McCormack *et al.* 2013). At RAPiD Genomics, the pooled library containing DNA from this sample was divided between two Illumina lanes and paired-end reads were generated, resulting in four sequence files per sample. Read One and Read Two files were each concatenated into a single file. Adapters and low-quality regions were then removed using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Mitochondrial DNA is a byproduct of UCE sequencing (Raposo do Amaral *et al.* 2015) and thus we assembled ND2 and cytb contigs from the resulting Illumina reads using Geneious (Version 8.0.4). Reads were mapped to ND2 (Genbank No. KT799371.1) and cytb (Genbank No. KT799276.1) sequences from FMNH 456485 using the 'map to reference' function set to medium-low sensitivity and iterated up to five times with no trimming. To identify potential contaminants, we constructed a neighbor-joining tree of the matched reads in PAUP* (v4.0a159; Swofford 2002) to insure that all reads mapped to the ingroup. We then generated a consensus sequence with a strict 50% threshold for each sequence fragment. In total, two fragments of cytb (207 bp and 122 bp) and 77 bp of ND2 were assembled from the UCE read files. We used the MUSCLE algorithm in SeaView v4.6.2 (Gouy *et al.* 2010) to align all selected reads to the other *Megascops* sequences. Each alignment was trimmed to the same length as the shorter toepad sequence and analyzed separately using a GTR+I+G model in RAxML. Furthermore, all three fragments were concatenated into a single alignment and analyzed using a GTR+I+G model in RAxML.

Phylogenetic analyses. We aligned sequences of all sampled *Megascops atricapilla—M. watsonii* individuals and outgroups using Sequencher v.4.8 (GeneCodes, Ann Arbor, Michigan, USA) or Bioedit v. 7.1.3 (Tom Hall, Ibis Biosciences, Carlsbad, CA, USA), and concatenated them into a single dataset using the Java exe command CONCAT. We reconstructed the phylogeny using Maximum Likelihood (ML) as implemented in RAxML (Stamatakis 2006), and Bayesian Inference (BI) as implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), and a multilocus coalescent Species Tree (ST) analysis, as implemented in *BEAST v. 1.4 (Drummond & Rambaut 2007). The best-fit models of sequence evolution for each gene were obtained with MrModeltest 2.3 (Nylander 2004), based on Akaike's information criterion (AIC), as follows: GTR+G (cytb); GTR+G (ND2); HKY+I (COI); HKY (MUSK); and HKY (CHD). These models were applied to BI and ST analyses.

We tested different partitioning schemes in BI using MrBayes 3.1.2: 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 combined 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 5,000 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. Using TRACER, version 1.5 (Drummond & Rambaut 2007), we determined that the chosen burn-in setting (25%) was sufficient for the log likelihood values of parallel runs to reach stationarity, with all parameters meeting benchmark effective sample-size values (>200).

Pairwise uncorrected genetic distances between lineages were calculated with MEGA 5 (Tamura *et al.* 2011), using only the mitochondrial data. To visualize genealogical relationships, haplotype networks of each sequenced gene were constructed in Haploviewer (Salzburger *et al.* 2011) based on the topologies recovered in the species tree analysis.

Operational Taxonomic Units in the trees were labeled according to the Area of Endemism (AOE) where they were collected (sensu Silva *et al.* 2005). These AOEs comprise regions in Amazonia separated by the main rivers, plus the Atlantic Forest region in eastern South America. The AOEs included in this study were: Guiana (east of the Negro and north of the Amazonas rivers); Imerí (between Negro and Japurá rivers); Napo (between Japurá and Solimões rivers); Inambari (between Solimões and Madeira rivers); Madeira (between Madeira and Tapajós rivers); Tapajós (between Tapajós and Xingu rivers); Xingu (between Xingu and Tocantins rivers); Belém (Amazonia east

of the Tocantins river); Pernambuco (Atlantic forest in eastern coastal Brazil from Paraiba to Alagoas); and southern Atlantic Forest (from Sergipe state southward).

Species delimitation. We ran a coalescent-based species delimitation method implemented in Bayesian Phylogenetics and Phylogeography v. 2.0 (BPP; Rannala & Yang 2003; Yang & Rannala 2010), which incorporates a Bayesian modeling approach to generate speciation probabilities of closely related taxa from multilocus sequence data (Rannala & Yang 2003; Yang & Rannala 2010). BPP uses Bayesian MCMC algorithms that accommodate the species phylogeny as well as coalescent processes in extant and extinct ancestral species (McKay *et al.* 2013). BPP also tests whether resolving the node into multiple species results in a statistically better fit to the data than collapsing the node into a single species. If resolving the node results in a better statistical fit, BPP recommends splitting that node into multiple species (McKay *et al.* 2013). BPP relies on a user-specified guide tree with individuals assigned to *a priori* defined putative species (McKay *et al.* 2013), which is used to reduce the number of possible species delimitations over which the program must integrate. At each node on the tree, the program assesses whether the sequence data are compatible with a one-species model or whether a two-species model has to be invoked to explain the data (Mckay *et al.* 2013). As a guide tree, we used the multilocus coalescent Species Tree generated by *BEAST (see Results). We implemented the approach of Leaché & Fujita (2010) and Smith *et al.* (2013) and performed analyses using three combinations of priors that represented 64 different population sizes and different ages for the root in the species tree: 1) large *Ne* and deep divergence: θ [theta] and τ [tau] gamma priors G (1, 10) and G (1, 10); 2) small *Ne* and shallow divergence: θ and τ gamma priors G (2, 2000) and G (2, 2000); and 3) large *Ne* and shallow divergence: θ and τ gamma priors G (1, 10) and G (2, 2000). BPP incorporates a model that includes the species divergence times (τ) and the population size parameter θ (see Leaché & Fujita 2010; Camargo *et al.* 2012; and McKay *et. al.* 2013 for summaries of how BPP treats these parameters). We ran the analyses for 200,000 generations, sampling every five generations, and specified a burn-in of the first 50,000 generations. We performed preliminary analyses using algorithms 0 and 1 with different fine-tune parameters. BPP generates a posterior distribution of speciation models differing in numbers of species. Speciation probabilities are estimated from the sum of probabilities of all models for speciation events at each node in the guide tree. Daughter lineages from nodes that had speciation probabilities of >0.95 under all three prior scenarios were classified as species (Leaché & Fujita 2010; Smith *et al.* 2013).

Species tree and molecular clock analysis. A multilocus coalescent Species Tree (ST) Analysis was run in *BEAST to infer divergence times among recovered lineages. An XML runfile file was created using Beauti (Drummond & Rambaut 2007), and then run in *BEAST. We used the best-fit models selected previously for each locus, and assumed a Relaxed Uncorrelated Lognormal Clock Model (Drummond *et al.* 2006) for all genes. We used a Yule speciation process for the tree prior and a widely used cytb rate of 0.0105 substitutions per million years (Weir & Schluter 2008) to calibrate the tree, whereas substitution rates for the other genes were estimated by the program. Four runs of 100 million generations, sampling every 10,000 generations, were performed on the CIPRES site (Miller *et al.* 2010). TRACER v. 1.6 (Rambaut *et al.* 2014) was used to visualize the posterior distributions for every parameter (burnin 10 million generations), and the results were combined and produced ESS values $\geq$200.

Ancestral area reconstruction. To reconstruct the ancestral biogeographic history of the genus *Megascops*, we reconstructed ancestral areas using BioGeoBEARS (BioGeography with Bayesian (and likelihood) Evolutionary Analysis in R Scripts; Matzke 2013; http://cran. rproject.org/web/packages/BioGeoBEARS/index.html). The program performs six analyses: A Dispersal-Extinction Cladogenesis Model (DEC), a likelihood version of the Dispersal-Vicariance Analysis ("DIVALIKE"), a version of the Bayesian inference of historical biogeography for discrete areas (BAYAREALIKE), and founder-event speciation. These biogeographic processes are implemented in a maximum likelihood (DEC, DEC+J, DIVALIKE, and DIVALIKE+J) or Bayesian (BAYAREALIKE, BAYAREALIKE+J) frameworks, as free parameters estimated from the data. We defined seven biogeographical areas based on the distribution of the taxa plus one outgroup: (1) Andes; (2) Guiana Shield; (3) Brazilian Shield; (4) Pernambuco Center; (5) Atlantic Forest (south of São Francisco River); (6) Northern Amazonian Sedimentary Basin; (7) Southern Amazonian Sedimentary Basin (modified from Conn & Mirabello 2007; Fig. S1). Our Bayesian relaxed-clock species 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).

Morphological analyses. We examined 252 study skins of *Megascops watsonii* and *M. atricapilla* collected throughout their ranges and housed in collections in Brazil and the USA (Fig. 1; Appendix). For all specimens in

good overall condition (i.e, without broken parts) and inferred as adults based on overall size and plumage patterns, we measured seven morphometric characters with an electronic caliper (to the nearest 0.01 mm) or a ruler to the nearest 1 mm: wing length; tail length; tarsus length (usually the right tarsus); bill length from the tip to the distal point of the nostrils; bill depth and width at the distal point of the nostril; and average width of ventral feather stripes (based on a subset of five randonmly chosen feathers). For each specimen, wing, tail, tarsus, and bill characters were measured twice and the average measure value was used in the analysis. We did not include weight data in the morphological analyses due to the lack of this information for most specimens. However, we performed a regression analysis on the available weight data for *M. atricapilla* (i.e., clades E and F; n=12), from measured specimens. We used Smithe (1975) to describe and study plumage coloration, which was scored qualitatively for each exemplar. We classified each individual in good plumage condition into a color morph (red, dark, brown, gray, or cinnamon) based mainly on its general back, chest and belly colors, using the codes (numbers) of the color guide.

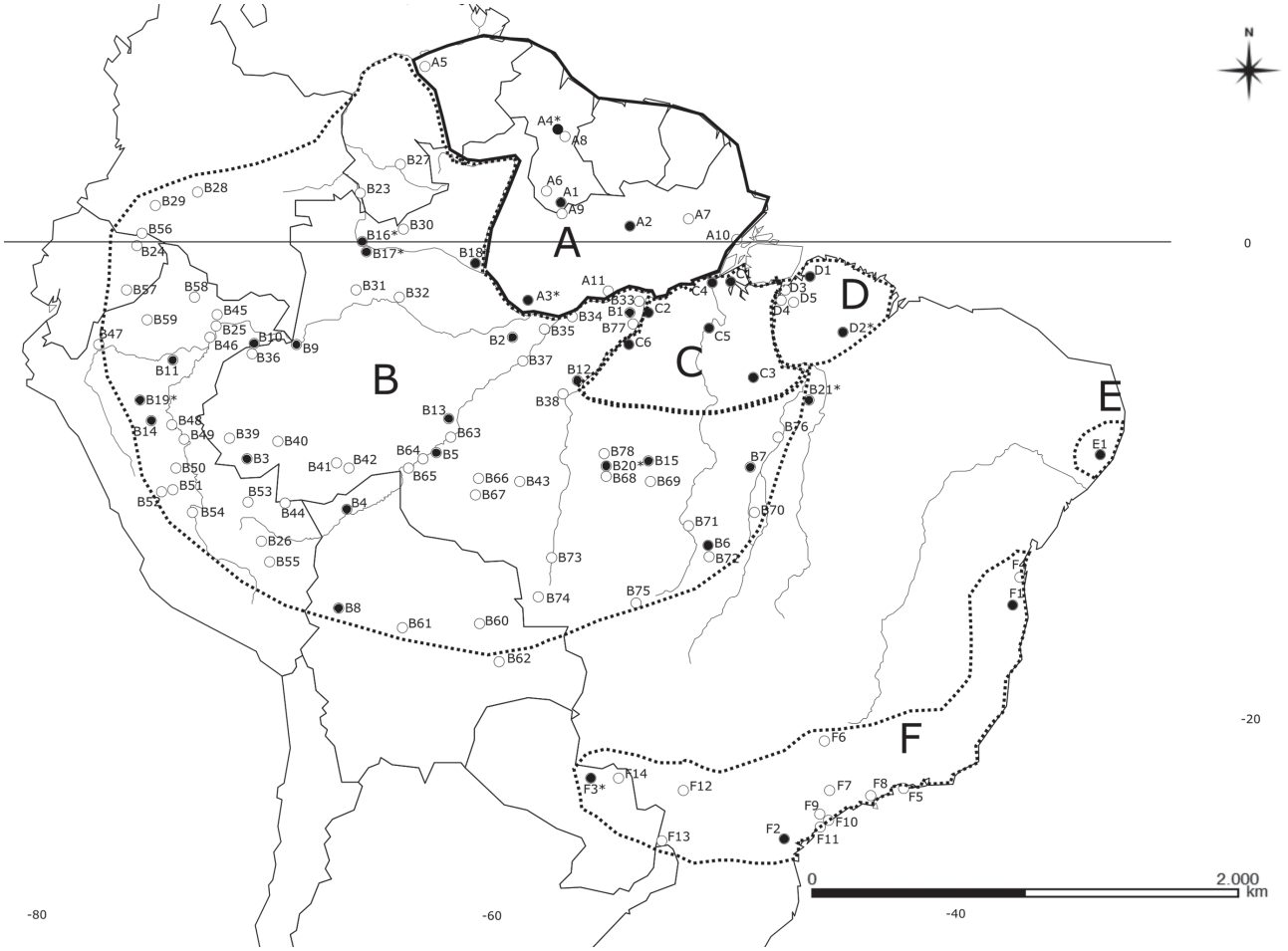

FIGURE 1. Geographic distribution of specimens of the *Megascops atricapilla-M. watsonii* complex examined and included in the analyses herein. White dots denote localities for which only skins were analyzed, whereas black circles represent sites from which both skins and tissues were examined. Black dots with Asterisks indicate localities for which only tissue samples were available. See Appendix for detailed locality and specimen information. Dotted polygons correspond to the distribution of the main clades (labelled A–F) recovered by the molecular analyses and plotted on the distribution ranges of *M. watsonii* and *M. atricapilla* as contained in Infonatura (2007), with some adaptations. Clade distributions were in a few cases also delineated based on vocal characters that were diagnostic among clades.

A Discriminant Function Analysis (DFA) was performed based on the measurements obtained for 235 specimens examined, which were classified *a priori* into natural groups (clades) according to the molecular phylogeny. Most of the measured specimens (study skins) were assigned to groups based on their congruent distributions within the ranges of the identified molecular clades (see results below) because only a small portion of specimens included in the morphological analyses were also included in the molecular analysis. Analyses were not performed separately

for each sex because most specimens studied were either males or unsexed. Analyses were performed in SYSTAT, version 12, for Windows (Systat Software, San Jose, CA, USA). In all tests, statistical significance was accepted at $P \leq 0.05$.

Vocal analyses. We analyzed 83 recordings of *M. watsonii* and *M. atricapilla* (Appendix), covering most of the distribution of these taxa (Fig. 2). It was not possible to determine the sex of the singing individuals, hence we did not take this variable into account. Whenever possible, we analyzed only one recording per locality, to guarantee sampling independence. In a few cases, we included more than one recording from a given area and in these cases we considered samples from the same location only if they corresponded to different individuals. Recordings were obtained by us directly in the field or provided by colleagues and the open sound archives xeno-canto and the Macaulay Library (Appendix). Only one sound bout (sequence) per recording was used in the analyses. Two types of vocalizations were analyzed, and are referred here as "longsongs" and "shortsongs". Longsongs consist of long series of similar notes uttered at an even pace throughout the whole sequence; they usually start softly and then become gradually louder, until reaching a more or less defined "plateau" of volume. Shortsongs consist on much shorter series of notes in two parts: 1) slower-paced; which suddenly turns into 2) faster-paced, gradually slowing down towards the end. Vocalizations were classified into longsongs (n=55) and shortsongs (n=28) aurally and by visual inspections of spectrograms, and analyzed separately in this study. In some cases, longsongs and shortsongs from the same individual were analyzed. The roles of these two main types of vocalizations are not well-established in *Megascops* (see Krabbe 2017), but apparently shortsongs are often associated with strong territorial responses to playback, and may be involved in aggressive interactions among individuals.

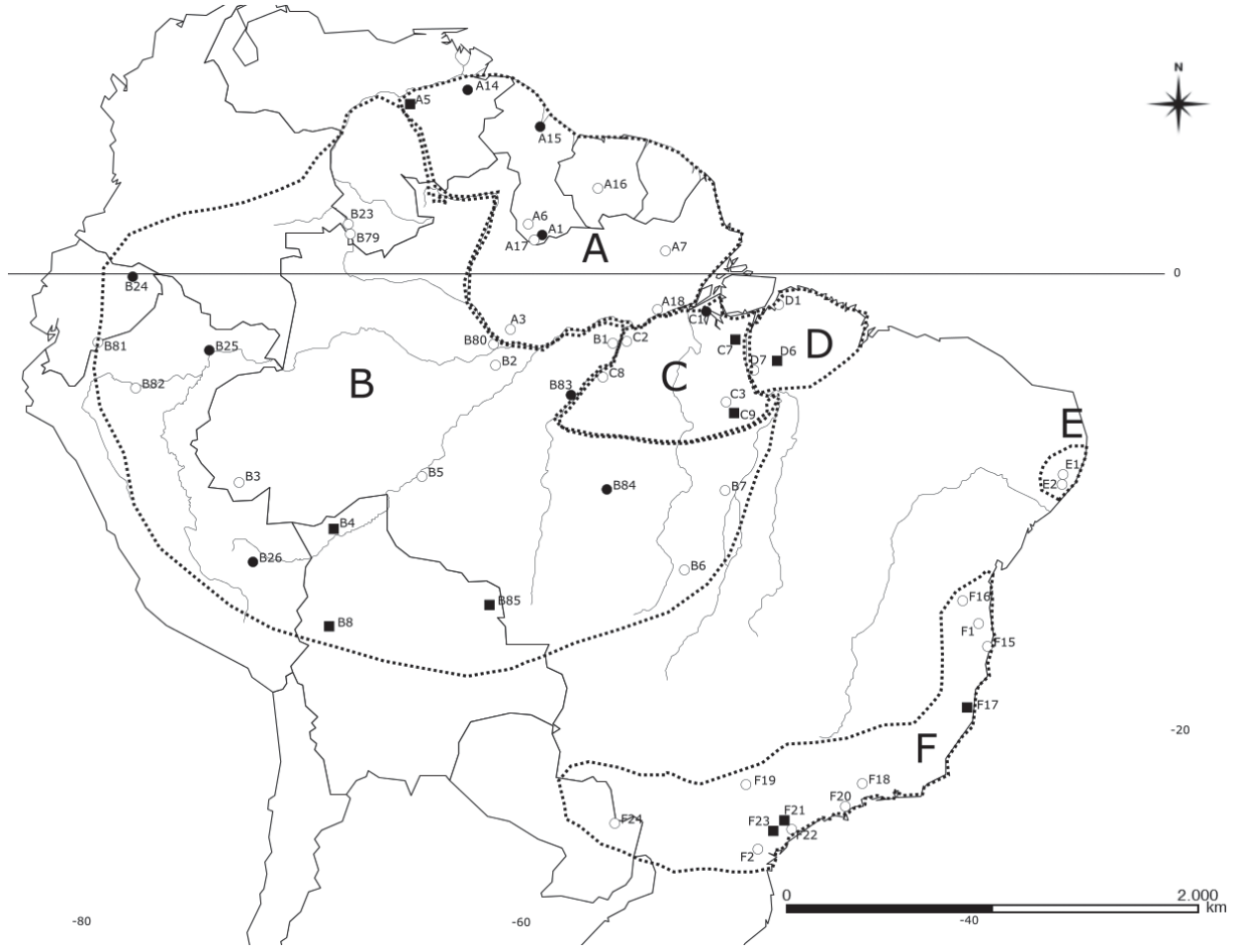

FIGURE 2. Geographic distribution of vocal samples of the *Megascops atricapilla-M. watsonii* complex examined herein. Different locality symbol types refer to distinct sampling of vocal characters, as follows: black circles = both long and shortsongs analyzed; white circles = only longsongs were analyzed; and black squares = only shortsongs were analyzed. See Appendix for detailed locality and recordings information. Dotted polygons correspond to the distribution of the main clades (labelled A–F) recovered by the molecular analyses and plotted on the distribution ranges of *M. watsonii* and *M. atricapilla* as in Infonatura (2007), with some adaptations.

The following vocal characters were measured for each analyzed recording: length of each note and the interval between them in seconds; pace (number of notes per second) of the entire song; peak frequency (in Hz) and Maximum Power (in dB) of each note. In longsongs, these characters were sampled only after amplitude stabilized in the "plateau" stretch of the song, where notes reached equivalent maximum frequencies. The total number of notes and the duration of longsongs and shortsongs are extremely variable among individuals, possibly in relation to the individual's motivational state when tape-recorded, and therefore we did not evaluate the number of notes and song duration in our analysis. Change in note length, note interval, peak frequency and maximum power were measured by calculating the ratio between these values in two adjacent notes. Change in pace was measured by dividing the sequence in three even parts, and calculating the ratio between the pace of two adjacent parts. Measurements were made using RAVEN PRO 1.3 (Cornell Laboratory of Ornithology, Ithaca, NY, USA).

A Discriminant Function Analysis (DFA) was performed based on the vocal characters measured for all recordings examined, which were classified *a priori* into natural groups (clades) according to the molecular phylogeny. Most of the recordings were assigned to groups based on their congruent distributions within the ranges of the identified molecular clades (see results below) because only a small portion of specimens included in the vocal analyses were also sequenced for their DNA. Analyses were performed in SYSTAT, version 12, for Windows (Systat Software, San Jose, CA, USA). In all tests, statistical significance was accepted at $P \leq 0.05$.

We followed the method of Isler *et al.* (1998) to test for statistically significant diagnostic vocal features among clades recovered in the molecular analyses. For each vocal character evaluated, the mean ($\bar{x}$) and standard deviation (SD) were calculated. We verified normality of the distribution of vocal characters using a Shapiro-Wilk test, and considered two continuous and normally distributed characters diagnostic between two clades only if their ranges did not overlap and if the means and SD of the clade with the smaller set of measurements (a) and the clade with the larger set of measurements (b) met the requirement: $\bar{x}_a + t_a\ SD_a \leq \bar{x}_b - t_b\ SD_b$, where t_i is the t score at the 97.5 percentile of the t distribution for $n - 1$ df (Isler *et al.* 1998).

Results

Molecular phylogenetics. We obtained a total of 3969 bp of DNA sequence data including Cytb (1035 bp), COI (379 bp), ND2 (1040 bp), BF5 (560 bp), MUSK (605 bp) and CHD (349 bp) for 56 *M. watsonii* and *M. atricapilla* specimens and outgroups (Appendix). ML, BI, and ST analyses based on concatenated mitochondrial and nuclear sequences placed *M. watsonii* as paraphyletic with respect to *M. atricapilla* (Figs. 3 and 4). ML and BI analyses resulted in identical phylogenies, recovering six well-supported geographic clades (labelled A–F, Fig. 3), whereas the ST analysis confirmed the same clades with high statistical support (Fig. 4). Clades A–D correspond to *M. watsonii* and clades E–F correspond to *M. atricapilla*.

Clade A contains samples from the Guiana Shield east of the Branco River (Fig. 1). Clade B includes samples distributed in part of Guiana (between the Branco and Negro rivers), in the Napo, Imerí, Inambari and Madeira AOEs, and the southern parts of the Tapajós and Xingu AOEs in southeastern Brazilian Amazonia (Fig. 1). Clade C groups samples from the lower Tapajós and Xingu AOEs (Fig. 1), whereas Clade D includes samples distributed in the Belém AOE (*sensu* Silva *et al.* 2005) east of the Tocantins River. Clades E and F, currently included in *Megascops atricapilla*, are distributed in the Atlantic Forest and are separated by the São Francisco River in northeastern Brazil (Fig. 1). All of these clades recognized in the *Megascops atricapilla-M. watsonii* complex are allopatric and separated by rivers or non-forest habitat, except for the parapatric B and C clades, which come into contact in the middle portions of the Tapajós and Xingu AOEs (Fig. 1).

Based on both ML and BI, nodal support for the relationships and reciprocal monophyly of clades A–F were high, except for the basal relationships among clades C, D, and E+F (Fig. 3), which were essentially unresolved. The ST also recovered statistically significant values for the reciprocal monophyly of most clades, except the basal relationships among clades C, D, E and F, which were not well supported, mirroring the results obtained in the ML and BI analyses (Fig. 4). Within Clade B, there were four statistically well-supported lineages with wide geographical overlap, including one clade comprising a single sample from the Department of La Paz, Bolivia (LSUMZ B947), a sample from São Gabriel da Cachoeira, Brazil (MPEG 77180) along the eastern northern bank of the Negro River, and a sample from Loreto, Peru (LSUMZ B2912), which grouped as sister to all remaining samples in this clade.

Pairwise genetic distances between clades A–F varied from 1.5% (between sister clades E and F) to 7.1% (between clades A and F; Table 2). Within individual clades, internal genetic distances were around 0.5% or lower, with

the exception of sample LSUMZ B947 from the Department of La Paz, Bolivia, which was 2.6% divergent from the remaining individuals of Clade B.

TABLE 2. Average pairwise uncorrected mitochondrial genetic distances between clades A–F of the *Megascops atricapilla-M. watsonii* complex recovered by the molecular phylogenetic analyses (see Figs. 1 and 3).

Clade	A	B	C	D	E
A	-	-	-	-	-
B	0.067	-	-	-	-
C	0.064	0.032	-	-	-
D	0.067	0.035	0.021	-	-
E	0.066	0.032	0.023	0.023	-
F	0.071	0.035	0.024	0.024	0.015

Haplotype networks for cytb, COI and ND2 closely reflected the results found by BI and ML analysis, with samples dividing into six or more "populations" (Fig. S2). Haploviewer recovered 44 haplotypes for cytb, 20 for COI, 39 for ND2, 6 for CHD, 22 for BF5 and 26 for MUSK (Fig. S2). Cytb, COI and ND2 networks showed similar structures to the ML and BI phylogenies, separating samples into six populations corresponding to clades A–F, although sample LSUMZ B947 was separated from the other Clade B samples in the cytb network. MUSK, BF5 and CHD haplotype networks exhibited little population structure (Fig. S2).

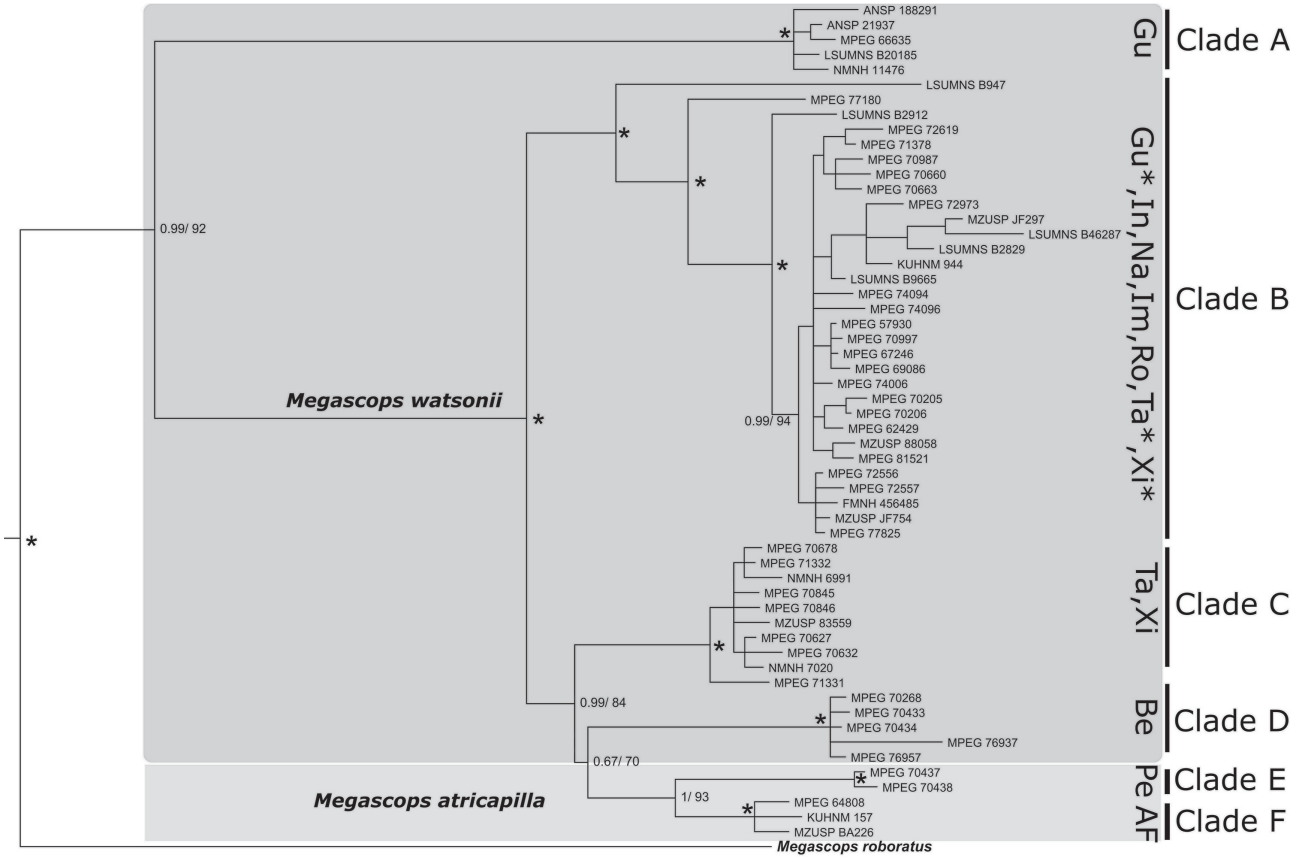

FIGURE 3. Results of ML and BI phylogenetic analyses based on 3969 bp of the concatenated mitochondrial and nuclear genes sequenced in this study. Numbers associated with nodes represent BI posterior probability and ML bootstrap values respectively. Asterisks (*) represent BI values > 0.95 and ML values > 95%. Main clades (labelled A–F) recovered by the molecular analyses are shown, along with the different areas of endemism where they occur, as follows: Gu (Guiana); Gu* (westernmost part of the Guiana area of endemism); Na (Napo); Im (Imeri); In (Inambari); Ro (Rondonia); Ta (Tapajós); Ta* (southernmost part of the Tapajós area of endemism); Xi (Xingu); Xi* (southernmost part of the Xingu area of endemism); Be (Belém); PE (Pernambuco); and AF (Atlantic Forest south of the Sao Francisco River). See also Fig. 1.

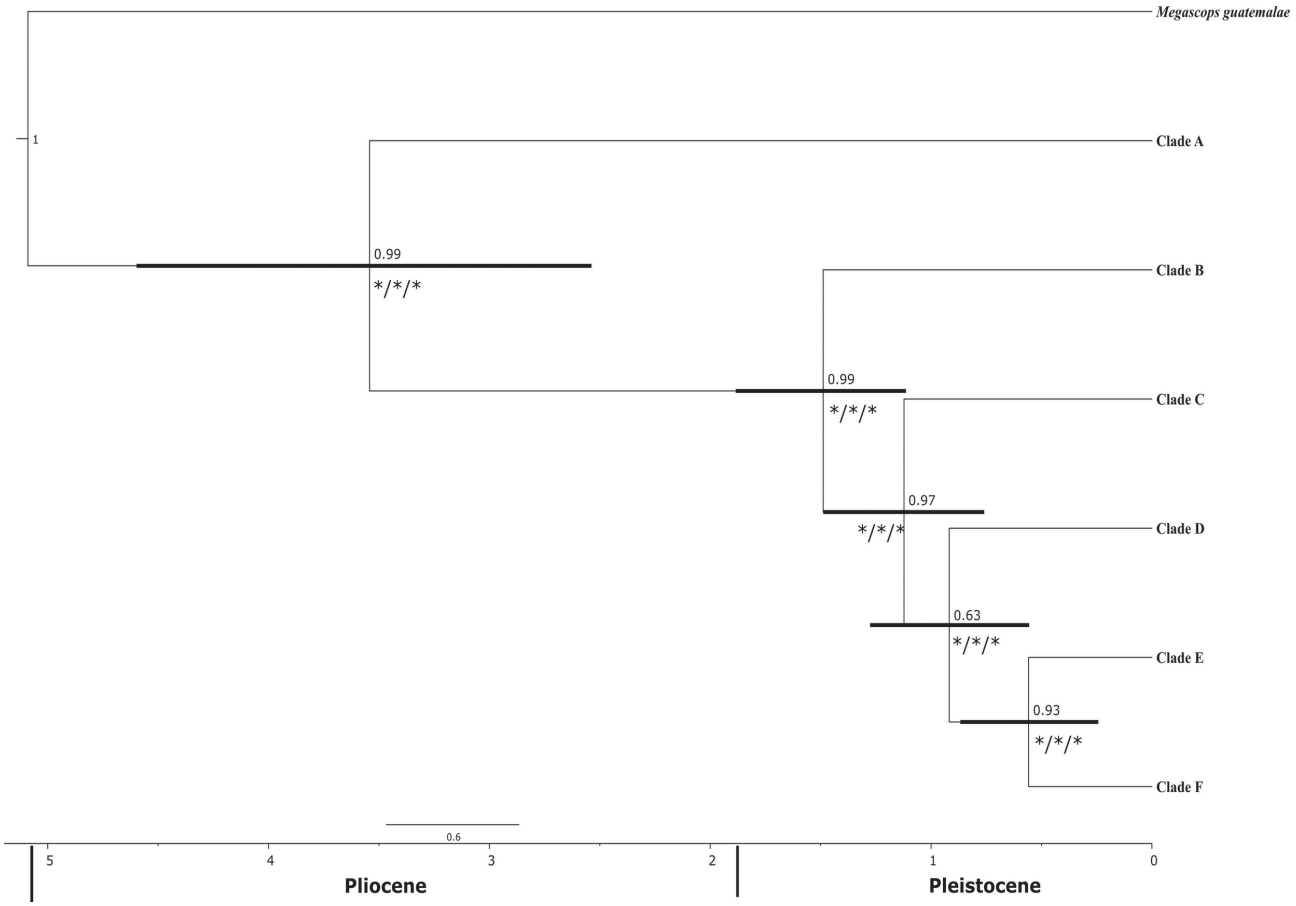

FIGURE 4. Species tree (ST) and BPP chronogram for the diversification of the *Megascops atricapilla-M. watsonii* complex. Main clades (labelled A–F) recovered by the molecular analyses are shown. Black bars associated with each node are 95% credibility intervals for node ages (in mya). Numbers above black bars denote nodal posterior probabilities and signs below the bars are speciation probabilities under the three prior distributions used for BPP analysis. Asterisks (*) indicate high speciation probabilities (PP > 0.95).

Both fragments of cytb and that of ND2 obtained for one of the lectotypes of *M. w. watsonii* (ANSP 2445) confidently placed this specimen within Clade A (endemic to the Guiana Shield), when individual fragments were analyzed separately (bootstrap values of 79; 95 and 99, respectively; trees not shown). Reinforcing this pattern, the concatenated cytb+ND2 dataset placed ANSP 2445 within Clade A with high statistical support (ML=100; Fig. 5). This concatenated dataset included all individual samples from the multilocus dataset analyzed for this paper, and placed each of these individuals into the same clade as reconstructed from the complete multilocus dataset. These results allow us to definitively place the lectotype ANSP 2445 of *Megascops watsonii* (Cassin, 1848) within Clade A.

Molecular clock analysis and BPP. According to the molecular clock analysis, all splits within *Megascops atricapilla-M. watsonii*-took place in the Plio-Pleistocene (4.5–0.8 million years ago [mya]; Fig. 4). The first split separated Clade A (from the Guiana Shield) from the remaining clades between 4.6 and 2.5 mya, and the second split isolated the broadly distributed Clade B, from the ancestor of clades C–F around 1.3 mya. Clade C split around 1.5 mya from the remaining clades, followed by a split between Clade D and the Atlantic Forest clades ca. 1.0 mya. Lastly, the two Atlantic Forest clades (E and F) split approximately 0.5 mya (Fig. 4). Under the coalescent-based species delimitation analysis (BPP), all terminal lineages had high speciation probabilities (PP>0.95) under all three prior distributions (Fig. 4).

Ancestral area reconstruction. The best ancestral area reconstructions identified by BioGeoBEARS were DEC+J (LnL=-12.94; AIC=31.87), DIVALIKE + J (LnL=-12.99 -; AIC=31.97), BAYAREALIKE+J (LnL=-12.99; AIC=32.00), and DIVALIKE (LnL=-20.68 -; AIC=45.37; Table 3). Taking into account the conceptual problems with the DEC model and the "jump speciation" +J parameter (Ree & Sanmartin 2018), we chose DIVALIKE as

the best estimate of the ancestral area relationships in the *Megascops atricapilla-M. watsonii* complex. The chosen reconstruction indicates that the first split in the complex took place ca. 3.7 mya, after a widespread Amazonian ancestor distributed across the Guiana and Brazilian shields became isolated into a northern (Guiana Shield) and a southern (Brazilian Shield) population, separating *M. watsonii* from the remaining clade members. Approximately 1.7 mya, a split within the southern (Brazilian Shield) group led to the origin of *M. usta* (whose ancestor spread to the southern and western sedimentary basins), and a clade that later (ca. 1 mya) split into two southeastern Amazonian populations (clades C and D), separated by the Tocantins River. The ancestor of the easternmost Amazonian Clade D colonized the Atlantic Forest in eastern South America, ultimately splitting into two forms (clades E and F), separated by the São Francisco River valley (Fig. 6).

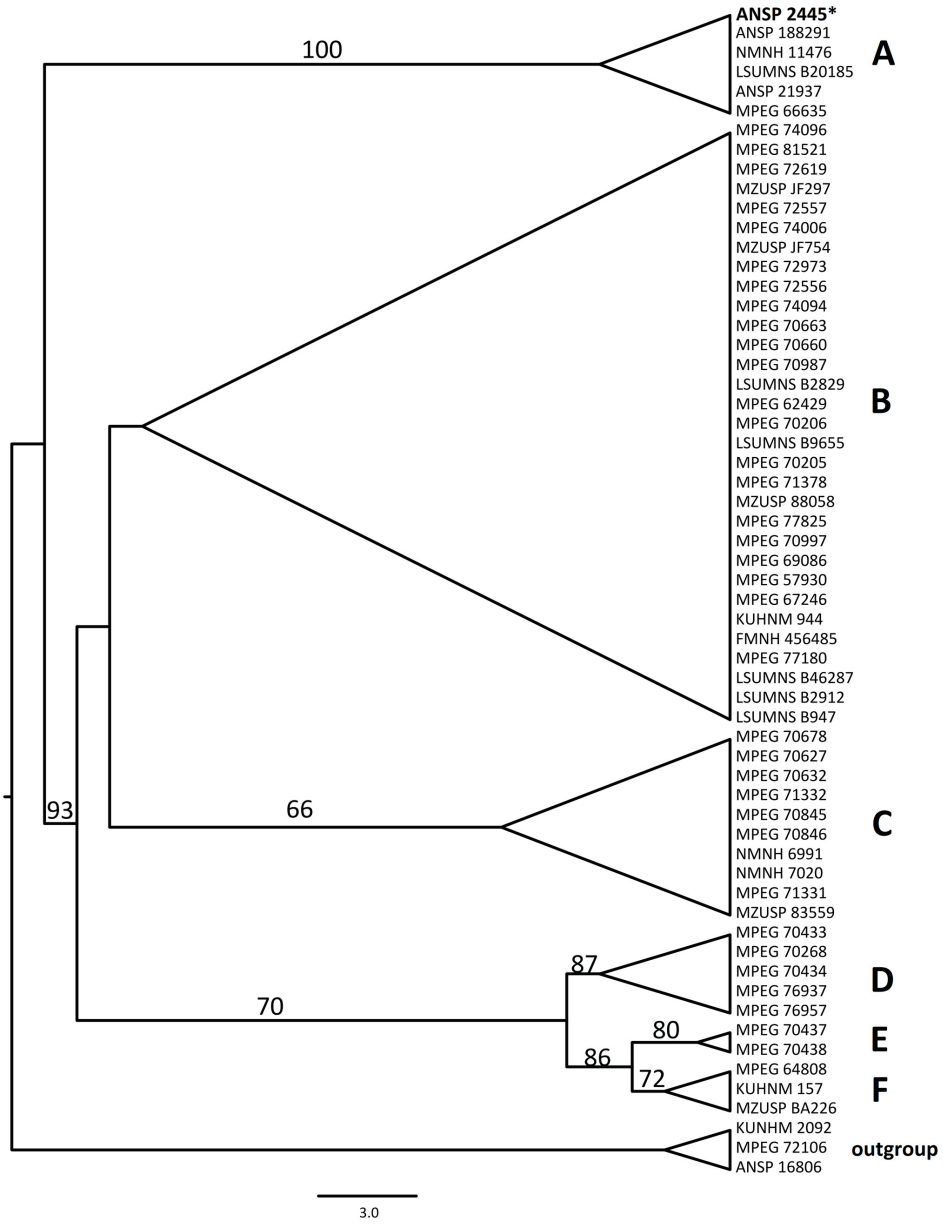

FIGURE 5. ML results for the position of the *Megascops watsonii* lectotype (ANSP 2445) with respect to the *Megascops atricapilla-M. watsonii* clades recovered in the study.

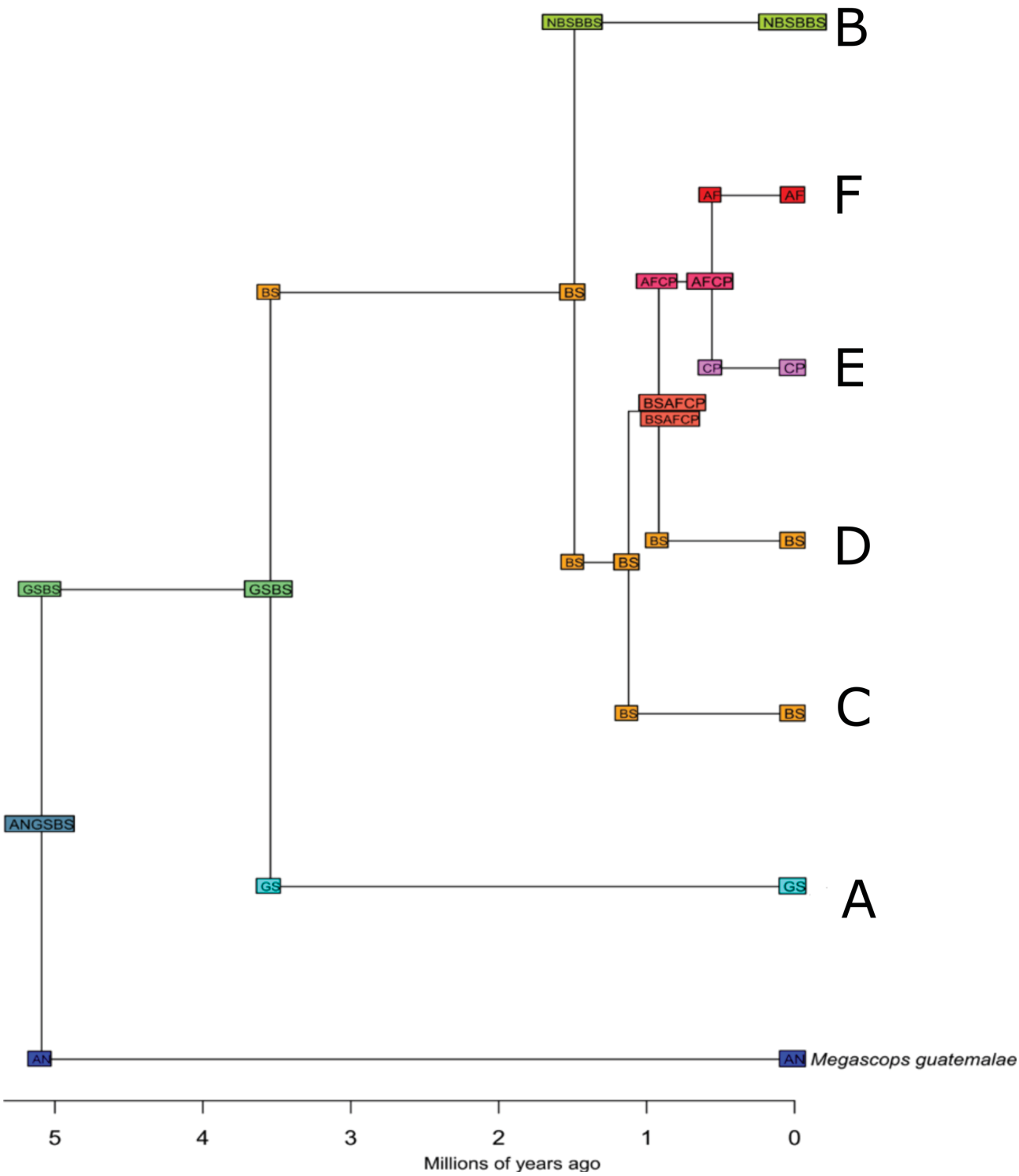

FIGURE 6. Ancestral area reconstruction based on the DIVALIKE model favored by BioGeoBEARS (see Table 3), derived from the Species Tree (see Figure 4). Most likely biogeographic areas are shown in the squares and are labeled as: AN = Andes; NB = Amazonian Sedimentary Basin (North bank); SB = Amazonian Sedimentary Basin (South bank); BS = Brazilian Shield; GS = Guiana Shield; AF = Atlantic Forest; CP = Pernambuco Center of Endemism.

Morphological analyses. A DFA analysis of morphometric characters among clades A–F recovered in the molecular phylogenies was significant (Wilk's Lambda=0.564; P=0.000). However, the classification matrix could not correctly assign most of the specimens to their respective clades based on the characters measured (Table 4), and there was broad clade overlap in the morphometric space (Fig. 7). One character (bill height) was not used in the analysis because it was highly correlated with other bill measurements (bill length=0.531; bill width=0.431;

p=0.354). The first two canonical discriminant variables accounted for 80.5% of total variation among clades. Specimens in Clade E were classified with 100% (or 75% in Jacknife matrix) confidence, but the sample size for this clade was small (n=4), consisting of individuals collected at the same site, and there was classification overlap with clades B and F (Table 4). Specimens in all other clades were classified with much less accuracy (less than 70% confidence), with Clade B having the lowest confidence value (26/23%). Clade B had the largest sampling and geographical range, and molecular analyses suggest subdivision within the clade, which could have influenced the values above. Total accuracy across all clades was low (38/32%; Table 4).

TABLE 3. Models and parameters from each of the analyses conducted using BioGeoBEARS including Dispersal (d), Extinction (e), Founder (j), values of Log-Likelihood (ln *L*) and Akaike Information Criterion (AIC) scores from each model implemented. The best-fitting model without a +J parameter chosen (DIVALIKE) is shown in in bold (see text for details).

Model	D	e	j	ln *L*	AIC
DEC	3.68	9.71	0.00	-21.47	46.94
DEC+J	1.00	1.00	2.37	-12.94	31.87
DIVALIKE	**3.97**	**4.24**	**0.00**	**-20.68**	**45.37**
DIVALIKE+J	1.00	1.00	1.71	-12.99	31.97
BAYAREALIKE	5.82	4.78	0.00	-22.95	49.9
BAYAREALIKE+J	1.00	1.00	1.00	-12.99	32.00

TABLE 4. Summary of classification accuracy of 235 specimens (study skins) distributed among clades A–F of the *Megascops atricapilla-M. watsonii* complex by a Discriminant Function Analysis (DFA) based on measurements of six morphometric characters (wing, tail, tarsus, bill length, bill width, ventral stripe width). Numbers before and after slashes are values obtained without and with jackknife procedures, respectively.

Clade	A (n=17)	B (n=151)	C (n=20)	D (n=11)	E (n=4)	F (n=32)	**% Correct**
A	7/4	2/3	2/2	4/6	0/0	2/2	41/24
B	36/38	39/35	28/29	25/25	11/12	12/12	26/23
C	0/0	4/5	13/11	1/2	0/0	2/2	65/55
D	3/4	0/0	3/3	5/2	0/0	0/2	45/18
E	0/0	0/1	0/0	0/0	4/3	0/0	100/75
F	1/2	2/2	2/2	2/3	3/3	22/20	69/63
Total	47/48	47/46	48/47	37/38	18/18	38/38	38/32

The striking variation in nearly all plumage characters evaluated makes diagnoses based on plumage among clades A–F nearly impossible. We distinguished red, gray, brown and dark morphs, which mainly differ in their predominant under- and upperparts colors (red, gray, brown and blackish), but many intermediate-colored individuals make distinction between morphs a purely subjective task in many cases. Reddish morphs in particular tend to have a less contrasting grayish wash on the chest than other morphs, and many red individuals have a red-striped or -spotted black crown, rather than a solely black or brownish crown as found in other color morphs. In most populations brownish, reddish, and intermediate red-brown morphs were more common than other morphs (Table 5).

Some trends in plumage variation were observed within particular clades, with proportions of different color morphs varying among populations within these clades. In clades A and D, most individuals are darker than the darkest morphs of other clades, especially in the overall back and crown color (Color 26, Clay color in Smithe 1975). Individuals in clades A and D also have more contrasting dark chest color. However, at least one Clade C individual (MPEG 70647, not included in the genetic analyses, from Carajás) closely approached this pattern and based on plumage coloration could be confused with individuals from clades A and D. Clade A dark birds tend to have a more uniform cinnamon-colored belly contrasting with the chest, and wider pectoral stripes than those found on dark individuals from Clade D. Some Clade B morphs possess distinctive cinnamon coloration on the underparts (Cinnamon, color 123A; Smithe 1975), which is less contrasting in other populations (except some clade A morphs). Apparently, reddish morphs are more frequent in Clade B than in other Amazonian clades, but similar-looking reddish morphs are also common in Clade C.

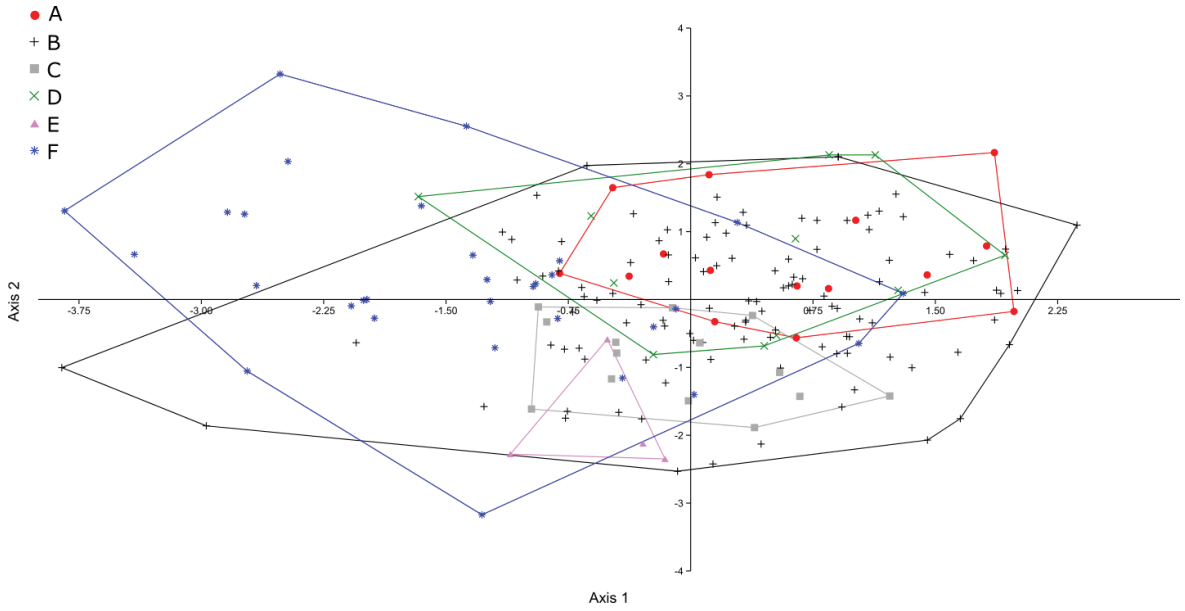

FIGURE 7. Graphic representation of scores for the first two factors of a Discriminant Function Analysis (DFA) separating 235 specimens (study skins) distributed among clades A–F of the *Megascops atricapilla-M. watsonii* complex based on measurements of six morphometric characters (wing, tail, tarsus, bill length, bill width, and ventral stripe width).

TABLE 5. Proportion (%) of color morphs among 224 specimens (study skins) belonging to clades A–F of the *Megascops atricapilla-M. watsonii* complex examined. Only specimens in good overall plumage condition were included in this analysis.

Color morphs	A (n=19)	B (n =141)	C (n=20)	D (n=11)	E (n=4)	F (n=29)	**Total**
brown	36.84	48.23	55.00	36.36	25.00	20.69	43.30
red	10.53	33.33	20.00	9.09	75.00	51.72	32.14
dark	52.63	-	10.00	36.36	-	3.45	7.59
gray	-	0.71	-	9.09	-	-	0.89
cinnamon	-	3.55	-	-	-	-	2.23
intermediate (red-brown)	-	8.51	10.00	-	-	10.34	7.59
intermediate (brown-gray)	-	3.55	-	9.09	-	13.79	4.46
intermediate (brown-cinnamon)	-	2.13	-	-	-	-	1.34
intermediate (brown-dark)	-	-	5.00	-	-	-	0.45

Individuals from Clade B exhibit additional local plumage variation. For example, some Peruvian specimens (FMNH 208178 and FMNH 222284) are grayer than others from the same population, and a very pale individual from Bolivia (LSUMZ 168771) is also smaller than others. Individuals of *Megascops atricapilla* from Bahia (Clade F in part) are more similar in plumage to *M. atricapilla* from Alagoas (Clade E) than they are to more southern members of Clade F. A regression based on weight data (not shown) from 12 *M. atricapilla* specimens from Clades E and F suggests a north-to-south pattern of clinal variation in body mass, with heavier southernmost individuals (R^2=0.684; p=0.001), and a high correlation (0.827) between geographic distance (from the northernmost sampling point) and weight.

Vocal analyses. We analyzed 55 longsong and 28 shortsong bouts of 65 *Megascops atricapilla-M. watsonii* individuals distributed as follows: Clade A (n=14); Clade B (n=27); Clade C (n=7); Clade D (n=4); Clade E (n=4); and Clade F (n=9). Longsongs in all clades consist of monotonous sequences of equally spaced notes delivered in a variable period of time, gradually rising in volume until reaching a "plateau" where note frequency stabilizes, and becoming slower at the end, usually in the last three to five notes (Fig. 8). However, sometimes longsong increases continually in frequency to the very end of the sequence, especially in Clade F birds. Note length, the

interval between notes, maximum frequency and other vocal traits are usually kept constant among different longsong bouts, sometimes varying very little in pitch. Notes are in general more uniformly shaped at the beginning of the longsong sequence, and can gradually change to slightly downslurred or upslurred notes towards the end of the sequence. Clade B populations have the slowest-paced longsongs on average (Table 6), with elongated notes that range from uniformly shaped or slightly "inverted-U" shaped (rising and falling in pitch) in the beginning to slightly downslurred or upslurred at the end (Fig. 8). Measurements of the nine longsong characters evaluated for clades A–F are presented in Table 6.

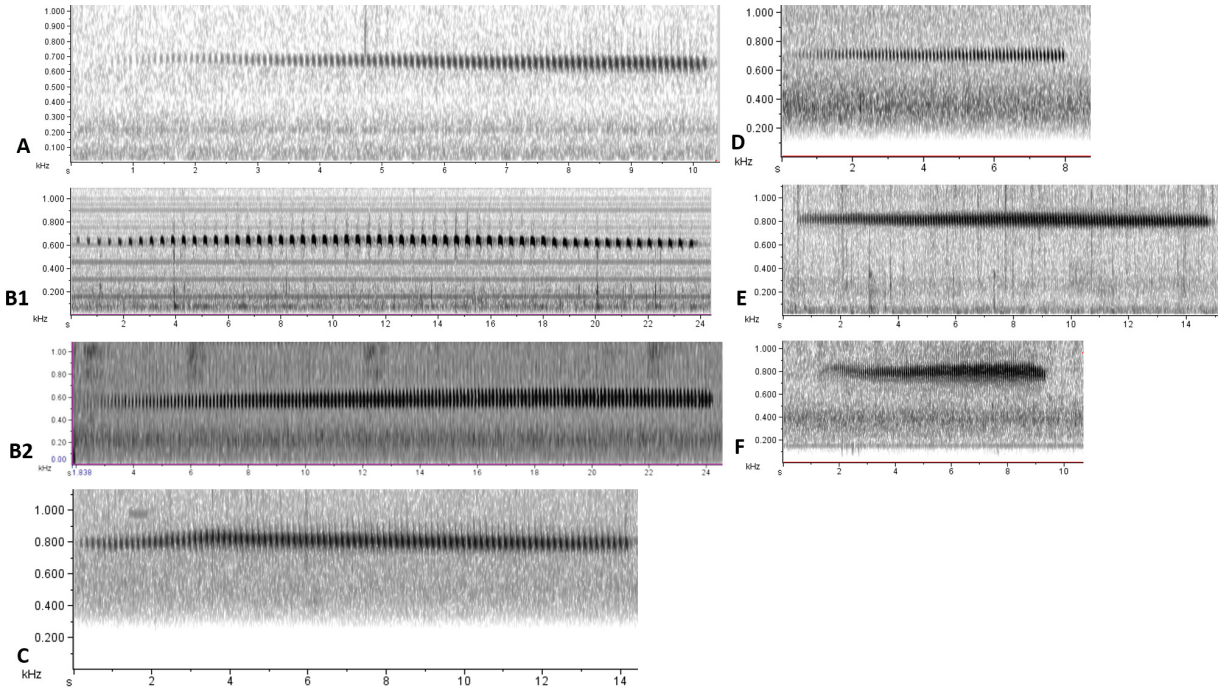

FIGURE 8. Examples of longsong audio spectrograms from clades A–F of the *Megascops atricapilla-M. watsonii* complex. (A) Clade A: Venezuela, Rio Cuyuni (Paul Schwartz, ML 59376); (B1) Clade B: Peru, Madre de Dios (Ted Parker III, ML 11496 = locality B26 in Fig. 1); (B2) Clade B: Venezuela, Rio Cassiquiare (Paul Schwartz, ML 59397 = locality B23 in Fig. 1); (C) Clade C: Brazil, Serra dos Carajás (Sidnei Dantas, XC 22514); (D) Clade D: Brazil, IPEAN, Belém (Paul Schwartz, ML 59395); (E) Clade E: Brazil, Usina Serra Grande, Ibateguara, Alagoas (Curtis Marantz, ML 127980); (F) Clade F: Brazil, Caetetus Ecological Station, São Paulo (Alexandre Aleixo, ML 94909). Each sonogram is typical of a given clade, except for Clade B, where a slow-paced song (less than 4 notes/s), and a fast-paced song (more than 6 notes/s) are shown. The slow-paced individuals are all from the south bank of the Amazon River, while most of the fast-paced Clade B specimens come from north of the Amazon, except for some samples from the lower Inambari and Rondonia areas of endemism in Brazil (see Fig. 9).

TABLE 6. Mean and standard deviation values of nine "longsong" characters measured for *Megascops atricapilla-M. watsonii* individuals belonging to clades A–F (see also Fig. 9).

Characters	A (n=14)	B (n=27)	C (n=7)	D (n=4)	E (n=4)	F (n=9)
Note length (sec)	0.05±0.01	0.12±0.05	0.08±0.01	0.07±0.01	0.06±0.005	0.05±0.01
Peak frequency (kHz)	0.65±0.06	0.65±0.04	0.71±0.05	0.68±0.04	0.78±0.02	0.81±0.05
Pace (n notes/sec)	10.34±1.3	4.66±2.0	7.25±0.89	9.54±0.53	10.62±0.1	13.11±0.9
Maximum power (dB)	87.12±15.0	96.25±12.17	92.77±9.2	78.3±8.54	90.2±13.67	103.31±20.8
Change in note length	1.01±0.01	1.01±0.01	1.01±0.01	1.01±0.0	1.01±0.01	1.01±0.0
Change in note interval	1.01±0.01	1.01±0.01	1.01±0.01	1.03±0.02	1.02±0.01	1.01±0.01
Change in peak frequency	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Change in maximum power	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Change in pace	1.01±0.01	1.01±0.02	1.03±0.02	1.01±0.0	1.01±0.01	1.01±0.02

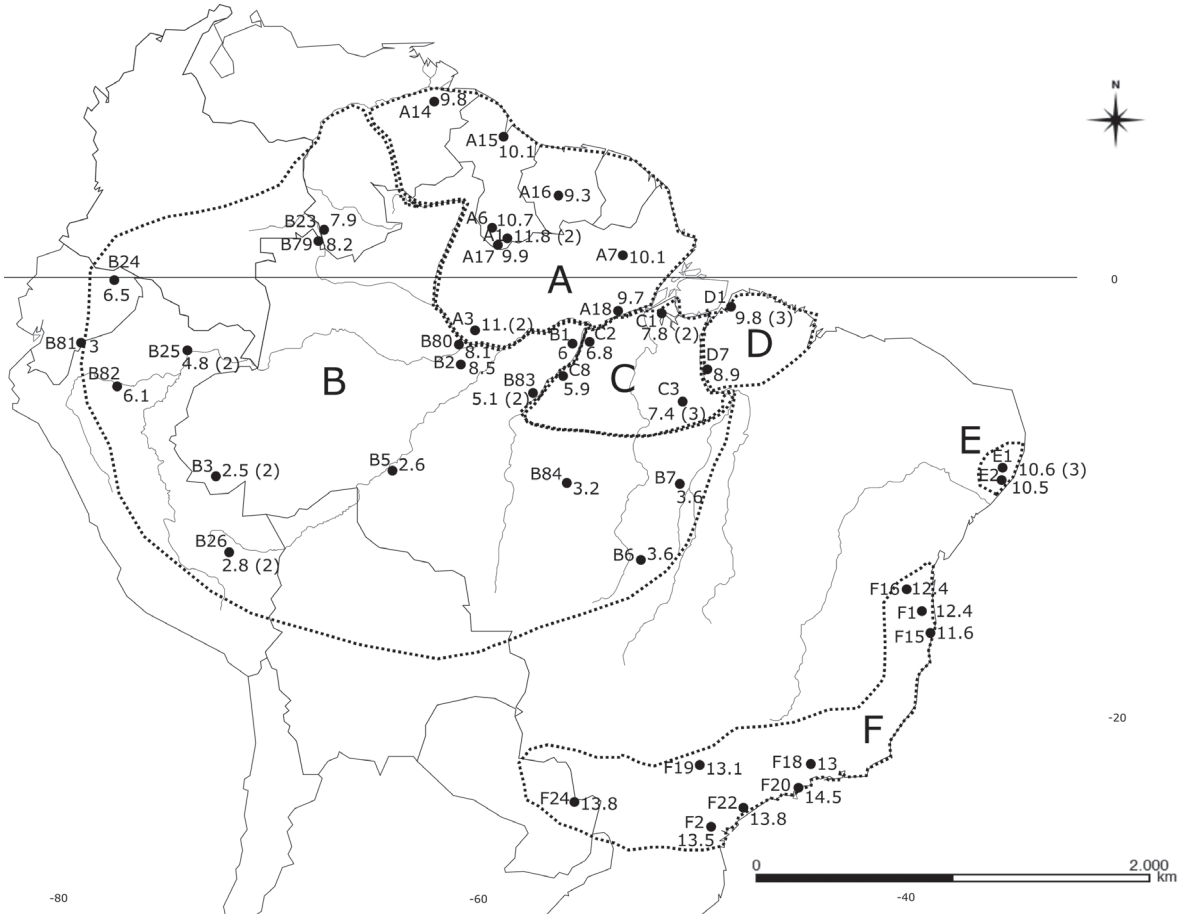

FIGURE 9. Geographic variation in longsong paces (notes/s) across clades A–F in the *Megascops atricapilla-M. watsonii* complex. Locality codes are given as letters followed by numbers and can be found in full in the Appendix. Numbers next to locality dots represent locally measured longsong pace values. When more than one individual was sampled at a given locality, the total number of longsongs measured is given in parentheses, with reported values representing averages.

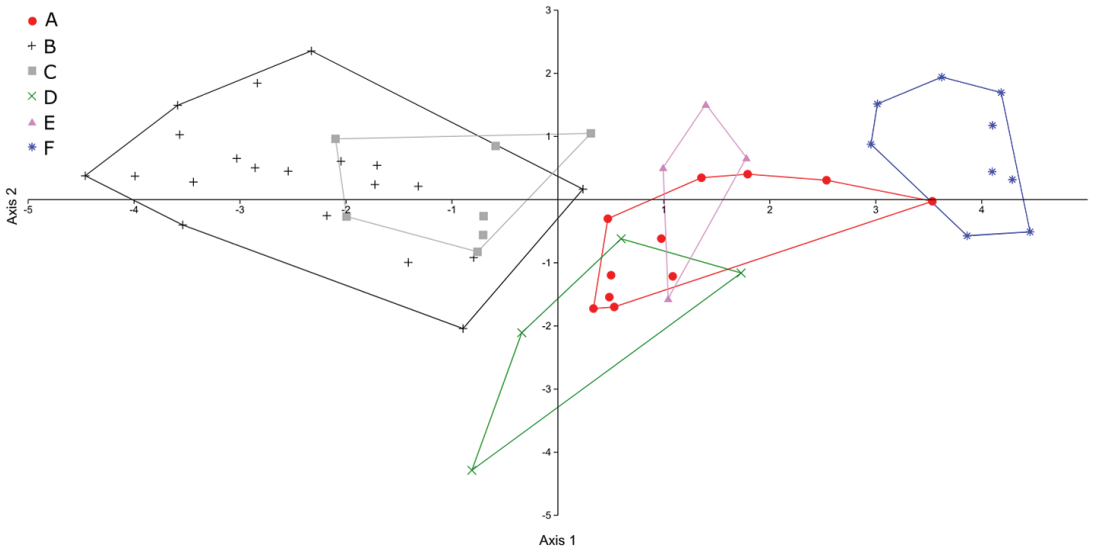

FIGURE 10. Graphic representation of the first two Discriminant Function Analysis scores separating *Megascops atricapilla-M. watsonii* clades A–F based on nine longsong character measurements (pace, note length, peak frequency, max power, change in note length, change in interval length, change in peak frequency, change in max power, and change in pace).

Some clades are extremely variable vocally. For example, in Clade B, the pace among individual longsong recordings varied from 2.5 notes.sec^{-1} to 8.5 notes.sec^{-1} (Fig. 9). However, there is some evidence of geographic structuring, with the slowest-paced longsongs found from southern Ecuador throughout the uppermost portions of the Inambari, Madeira, Tapajós, and Xingu AOEs (3.03±0.5 notes/s), whereas the fastest-paced longsongs occur north of Amazon River and in the lowermost portions of Inambari and Madeira AOEs (6.7±1.3 notes/s; Fig. 9). An ANOVA for longsong pace between Clade C and Clade B from Tapajós and Xingu AOEs, where both clades occur in parapatry (10 samples from 7 localities in the states of Pará and Mato Grosso, Brazil, i.e., Alta Floresta, B84; Santana do Araguaia, B7; Canarana, B6; Carajás, C3; Trairão, C8; Caxiuanã, C1; and Santarem, C2; Fig. 9), showed a significant difference (F=47.989, p=0.000), with Clade B birds having a significantly slower-paced longsong. With respect to sister clades E and F distributed throughout the Atlantic Forest, a simple regression analysis revealed a gradual latitudinal variation in the pace of longsongs (R^2=0.825, p=0.000; Fig. 9), with this vocal character highly correlated with geographic distance from the northernmost sample point (0.908).

A DFA with longsongs from birds in clades A–F found that individuals from Clade F differ significantly from all the others, despite limited overlap in measurements with individuals from the distantly related Clade A, with the first two canonical discriminant variables accounting for 93.6% of the total variation (Wilk´s lambda=0.062, P=0.000, Fig. 10). The classification matrix correctly assigned 64–77% of the specimens to their respective clades based on the vocal characters measured (Table 7). Variation was explained mostly by pace (F=17.078), maximum power (F=3.159), and change in note interval (F=2.434). Otherwise, there is broad overlap among longsongs of individual birds in the non-sister and parapatric clades B and C, and the distantly related and allopatric clades A, D, and E (Fig. 10). Hence, three main types of longsong were recovered by the DFA: one unique to individuals in Clade F, a second shared by individuals in clades B and C, and a third given by individuals in clades A, D, and E. These three major longsong types overlap only marginally with each other (Fig. 10). This is also evident when the analyses are restricted to individuals in the closely related clades C–F, with the resulting DFA (the first two first canonical discriminant variables accounting for 99.2% of the differences) showing a clear separation among longsongs of individuals in clades C and F with respect to those in clades D and E, which overlap with each other (Wilk´s lambda=0.006, P=0.000; graph not shown).

TABLE 7. Summary of classification accuracy of recordings among *Megascops atricapilla-M. watsonii* individuals from clades A–F based on a Discriminant Function Analysis (DFA) of measurements for nine longsong characters (pace, note length, peak frequency, max power, change in note length, change in interval length, change in peak frequency, change in max power, and change in pace). Most of the recordings were assigned to groups based on their congruent distributions within the ranges of the identified molecular clades. Numbers before and after slashes were obtained without and with jackknife procedures, respectively.

Clade	A (n=11)	B (n=21)	C (n=7)	D (n=4)	E (n=4)	F(n=9)	% correct
A	9/7	0/0	0/0	0/2	1/0	1/2	82/64
B	0/1	14/14	4/3	3/3	0/0	0/0	67/67
C	0/1	1/2	6/3	0/1	0/0	0/0	86/43
D	0/0	0/0	0/1	2/1	2/2	0/0	50/25
E	0/0	0/0	0/0	1/1	3/3	0/0	75/75
F	0/1	0/0	0/0	0/0	0/0	9/8	100/89
Total	9/10	15/16	10/7	6/8	6/5	10/10	77/64

Sample sizes of shortsongs were too small to assess for clades D and E, and therefore these were excluded in the analyses. In most cases, shortsongs are monotonous sequences of short notes, upslurred (inverted U- or V-shaped) towards the end (Fig. 11). For clades A and F, shortsongs were composed of very short notes, downslurred (U or V shaped) or more commonly upslurred, with a sharp inflection in the middle, making the note A-shaped (Figs. 11A, 11D). The inflection is more evident in notes from the second part of the shortsong. In Clade B, the shortsong is composed of much longer and less inflected notes, which can be upslurred, downslurred or more uniformly shaped (Fig. 11B). In fast-paced populations of Clade B and in Clade C, shortsong notes are shorter, although not as much as in clades A and F, and the notes are more inflected in the second part of the shortsong (Fig. 11C). Measurements of note length, peak frequency and pace values from shortsongs of clades A–F are found in Table 8.

TABLE 8. Mean and standard deviation values of nine "shortsong" characters measured for *Megascops atricapilla-M. watsonii* individuals belonging to clades A–F.

Characters	A (n=7)	B (n=14)	C (n=4)	F (n=3)
Note length (sec)	0.07±0.01	0.17±0.05	0.10±0.01	0.06±0.01
Peak frequency (Hz)	0.61±0.05	0.64±0.17	0.67±0.03	0.74±0.15
Pace (n notes/sec)	7.97±0.43	3.21±1.21	4.70±0.30	8.87±1.11
Maximum power (dB)	84.44±21.07	84.38±16.4	101.45±43.33	86.06±7.09
Change in note length	1.01±0.01	1.0±0.03	1.02±0.03	1.03±0.03
Change in note interval	1.03±0.01	1.06±0.03	1.1±0.03	1.06±0.02
Change in peak frequency	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Change in maximum power	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Change in pace	0.88±0.03	0.74±0.1	0.82±0.16	0.82±0.05

A DFA conducted with shortsongs of clades A, B, C, and F indicated a strong separation of clades A and F from Clade C (Wilk´s-lambda=0.029, P=0.000), with the first two canonical discriminant variables accounting for 98.8% of the differences. The DFA correctly classified 89% of the samples (Table 9). Among these four clades there is some overlap between the allopatric clades A and F and the parapatric clades C and B (Fig. 12).

Six *Megascops atricapilla-M. watsonii* clade pairs were significantly diagnosable from each other by at least one vocal (longsong and/or shortsong) trait according to the diagnosability test employed (Table 10). Four clade pairs (A–B, B–E, C–F and E–F) differed by one character, two clade pairs (A–C and B–F) differed by two characters, and one clade pair (C–E) differed by three characters (Table 9).

TABLE 9. Summary of classification accuracy of recordings among *Megascops atricapilla-M. watsonii* clades A, B, C, and F by a Discriminant Function Analysis (DFA) based on measurements of nine shortsong characters (pace, note length, peak frequency, max power, change in note length, change in interval length, change in peak frequency, change in max power, and change in pace). Clades D and E were not included in this analysis due to their low sample sizes. Numbers before and after slashes are values obtained without and with jackknife procedures, respectively.

Clade	A (n=3)	B (n=4)	C (n=14)	F (n=7)	% correct
A	7/6	0/0	0/0	0/1	100/86
B	0/0	12/11	2/3	0/0	86/79
C	0/0	0/3	4/1	0/0	100/25
F	1/2	0/0	0/0	2/1	67/33
Total	8/8	12/14	6/4	2/2	89/68

Discussion

Genetic variation and species limits in Megascops atricapilla-M. watsonii. Results from the molecular analyses revealed a phylogenetic history that is more diverse and complex than the current taxonomic treatment applied to the *Megascops atricapilla-M. watsonii* complex (Marks *et al.* 1999; König & Weick 2008; Dickinson & Remsen 2013; Piacentini *et al.* 2015; Clements *et al.* 2019; Gill *et al.* 2020; Remsen *et al.* 2020). The traditional taxonomy of this group, based mostly on poorly understood variation in plumage and vocal attributes (Chapman 1928; Marshall 1991; but see Heidrich *et al.* 1995), is in need of revision, in particular because *Megascops watsonii*, as currently recognized, is paraphyletic with respect to *M. atricapilla* (Dantas *et al.* 2016). Herein, our ML and BI trees were virtually identical, with most relevant nodes statistically well-supported (>0.95), and again recovering the paraphyly of *M. watsonii* with respect to *M. atricapilla* (Fig. 3). We documented six reciprocally monophyletic and genetically divergent groups within *Megascops atricapilla-M. watsonii* (labeled A–F; Figs. 3 and 4), at least three of which appear allopatric or parapatric in Amazonia and the Atlantic Forest (D–F). Clades B and C come into contact in the central portion of the Xingu and Tapajós AOEs, and clades A and B are potentially in contact near the Branco River, in northern Amazonia.

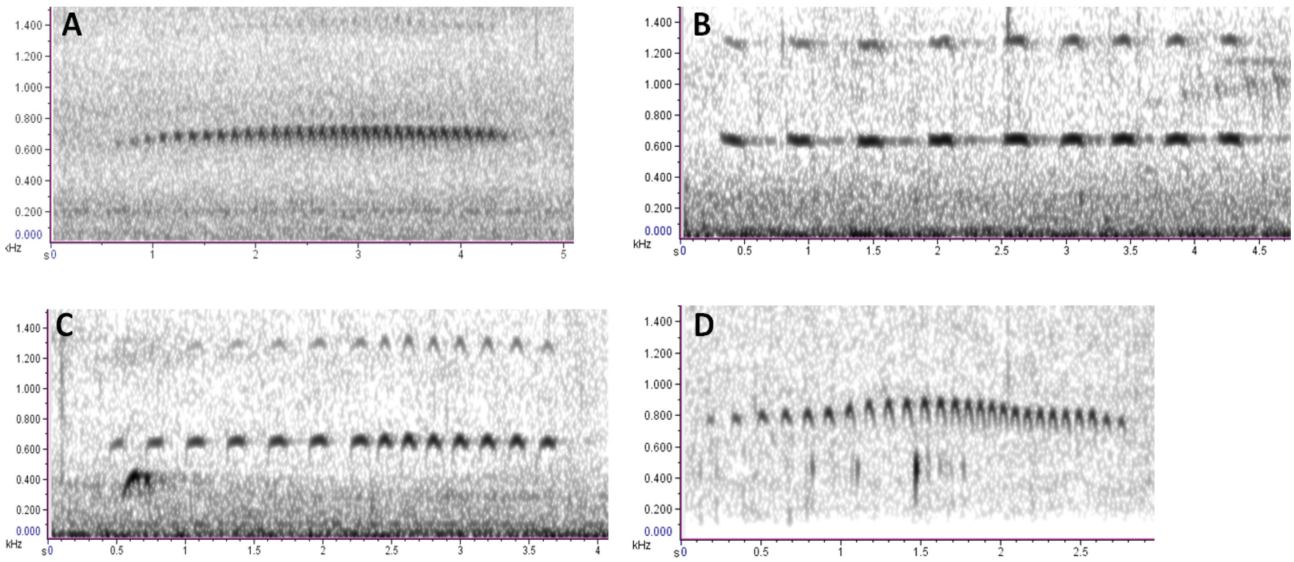

FIGURE 11. Audiospectrograms of shortsongs of *Megascops atricapilla-M. watsonii* clades A, B, C, and F. (A) Clade A: Venezuela, Rio Grande (Paul Schwartz, ML 59359); (B) Clade B: Brazil, Mato Grosso (Curtis Marantz, ML 126830); (C) Clade C: Brazil, Serra dos Carajás (Curtis Marantz, ML 126657); and (D) Clade F: Brazil, Parque Estadual Intervales, São Paulo (Dan Lane, XC 75524).

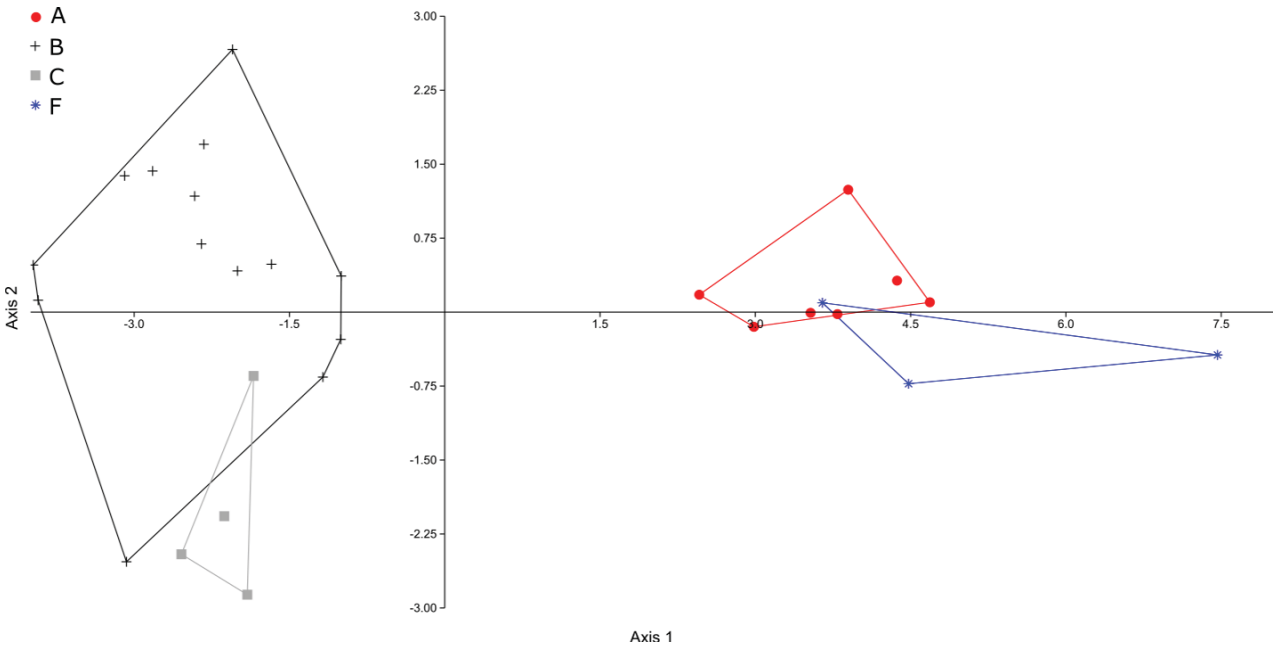

FIGURE 12. Graphical representation of scores for the first two DFA factors separating *Megascops atricapilla-M. watsonii* clades A, B, C, and F based on measurements of nine shortsong characters (pace, note length, peak frequency, max power, change in note length, change in interval length, change in peak frequency, change in max power, and change in pace).

No evidence of widespread gene flow could be detected among the six clades recovered by the concatenated molecular data (i.e., no groups were poly- or paraphyletic in mtDNA; Figs. 3 and 4). Furthermore, the estimated species tree also indicated advanced patterns of coalescence amongst clades A, B, and a major clade grouping clades C–F. Within the C+D+E clade, basal relationships mirrored those estimated by the concatenated multi-locus Bayesian tree, but without strong statistical support, suggesting either ongoing (albeit reduced) gene flow or lack of lineage sorting. The fact that clades C+D+E+F are either parapatric (C and D; separated by the Tocantins River) or allopatric (clades C+D with respect to clades F+E, separated by the *Caatinga* and *Cerrado* biomes, and clades E and F separated by the São Francisco River) favors the hypothesis of ancestral polymorphism among diverged

lineages across riverine and ecological barriers rather than recent or ongoing gene flow. Therefore, a combination of the concatenated phylogenetic analysis and the species tree analyses are consistent with limited or no geneflow among clades A–F, suggesting that all six could be treated as separate species-level taxa (Fig. 4).

In sharp contrast to these findings, Harvey *et al.* (2017) analyzed Single Nucleotide Polymorphisms (SNPs) obtained from 2286 loci of ultraconserved elements (UCEs) and exon sequences belonging to 11 specimens of *M. watsonii*, encompassing clades A, B, and C identified herein. Harvey *et al.* (2017) did not detect any phylogenetic structure amongst these clades when the nuclear data was considered, although two major groups emerged when their mitogenome amplified sequences (recovered as a byproduct of sequencing UCEs and exons) were considered. However, one of their mitogenome groups clustered specimens of clades A, B, and C (respectively, LSUMZ B20185, LSUMZ B947, USNM B6991, which were all also sequenced by us; Fig. 3), while the other grouped eight specimens from the ranges of clades A and B, including three specimens also sequenced by us (MPEG 66635, Clade A and MPEG 62429 and FMNH 456485, Clade B: see Fig. 3). Therefore, despite the detection of a major division in the mitogenome of *M. watsonii*, which is consistent with our results showing a major split separating Clade A from clades B–F (Fig. 3), membership in each mitogenome group identified by Harvey *et al.* (2017) did not match the clade limits recovered herein. These findings seem puzzling, particularly considering that most specimens analyzed in Harvey *et al.* (2017) were also included herein. It is important to emphasize that while the present study was entirely focused on the *Megascops atricapilla-M. watsonii* complex, that of Harvey *et al.* (2017) assessed comparative levels of genetic differentiation across 40 species of birds belonging to distant phylogenetic groups ranging from tinamous to oscine passerines. Due to the comparative nature of their study among deeply divergent avian lineages, Harvey *et al.* (2017) used highly conserved markers that could be amplified across the avian tree of life, employing SNP filtering strategies which contributed little phylogenetically informative genomic variation within *M. watsonii*. Similarly, the assembly of their mitochondrial reads relied on the full mitochondrial genome of a distantly related species, *Strix leptogrammica* Temminck; and issues such as duplication of some mitochondrial genes (see Hanna *et al.* 2017) could have caused the discrepancies discussed above with respect to the patterns of differentiation revealed by the mitogenome. Therefore, despite their greater coverage in terms of genetic loci, we believe that the results presented by Harvey *et al.* (2017) are less informative under a phylogenetic and phylogeographic perspective than the results presented herein, which have greater sampling density and geographic scope, providing a better resolved estimate on the spatio-temporal pattern of diversification in the *Megascops atricapilla-M. watsonii* complex.

Patterns of phenotypic variation in Megascops atricapilla-M. watsonii. In contrast with our molecular results (Figs. 3 and 4), morphological analyses did not detect any fully diagnosable groups within *Megascops atricapilla-M. watsonii* due to the combination of morphological overlap among clades and extreme variation in plumage patterns within and among clades (Fig. 7; Table 4). However, some color morphs are apparently more common in some clades than in others. In particular, dark morphs of clades A and D have a more contrasting chest color, and are in general darker than in other populations (Table 5). In general, variation in plumage and morphological traits in owls are thought to be linked in part to environmental variables rather than to phylogeny alone (Fuchs *et al.* 2007; Roulin *et al.* 2011) and therefore, the lack of diagnosability based on plumage coloration and pattern is not surprising. In at least one clade (Clade F, *M. atricapilla*) we found clinal variation in body mass, which increases from lower to higher latitudes, mirroring Bergmann´s rule (Bergmann 1847). Latitudinal clines in size and plumage are also known for other screech-owls, such as *M. asio* and *M. kennicottii* (Proudfoot *et al.* 2007).

With respect to vocal characters, six pairs of well-supported clades recovered by the molecular analyses are also reciprocally distinguishable by longsong and shortsong features, also indicating independent evolutionary histories among them, and thus more subdivided species limits than currently recognized (Table 10). The DFA and the diagnosability test employed indicated significant differences in at least one vocal character among all clades (Table 10; Figs. 10 and 12), except Clade D (for which only four longsongs were analyzed). In most cases, for both longsongs and shortsongs, only one vocal trait was significantly diagnosable among clades, whereas in two instances, two characters diagnosed clades, and in one instance two clades were diagnosed by three traits (Table 10). Despite these differences, no clade could be uniquely diagnosed from all the remaining ones by the vocal characters measured, which suggests a considerable level of homoplasy, or perhaps retention of ancestral polymorphism in longsong and shortsong structures through the evolutionary history of the *Megascops atricapilla-M. watsonii* complex. This is further illustrated by the high level of vocal variation within the well-supported and widely distributed Clade B, which matches the higher (albeit non-geographically structured) degree of genetic differentiation found within this clade in comparison to all the others (Figs. 3 and 9; see also Krabbe 2017).

TABLE 10. Results of pairwise diagnosability tests employed among *Megascops atricapilla-M. watsonii* clades A–F based on the longsong and shortsong character measurements (see text for details).

Clade	A (n=18) [1]	B (n=35)	C (n=11)	D (n=4)	E (n=4)
B	(1)[2] pace shortsong	-	-	-	-
C	(2) pace shortsong note interval shortsong	-	-	-	-
D	- [3]	-		-	-
E	-	(1) pace longsong	-	-	-
F (n=12)	-	(2) pace longsong peak frequency longsongs	(3) pace longsong note interval longsong note interval shortsongs	-	(1) pace longsong

[1] Sample sizes for each clade.

[2] Total number of vocal characters found to differ significantly according to the test results, followed by specific vocal trait(s) diagnosing any two clades.

[3] Pairwise comparison not significantly diagnosable.

Isler *et al.* (1998) showed that sympatric species pairs of antbirds (Thamnophilidae) usually differ diagnostically by at least three vocal features, which prompted those authors to propose this as a minimum number of diagnostic vocal characters to rank taxa as biological species rather than subspecies. However, Chaves *et al.* (2010) concluded that only two vocal traits were sufficient to consider two taxa of antbirds then assigned to the genus *Myrmeciza* Gray as full species, yet these were largely sympatric, replacing each other elevationally. Carneiro *et al.* (2012) went even further and found only one statistically diagnosable vocal trait distinguishing reciprocally monophyletic populations of *Hylopezus* Ridgway antpittas. Reciprocal monophyly and concordant vocal diagnoses are indicative of species level differences under the Phylogenetic Species Concept (PSC) or the General Lineage Species Concept (GLSC; de Queiroz 1998), providing the basis for considering those *Megascops atricapilla-M. watsonii* clades diverging from each other by at least one significantly diagnosable vocal trait as separate species (i.e., clades A, B, C, E, and F). However, in the case of Clade D, which is not vocally diagnosable from any other clade in the *Megascops atricapilla-M. watsonii* complex, the lack of vocal diagnosability is at odds with statistically significant reciprocal monophyly in mtDNA and relatively high average pairwise genetic p-distance, ranging from 2.1% (to Clade C) to 6.7% (to Clade A) with respect to other *Megascops atricapilla-M. watsonii* clades (Fig. 3, 4, and 9; Table 2). It is difficult to determine whether this lack of vocal diagnosability of Clade D birds is the result of a very small sample size (n=4), but the same number of longsongs was available for Clade E birds, which were significantly vocally diagnosable from clades B, C, and F.

There was a lack of samples from most of the area where clades B and C meet in the middle part of the Tapajós and Xingu interfluves, and from the upper Branco River and in southern Venezuela, where clades A and B also likely meet. Hence, it is possible that our sampling could have missed some possible introgressed individuals. Both fast and slower longsong types have been reported in zones of potential contact between clades B and C (V. Piacentini, pers. comm.; S. Dantas, pers. obs.), and further collecting and recording is needed from these localities. But even if present, introgression is not extensive, given the high statistical support for the reciprocal monophyly and divergence between clades B and C according to both the concatenated multi-locus Bayesian tree and the multi-locus coalescent species tree. Clades B and C are separated by a relatively high average uncorrected mitochondrial distance of 3.2%, similar to that separating the sister species pair *Megascops choliba* (Vieillot) and *M. koepckeae* (Hekstra) (Dantas *et al.* 2016), which indicates separate evolutionary trajectories for a significant amount of time (see below). In North America, populations of *Strix occidentalis* (Xántus), a sedentary species, exhibit evidence of limited introgression away from immediate zones of contact (Barrowclough *et al.* 2005). Presumably also sedentary, non-sister and parapatric clades B and C would have limited introgression due to competitive exclusion, as verified for antbirds sharing the same contact zone in southeastern Amazonia (Pulido-Santacruz *et al.* 2018). These two clades of *Megascops* differ in their longsongs, with Clade C being significantly faster-paced than the parapatric populations of Clade

B (Fig. 9). The different voices of these potentially sympatric *Megascops* lineages, coupled with their reciprocal monophyly and high genetic divergence, suggests vocal traits may be important species-specific recognition cues, as suggested for other owls (Marshall 1967; Marks *et al.* 1999; König & Weick 2008). In the Bolivian Andes, *M. marshalli* (Weske & Terborgh) longsong is different from that of the sympatric *M. ingens* (Salvin), but similar to the allopatric *M. petersoni* (Fitzpatrick & O'Neill) longsong (Herzog *et al.* 2009; Krabbe 2017). Interestingly, longsongs of allopatric populations of clades B and C (from opposite sides of the Tapajós River) sound more similar to one another than where they are near potential contact zones such as in Carajás and Santana do Araguaia, ca. 300 km apart (Fig. 9). Herzog *et al.* (2009) reported a geographic cline in the longsong of *M. ingens*, although this may be related to genetic structure in this species (Dantas *et al.* 2016), and we also found apparent clinal variation in the longsong of *M. atricapilla*, also linked to possible genetic structure. Marshall (1978) noted that form and quality of song "…have not proved valuable as generic characters" of *Otus* screech-owls in Asia, and Fuchs *et al.* (2008), in a study on scops-owls (*Otus*) from Indian Ocean islands found that "…vocal and morphological differences are indeed associated with distinct evolutionary lineages, but suggest that they are not related in any simple or obvious way to the evolutionary distance between these lineages, and therefore must be used with caution in identifying affinities between taxa (or lineages)". Our results showed similar patterns in the *Megascops atricapilla-M. watsonii* complex that will warrant more study in the future.

In particular, longsong traits in *Megascops atricapilla-M. watsonii* were useful in distinguishing four of the six clades recovered by the molecular phylogenetic analysis (i.e., clades B, C, E, and F), with shortsong traits further diagnosing Clade A (which was not diagnosed by any longsong feature) from the other two clades (Table 10). Thus, a combination of longsong and shortsong features is useful for separating five of the clades recovered in *Megascops atricapilla-M. watsonii*. The only clade that could not be diagnosed from other clades by any vocal character was Clade D, which is endemic to the Belém AOE in easternmost Amazonia. When only longsong pace, the most important trait in distinguishing species pairs according to the diagnosability test employed (Table 10), is considered, the average values obtained for Clade D are intermediate between those of clades C and A + E, which could potentially explain this lack of significant diagnosis (Fig. 9). A larger sample for Clade D would perhaps clarify this question.

Timing of diversification and biogeography. Speciation within the *Megascops atricapilla-M. watsonii* complex took place throughout the Plio-Pleistocene (4.5–0.5 mya), a time frame that overlaps with other Amazonian vertebrate diversification events (Weir & Price 2011; Alfaro *et al.* 2012; Silva *et al.* 2019). Spatio-temporal patterns of avian diversification in Amazonia vary greatly among groups (Miller *et al.* 2008; Patel *et al.* 2011; d´Horta *et al.* 2013; Silva *et al.* 2019), due to multiple geological and climatic events, and biological and ecological differences among taxa. Many hypotheses have been proposed to explain these patterns, such as the refuge (Haffer 1974) and the riverine barrier (Gascon *et al.* 2000) hypotheses. Recently, Silva *et al.* (2019) proposed a paleobiogeographic model for biotic diversification within Amazonia, based on phylogeographies of 23 upland terra firme avian lineages, including *M. watsonii*. Along with 16 other lineages, *M. watsonii* shared the predominant "counterclockwise" pattern of diversification observed, whereby earlier splits occurred on the Guiana Shield before 2 mya ago, followed by divergences across western-central Amazonia around 1 mya ago, and into the Brazilian Shield in southeastern Amazonia around 0.8 mya ago (Silva *et al.* 2019). This study suggested that a combination of climate-driven refugial dynamics and dynamic riverine barriers led to a pattern where older lineages in the wetter western and northern parts of the Amazon gave origin via dispersal and subsequent vicariance to lineages in the drier southern and eastern parts of Amazonia (see also Bicudo *et al.* 2019). The time tree estimated herein for *Megascops atricapilla-M. watsonii* included a greater sample of specimens (56 versus 52) and nuclear markers (FIB5, CHD, and MUSK versus just FIB5) than that analyzed in Silva *et al.* (2019), and the results obtained by each analysis differed in some important aspects. The major difference was that in Silva *et al.* (2019), clades B and C were recovered as forming a clade, although with no statistical support, whereas we found Clade C to be closer to clades D, E, and F, with high statistical support (Fig. 4). Another important difference was that average splitting dates among lineages of *Megascops atricapilla-M. watsonii* were younger in Silva *et al.* (2019). Possibly, the greater number of genetic loci and specimens in clades B, C, and F analysed herein, coupled with differences in the rooting strategy of the species tree adopted by each study, could explain these differences. Because the current analysis included a more representative sample of specimens and loci, it can be regarded as more robust; therefore, we follow it in the remaining discussion (Fig. 4).

The most important rivers for the differentiation of Amazonian *Megascops atricapilla-M. watsonii* were (in chronological order from earliest) the Amazonas (lower Amazon), Negro-Branco, Tapajós and Tocantins. Our study of the *Megascops atricapilla-M. watsonii* complex differs from Ribas *et al.* (2012) on *Psophia* Linnaeus trumpeters

in that populations from north of the upper Amazon-Solimões (Napo and Imerí AOEs) were more closely related to lineages south of this river than to those found in the Guiana Shield (Clade A). In fact, populations of *M. w. usta* (Clade B) separated by the upper Amazon River were not genetically distinct from one another, despite vocal differences between them. Also, the Branco River, a tributary of the Negro River, separated clades A and B. Recently, the Branco River has been documented as an important barrier separating populations of many bird taxa (Naka 2011; Naka *et al.* 2012; Fernandes *et al.* 2013, 2014).

Rivers have played an important role in separating some clades of *Megascops atricapilla-M. watsonii*, but not others. Clade B, for example, crosses the middle and upper Tapajós River and is parapatric with regard to Clade C in the Tapajós-Xingu and Xingu-Tocantins interfluves (Tapajós and Xingu AOEs; Fig. 1). Similar patterns of phylogeographic breaks away from the course of main Amazonian rivers have been documented for several avian taxa along the Tapajós River, including *Glyphorynchus spirurus* (Vieillot) (Fernandes *et al.* 2013), *Hylophylax naevius* (Gmelin) (Fernandes *et al.* 2014), and several others (Silva *et al.* 2019). One possible explanation for parapatry not related to the main Amazonian tributaries posits secondary contact via expansion of at least one of the clades (Haffer 1997; Fernandes *et al.* 2012). Thus, expansion of an originally western Amazonian Clade B into eastern Amazonia is a possibility and is supported to some extent by the higher level of phylogeographic structure detected among western populations of this species, where an individual from the Andean foothills in the Department of La Paz, Bolivia (LSUMZ B947; locality B8 in Fig. 1), is separated by an average uncorrected mitochondrial distance of 2.6% from the remaining populations of the same clade, a distance which equals or surpasses uncorrected p-distance between some clades within the *Megascops atricapilla-M. watsonii* complex (clades C-D-E-F; see also Silva *et al.* 2019). However, denser sampling of Clade B, particularly along the foothills of the Andes, is necessary to test this expansion scenario in a robust way. Clades C and D split around 1 mya (Fig. 6), and are separated by the Tocantins River. This more recent divergence timing coincides with divergence among lineages of *Psophia* (Ribas *et al.* 2012) and *Dendrocolaptes certhia* (Boddaert) (Batista *et al.* 2013) split by the same river and thus indicates that the Tocantins River had a later influence on the divergence among lineages of some Amazonian birds compared with other rivers in the region (Silva *et al.* 2019; Soares *et al.* 2019).

The split between Amazonian and Atlantic Forest *Megascops atricapilla-M. watsonii* lineages was estimated to have occurred between 0.7 and 1.3 mya. Two or three historical connections between Amazonian and Atlantic Forest have been suggested, including an older Miocene connection linking the Andes to southern Atlantic Forest, and one or two more recent Pliocene to Pleistocene connections linking southeastern Amazonia to the northern sector of the Atlantic Forest (Batalha-Filho *et al.* 2013; Capurucho *et al.* 2018). These younger connections involved the easternmost Amazonian populations (i.e. those from Belém AOE) and northernmost Atlantic Forest populations, such as the Pernambuco AOE population. Atlantic Forest clades of the *Megascops atricapilla-M. watsonii* complex (clades E and F) are closer to clades C and D from southeastern Amazonia (Tapajós, Xingu and Belem AOEs), and is therefore consistent with a younger (Pleistocene) pathway theory (see also Thom & Aleixo 2015) between these biogeographic regions. The Atlantic Forest clades E and F likely descended from an ancestor colonizing the Atlantic Forest from southern Amazonia (Fig. 6). Again, denser sampling of clades E and F is necessary to further test this hypothesis.

The timing of the Amazonian and Atlantic Forest split within the *Megascops atricapilla-M. watsonii* complex was similar to the splits documented for *Ramphastos* Linnaeus (0.77–1.4 mya; Patané *et al.* 2009) and *Pionus* Wagler (0.15–1.41 mya; Ribas *et al.* 2007), and younger than the timing of these same biogeographic splits for taxa such as *Brotogeris* Vigors (Ribas *et al.* 2009), *Xiphorhynchus* Swainson (Cabanne *et al*. 2008), *Dendrocincla* Gray (Weir & Price 2011), *Pteroglossus* Illiger (Patel *et al*. 2011) and *Pyrilia* Bonaparte (Eberhard & Bermingham 2005), which occurred between 3 and 10 mya. Although these spatio-temporal patterns of diversification are more commonly found between Amazonian-Atlantic forest lineages, broader sampling in some lineages suggests a more complex evolutionary history in these regions, as illustrated by the case of the *Xiphorhynchus guttatus* (Lichtenstein)-*guttatoides* (Lafresnaye)-*sussurrans* (Jardine) complex (Rocha *et al.* 2015), in which the Atlantic Forest lineages are closely related to the Guianan AOE populations and not to the southeastern Amazonian populations.

Atlantic Forest lineages (clades E and F) appear to be sister to Clade D, although this relationship is not statistically well supported according to the phylogeny estimates obtained (Figs. 3 and 4). With development of the Tocantins River (which separates clades C and D), divergence of Amazonian and Atlantic Forest clades may have occurred at approximately the same time (1.0 mya) as in other avian groups (Ribas *et al.* 2012; Batalha-Filho 2012). The split amongst clades C–F occurred in a short time interval (Fig. 4). This combination of a recent and concordant

split among these clades would explain the phylogenetic uncertainty among lineages from these regions. Sampling additional loci and individuals from these regions may help clarify the history of these recent divergence events.

Within the Atlantic Forest there are two clades (E and F) of *M. atricapilla* separated by the São Francisco River valley. This large eastern Brazilian river separates many distinct avian taxa in the Atlantic Forest, isolating the Pernambuco AOE to the north. *Dendrocincla turdina* (Lichtenstein) and *D. fuliginosa taunay* Pinto, strongly supported as sister taxa (Weir & Price 2011; Schultz *et al.* 2019), are also separated by the São Francisco River. These *Dendrocincla* species are estimated to have diverged between 0.5 and 2.0 mya. Other sister taxa separated by this river include *Xiphorhynchus fuscus* (Vieillot)-*X. atlanticus* (Cory) (Cabanne *et al.* 2008) and *Tangara seledon* (Müller)-*T. fastuosa* (Lesson) (Burns & Naoki 2004), and these splits may have taken place around the same time. The confidence interval obtained for the divergence between *M. atricapilla* clades E and F also overlaps with this time frame. These results reinforce the suggestions of Weir & Price (2011) that a common biogeographic event may have simultaneously affected populations among multiple taxa across the São Francisco River. Carnaval *et al.* (2009) suggested that separate humid forest refugia existed to the north and south of São Francisco River during Pleistocene glacial maxima, and this could have promoted speciation.

Taxonomic recommendations. The combined data sets presented and discussed here provide resolution into the diversification of the *Megascops atricapilla-M. watsonii* complex, and show that currently recognized species limits in this group are at odds with the complex's evolutionary history. Different species delimitation strategies exist (De Queiroz 1998), but they are more properly applied when explicit evaluations of interspecific limits are available under an evolutionary perspective. Herein, we formally tested for the first time interspecific limits in the *Megascops atricapilla-M. watsonii* complex with an objective and robust coalescent-based method (i.e., BP&P; see Leaché *et al.* 2019 and references therein). BP&P supported the existence of six distinct lineages (called clades A–F) in the *Megascops atricapilla-M. watsonii* complex that have coalesced extensively with respect to each other for the genes analyzed under a wide array of distinct demographic and divergence scenarios (Fig. 4), a precondition for their ranking as separate basal level taxa rather than members of a single reproductive community (Gill 2014). We also gathered vocal data showing important differences across these clades.

Except for Clade D, pairwise diagnosability tests applied to vocal characters show that all remaining significantly coalesced lineages in the *Megascops atricapilla-M. watsonii* complex (clades A, B, C, E, and F) were also diagnosed from at least another lineage in the group, although none could be uniquely distinguished by the vocal characters measured (Table 10). Even though vocal characters are thought to play an important role in species-specific mate recognition in birds in general and owls in particular (Fjeldså *et al.* 2012; Rasmussen *et al.* 2012; Sangster *et al.* 2013; Krabbe 2017), our analyses supported significant homoplasy in longsong and shortsong evolution in the *Megascops atricapilla-M. watsonii* complex, as also demonstrated for other owl lineages (Marshall 1978; Fuchs *et al.* 2008; Herzog *et al*. 2009). This overall lack of phylogenetic structure in vocal character variation for the *Megascops atricapilla-M. watsonii* complex is in sharp contrast with the recovered patterns of highly structured genetic variation in this group, which rules out the existence of a single, panmictic population with free interbreeding, as implied by current taxonomy (Dickinson & Remsen 2013; Clements *et al.* 2019; Gill *et al.* 2020). However, in at least one instance where two such lineages are in direct contact away from any major riverine barrier (i.e., clades B and C), sharp differences in longsong pace exist that seem consistent with clade boundaries, reinforcing the view that these evolutionary units cannot be treated as a single reproductive entity (Fig. 9). In this particular case, at a broader geographic scale, vocal characters are plastic throughout the range of the *Megascops atricapilla-M. watsonii* complex. Nonetheless, in areas of sympatry between recovered lineages, existing vocal differences can be locally reinforced due to reproductive selection, with longsong pace for instance functioning as a "cue" directing assortative mating among individuals belonging to the same clade (see also Herzog *et al*. 2009). Interestingly, no striking differences in longsong pace exist between clades B and C across the lower Tapajós River, where these lineages are separated by a few dozen kilometers from each other (Fig. 9). We hypothesize that the significant degree of coalescence recovered herein among *Megascops atricapilla-M. watsonii* clades A–F (Fig. 4) is due to a combination of geographic isolation and assortative mating among clades, and that longsong pace is under character displacement selection in areas of direct contact between clades, but not between populations separated by major physical and ecological barriers.

Therefore, when interpreted in concert, both genetic and vocal characters suggest that clades A–F recovered herein as significantly coalesced units already exhibit some level of reproductive isolation as opposed to free interbreeding (Gill 2014), and also suggest mutation order speciation (Winger & Bates 2015), supporting their ranking

as separate species-level taxa. Below, we provide a taxonomic review of the *Megascops atricapilla-M. watsonii* complex based on our integrative molecular, vocal, and morphological datasets. Although available taxon names can be assigned to clades A, B, D, and F, clades C and E represent new taxa that we describe, respectively, as:

***Megascops stangiae,* sp. nov.**
Xingu Screech-Owl

corujinha-do-xingu (Portuguese)
Otus watsonii usta (Sclater, 1858): Marks *et al.* (1999); Weick (2006; part: specimens between lower Tapajós and lower Tocantins rivers).
Megascops usta (Sclater, 1858): König *et al.* (1999); König & Weick (2008; part: specimens between lower Tapajós and lower Tocantins rivers).
Megascops watsonii usta (Sclater, 1858): Dickinson & Remsen (2013); Clements *et al.* (2019); Gill *et al.* (2020; part: specimens between lower Tapajós and lower Tocantins rivers).

Corresponding to Clade C recognized in this study, *M. stangiae* is endemic to Brazil and distributed along the lower parts of the Tapajos-Xingu and Xingu-Tocantins interfluves, and may not cross the Araguaia River. The southern limits of this taxon are unclear, but extend at least as far as the Serra dos Carajás (01º44'S, 51º27"W).

Holotype: MPEG 70627 Skin. A male collected on 4 August 2010 at Serra dos Carajás, Parauapebas, Pará State, Brazil (05º46'12.5"S; 50º29'54.9"W), and deposited at the Museu Paraense Emilio Goeldi.

Paratypes: MZUSP 93276, unsexed, collected on 1 July 2010 at Porto de Moz, Pará, Brazil (2°10'57,04"S; 52°16'14,16"W); MZUSP 83558, female, 110 g, collected on 12 December 2008 at Porto de Moz; MPEG 53840, female, smooth ovary 3x1 mm, 125g, collected on 21 August 1997 at the Floresta Nacional do Tapajós, Belterra, Pará (3°05'43.96"S, 54°55'48.12"W); MPEG 70684, male, testes 15x10 mm, 130 g, brown iris, collected on 14 September 2010 at Floresta Nacional do Tapajós; MPEG 70678, male, testes 15x10 mm, brown iris, 125 g, collected on 13 September 2010 at Floresta Nacional do Tapajós; MZUSP 64307, female, widest ovum 2 mm, 141 g, amber iris, collected on 26 September 1986 in the municipality of Altamira, Pará, Brazil (03°39'S, 52°22' W); MPEG 65676, male, testes 10x5 mm, 130 g, brown iris, collected on 24 July 2008 at Floresta Nacional do Crepori, Jacareacanga, Pará, Brazil (6°34'45.67"S, 57° 9'4.91"W); and MPEG 70846, male, brown iris, collected on 9 March 2010 in the district of Mirituba, Itaituba, Pará, on the right (east) bank of the Tapajós River (4°17'51.04"S; 55°57'19.63"W).

Description of the holotype: A brown morph of the *Megascops atricapilla-M. watsonii* complex with Light brown upperparts (121D; Smithe 1975) and a Dark brown cap (219; Smithe 1975). Chest speckled with Dark brown (219) fishbone-shaped stripes in a light brown background, and belly Tawny-olive (223D; Smithe 1975) with a few dark brown fishbone-shaped stripes. Yellowish-brown underwing and tarsi coverts. Tail light Brown (119; Smithe 1975). Iris orange, bill lead-colored, tarsi whitish. Measurements: Wing length 168 mm; tail length 90.3 mm; tarsus length 29.1 mm; bill length at anterior end of nostrils 14.9 mm; bill width 8.5 mm; bill height 11.4 mm; body mass 140 g.

Variation in the type series: The type series is highly variable in overall color, as well as in the amount and shape of ventral stripes. It includes brown, red, and red-brown morphs, and some dark individuals are similar to dark morph Clade D birds (e.g. MPEG 70647; see below). Red morphs tend to have less stripes on underparts, and these may not look "fishbone-shaped". Black on the crown can be reduced to spots or longitudinal stripes in red morphs.

Diagnosis: As Clade C birds, uniquely distinguished from all other lineages and taxa in the *Megascops atricapilla-M. watsonii* complex by six fixed (unvariable) synapomorphic (shared-derived) mutations (five transitions and one transversion) in sequences of the mitochondrial genes COI (positions 891, 948, 963, and 990 in the supplied alignment; Supplementary file 1) and cytb (positions 258 and 669 in the supplied alignment; Supplementary file 2). From a phenotypic perspective, no reliable morphological diagnosis exists with respect to other species in the complex, particularly among the Amazonian ones. Similarly, no single vocal character distinguishes *M. stangiae* from all other taxa in the *Megascops atricapilla-M. watsonii* complex, although pairwise diagnosability tests supported reciprocal vocal diagnoses with respect to most taxa, except clades D (*Megascops ater*, see below) and E (*Megascops* **sp. nov.**, see below), as follows. *Megascops stangiae* is vocally distinguishable from *M. atricapilla* (Clade F,

see below) and *M. usta* (Clade B, see below) by longsong pace (7.25±0.89 notes per second vs. 13.11±0.9 notes per second and 4.66±2.0 notes per second) and from *M. watsonii* (Clade A, see below) and *M. atricapilla* by shortsong pace (4.70±0.30 vs. 7.97±0.43 and 8.87±1.11 notes per second). No recording was obtained for the *M. stangiae* holotype, but longsong and shortsong recording from the same locality are available in xeno-canto under the numbers XC 22514 (longsong), obtained on 13 August 2008, and XC 26115 (shortsong), obtained on 30 November 2008, both by S.M. Dantas. Longsongs consist of monotonous sequences of equally spaced notes delivered during a variable period of time, gradually rising in volume until frequency stabilizes, becoming lower towards the end (Fig. 8c). Shortsongs are monotonous sequences of short notes, with upslurred (inverted U or V shaped) notes towards the end, with two parts, a slower-paced and a faster-paced that gradually slows down towards the end (Figure 11c).

Etymology: We name this species in honor of the late Sister Dorothy Mae Stang (1931–2005), who had worked on behalf of poor farmers and the environment in the Brazilian Amazon region since the 1960s until she was brutally murdered by ranchers in Anapú, Pará State. The common names Xingu Screech Owl (English) and Corujinha do Xingu (Portuguese) refer to the area where the species is found, between the Tapajós and Xingu rivers, where Dorothy was very active as a community leader and ultimately was killed.

Habitat: The new species inhabits *terra firme*, *igapó* or *várzea* forests, from sea level to about 700 m (Serra dos Carajás). Apparently more common near the edge of the forest, and usually the most abundant nocturnal forest bird where it occurs. It perches from undergrowth to near canopy, and roosts by day inside holes or frequently inside bundles of dead leaves in the undergrowth (SMD, pers. obs.).

Remarks: Average uncorrected pairwise p-distances between *M. stangiae* and the remaining species in the *Megascops atricapilla-M. watsonii* complex were as follows: 6.4% (*M. watsonii*); 3.2 % (*M. usta*); 2.1% (*M. ater*); 2.3 % (*Megascops.* **sp. nov.**, see below); and 2.4% (*M. atricapilla*).

***Megascops alagoensis*, sp. nov.**
Alagoas Screech-Owl

corujinha-de-alagoas (Portuguese)
Megascops atricapilla (Temminck, 1822): Roda & Pereira (2006).

Corresponding to specimens in Clade E, *M. alagoensis* is restricted to isolated forest patches in the Atlantic Forest north of the São Francisco River, in the states of Alagoas and Pernambuco, Brazil.

Holotype: MZUSP 79947. An unsexed individual, yellow iris, gray bill and feet, collected at Engenho Coimbra, Ibateguara, Alagoas State, Brazil (9ºS, 35º31'W) on 30 February 2003, and deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Paratypes: All collected at the same locality as the holotype. MZUSP 79948, a male collected on 17 May 2004, 101 g; MPEG 70437, male, collected on 7 June 2010, testes 6x6 mm, yellow eyes, greenish bill and feet, 105 g; MPEG 70438, male, collected on 10 June 2010, brown eyes, green bill, greyish-pink feet, 95 g.

Description of the holotype: A red morph, with a general red color of upperparts with darker longitudinal stripes across the back. Cap Red (37; Smithe 1975) with dark stripes, and a light brownish half-collar surrounding it. Chest reddish with dark red longitudinal and thin stripes, not forming a fishbone shape. Belly yellowish with dark brown longitudinal stripes with some horizontal stripes crossing them. Some whitish or white spots between the ventral horizontal stripes. Uppertail tawny brown with lighter cross-stripes, undertail gray with faint barring. Yellow iris, gray tarsi and bill. Measurements: Wing length 164.7 mm; tail length 72.3 mm; tarsus length 23.4 mm; bill length at anterior end of nostrils 13.4 mm; bill width 7.1 mm; bill height 11.2 mm.

Diagnosis: As Clade E birds, uniquely distinguished from all other taxa in the *Megascops atricapilla-M. watsonii* complex by eight fixed (invariable) synapomorphic (shared-derived) mutations (all transitions) in sequences of the mitochondrial genes ND2 (positions 234, 366, 508, and 571 in the supplied alignment; Supplementary file 3), COI (position 1047 in the supplied alignment; Supplementary file 1), and cytb (positions 462, 786 and 987 in the supplied alignment; Supplementary file 2). From a phenotypic perspective, no reliable morphological diagnosis exists with respect to other species in the complex. Vocally, no single character distinguishes *M. alagoensis* from all other taxa in the *Megascops atricapilla-M. watsonii* complex, although pairwise diagnosability tests supported reciprocal diagnoses in longsong pace with respect to *M. usta* (10.62±0.1 vs. 4.66±2.0 notes/sec) and its sister species, *M. atricapilla* (10.62±0.1 vs. 13.11±0.9 notes/sec).

Longsong as in other taxa of the *Megascops atricapilla-M. watsonii* complex, consisting of sequences of equally spaced notes delivered during variable periods of time, which gradually rise in volume until frequency stabilizes, lowering towards the end (Fig. 8e).

Variation in the type series: Red and brown morphs are found in the species. Three of the four individuals examined were red morphs and one was brown with a reddish wash on the chest. Two of the red morphs (MZUSP 79947 and MPEG 70437) had yellow irides and the brown one (MPEG 70438) had brown irides. This pattern also holds for *M. atricapilla* in which red morphs in general have a yellow iris, but there are also brown-eyed *M. atricapilla* red morphs known.

Etymology: We name the species after the Brazilian state of Alagoas, where it was recorded for the first time in February 2001 by Curtis A. Marantz (original tape recording available from the Macaulay Library under ML 127829: see Appendix) and where most of the known population remains (Roda & Pereira 2006). The common names Alagoas Screech Owl (English) and Corujinha-de-Alagoas (Portuguese) also refer to that location.

Habitat: Atlantic Forest patches in Alagoas and in southern Pernambuco states in Eastern Brazil. This taxon is known from only four localities in Alagoas and one in Pernambuco (Roda & Pereira 2006; Malacco 2013). It perches from undergrowth to canopy. Given the extensive forest fragmentation in the region, the species should be considered critically endangered, along with numerous other localized forest bird species found there (Roda & Pereira 2006; Pereira *et al.* 2014).

Remarks: Average uncorrected pairwise mtDNA p-distances between *M. alagoensis* and the remaining species in the *Megascops atricapilla-M. watsonii* complex were as follows: 6.6% (*M. watsonii*); 3.2 % (*M. usta*); 2.3% (*M. stangiae*); 2.3 % (*M. ater*); and 1.5% (*M. atricapilla*).

In addition to the new taxa described above, we also recommend the treatment of the other lineages of the *Megascops atricapilla-M. watsonii* identified herein as species-level taxa, as follows:

***Megascops watsonii* (Cassin, 1848)**

Guianan Screech-Owl

corujinha-das-guianas (Portuguese)

Ephialtes watsonii Cassin, 1848 (lectotypes at ANSP examined)

Otus watsonii (Cassin, 1848): Cory (1918; part: specimens east of the Negro and Amazon rivers including southern Venezuela, northern Brazil, Guiana, Surinam, and French Guiana).

Otus watsonii watsonii (Cassin, 1848): Chapman (1928); Peters (1940); Marks *et al.* (1999); Weick (2006): 84 (part: specimens east of the Negro and Amazon rivers including southern Venezuela, northern Brazil, Guiana, Surinam, and French Guiana).

Megascops watsonii (Cassin, 1848): König *et al.* (1999); König & Weick (2008; part: specimens east of the Negro and Amazon rivers including southern Venezuela, northern Brazil, Guiana, Surinam, and French Guiana).

Megascops watsonii watsonii (Cassin, 1848): Dickinson & Remsen (2013); Clements *et al.* (2019); Gill *et al.* (2020) (part: specimens east of the Negro and Amazon rivers including southern Venezuela, northern Brazil, Guiana, Surinam, and French Guiana).

The type locality of this taxon is defined as "Orinoco River, Venezuela" (Chapman 1928) and it corresponds to Clade A recovered herein as the lineage of the *Megascops atricapilla-M. watsonii* complex restricted to the Guiana Shield, east of the Branco and Negro rivers, in Brazil, through Venezuela, Guyana, French Guiana, and Surinam. As reported above, sequences obtained from one of the lectotypes of *M. watsonii* (ANSP 2445) confidently placed it within Clade A (Fig. 5). Statistical support for the reciprocal monophyly and significant coalescence of *M. watsonii* with respect to the other species in the complex was high in all analyses (Figs. 3 and 4). Average corrected pairwise p-distances between *M. watsonii* and the remaining species in the *Megascops atricapilla-M. watsonii* complex were as follows: 6.7% (*M. usta*); 6.4 % (*M. stangiae*); 6.7% *(M. ater*); 6.6 % (*M. alagoensis*); and 7.1% (*M. atricapilla*).

Dark morphs of this species tend to be darker than individuals from other species in the *Megascops atricapilla-M. watsonii* complex, except for *M. ater* (see below), from which it differs mainly in back color. In some individuals of *M. watsonii* (e. g. MPEG 66635), the back is black. The darker individuals also tend to have wider and bolder underparts striping than in other species of the complex, which distinguishes them from *M. ater* dark morphs. How-

ever, as is usual within this genus, the tremendous individual variation in *M. watsonii* makes any generalization about diagnostic morphological characters impossible.

Longsong distinguishable from slower-paced longsongs of *M. stangiae* and *M. usta* and faster paced longsongs of *M. atricapilla* (Fig. 9: Tables 6 and 10). Shortsong faster-paced than in *M. usta* and *M. stangiae* (Table 8).

Megascops usta (Sclater, 1858)

Variable Screech-Owl

corujinha-relógio (Portuguese)
Scops usta Sclater, 1858 (type not examined).
Pisorhina watsonii (Cassin, 1848): Hellmayr (1907).
Otus watsonii (Cassin, 1848): Hellmayr (1910).
Pisorhina usta (Sclater, 1858): Snethlage (1914).
Otus watsonii usta (Sclater, 1858): Chapman (1928); Peters (1940); Hekstra (1982); Marks *et al.* 1999; Weick (2006; part: specimens in southernmost Venezuela and Amazonian Colombia, Ecuador, Peru, Bolivia, and Brazil between the western banks of the Negro and the lower Tapajós and upper Xingu rivers).
Otus atricapillus morelius Hekstra, 1982: Browning (1989); holotype at AMNH examined.
Otus atricapillus inambarii Hekstra, 1982: Browning (1989); holotype at FMNH examined.
Otus atricapillus fulvescens Hekstra, 1982: Browning (1989); holotype at AMNH examined.
Megascops usta (Sclater, 1858): König *et al.* (1999); König & Weick (2008; part: specimens in southernmost Venezuela and Amazonian Colombia, Ecuador, Peru, Bolivia, and Brazil between the western banks of the Negro and the lower Tapajós and upper Xingu rivers).
Megascops watsonii usta (Sclater, 1858): Dickinson & Remsen (2013); Clements *et al.* (2019); Gill *et al.* (2020; part: populations in southernmost Venezuela and Amazonian Colombia, Ecuador, Peru, Bolivia, and Brazil between the western banks of the Negro and the lower Tapajós and upper Xingu rivers).

With type locality referred as Tefé, Amazonas, Brazil (Sclater, 1858), and corresponding to specimens in Clade B, *M. usta* is distributed over a wide geographic area in Amazonia, ranging from west of the Branco-Negro rivers throughout the Imerí, Napo, Inambari, Madeira and upper stretches of the Tapajos and Xingu AOEs. Strong statistical support for reciprocal monophyly, high degree of coalescence, and high uncorrected pairwise p-distances ranging from 2.1% (*M. ater*) to 6.4% (*M. watsonii*) differentiates this taxon from others in the *Megascops atricapilla-M. watsonii* complex. Morphologically variable with multiple morphs, mainly brown or red, but also gray, and not safely distinguishable solely based on morphology from the other species in the complex. There is geographical variation in the frequency of different morphs among populations. Apparently does not include morphs as dark in color as those found in *M. watsonii* and *M. ater*. Vocally distinct from *M. watsonii, M. stangiae* and *M. atricapilla* by on average slower-paced longsongs and shortsongs (Fig. 9; Tables 6, 8, and 10).

Megascops ater (Hekstra, 1982)

Belém Screech-Owl

corujinha-de-belém (Portuguese)
Otus atricapillus ater Hekstra, 1982: Browning (1989); holotype at USNM examined.
Megascops watsonii watsonii (Cassin, 1848): Dickinson & Remsen (2013).

This taxon name (type locality Belém, Pará, Brazil) corresponds to Clade D identified herein, being restricted to the Belém AOE, east from the Tocantins River to Maranhão, Brazil. Statistical support for reciprocal monophyly and significant degree of coalescence of *M. ater* with respect to the other species in the complex was high in all analyses. Average uncorrected pairwise p-distances between *M. ater* and the other sampled lineages ranges from 2.1% (*M. stangiae*) to 6.7% (*M. watsonii*). Morphologically highly variable, with brown and dark morphs predominant. Dark morphs in *M. ater* similar to *M. watsonii* dark morphs, but with narrower ventral stripes. Vocally not safely distinguishable from any of the other species in the complex recognized herein. The proposed common name is a reference to the Area of Endemism in which this species is endemic. The Belém AOE is the most deforested sector of the entire Amazon (Silva *et al.* 2005), with deforestation still ongoing. Therefore, the conservation status of *M. ater* must be urgently evaluated, as it could already be endangered.

***Megascops atricapilla* (Temminck 1822)**
Black-capped Screech-Owl

corujinha-sapo (Portuguese)
Strix atricapillus Temminck, 1822 (type not examined).
Otus atricapillus (Temminck, 1822): Cory (1918); Peters (1940); Marks *et al.* (1999); Weick (2006).
Megascops atricapillus (Temminck, 1822): König *et al.* (1999); König & Weick 2008.

The type locality is referred to simply as "Brazil" (Hoek Ostende *et al.* 1997). This taxon corresponds to Clade F recovered herein, which is distributed in the Atlantic Forest from coastal Bahia, southern Goiás, southeastern Brazil, Mato Grosso do Sul, Paraná, and Santa Catarina through northeastern Argentina and eastern Paraguay. Strong statistical support for reciprocal monophyly and significant degree of coalescence with respect to all other species in the complex, and high average pairwise uncorrected mtDNA p-distances ranging from 1.5% (*M. alagoensis*) to 6.7% (*M. watsonii*). Morphologically as variable as the other species in the group, with a cline in body size from north to south, with southernmost individuals being heavier than northern ones, and a north-to-south cline in longsong pace, with southern individuals possessing faster longsongs than northern ones (Fig. 9). Despite this variation, vocally diagnosable from *M. usta, M. stangiae* and *M. alagoensis* by significantly faster longsong and shortsong paces (Fig. 9: Tables 6, 8, and 10). Interestingly, the fastest-paced southernmost populations of *M. atricapilla* are sympatric with *Megascops sanctaecatarinae*, whose longsong is slower-paced, suggesting another instance of character displacement (See Herzog *et al.* 2009).

Acknowledgments

We are grateful to the following institutions, which made this work possible through tissue loans and access to specimens under their care: American Museum of Natural History (Paul Sweet, Thomas Trombone); Academy of Natural Sciences of Drexel University (Nate Rice); Field Museum of Natural History (Dave Willard, Ben Marks); Kansas University Natural History Museum (Mark Robbins); Lousiana State University Museum of Natural Sciences (Donna Dittmann, J. V. Remsen, Jr., Robb Brumfield, and Fred Sheldon); Museu de História Natural Capão da Imbuia (Ligia Abe); Museu Nacional-RJ (Marcos Raposo); Museu Paraense Emílio Goeldi; Museu de Zoologia da Universidade de Sao Paulo (Luis Fabio Silveira); National Museum of Natural History (James Dean); University of Washington Burke Museum (John Klicka). The Macaulay Library, xeno-canto, Christian Andretti and Gregory Thom generously made their recordings available for this study. During data collection and analysis SMD was supported by a doctoral fellowship from "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq #142211/2009-5) and a "sandwich" PhD scholarship from CAPES/Fulbright Brazil (#BEX 3424-10-3). The laboratory work was conducted in the Pritzker laboratory in Field Museum of Natural History and in the Molecular Biology Laboratory in the Museu Paraense Emilio Goeldi (MPEG). Jake Russel (Drexel University) generously provided laboratory access for sequencing the toepad sample. This work was supported in part by grants from CNPq (#310593/2009-3, ''INCT em Biodiversidade e Uso da Terra da Amazônia'' # 574008/2008-0, # 471342/ 2011-4, and a research productivity fellowship to AA, US NSF DEB-1503804 to JDW, and NSF Dimensions US-BIOTA-Sao Paulo-DEB-1241075 to JMB). Permits for the collection of specimens were provided by IBAMA (Instituto Brasileiro do Meio Ambiente). We thank Pamela Rasmussen, and two anonymous reviewers for their criticism, which significantly improved this manuscript.

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APPENDIX. Detailed information on tissues, skins, and vocal samples of the *Megascops atricapilla / watsonii* complex analyzed in this study.

Locality code [1]	Tissue [2]	Skin [3]	Song [4]	Locality name	Latitude [5]	Longitude
A1	USNM 11476 [6]	USNM 625366	ML 134352, ML73042; ML 73045; ML 77917; ML 87589	Guyana, west bank upper Essequibo river	1,65	-58,62
A2	MPEG 66635	MPEG 66635; MPEG 66608; FMNH 101573		Brazil, Pará, Óbidos, ESEC Grão-Pará	0,63	-55,72
A3	LSUMZ 20185		XC120065; ML 59394	Brazil, Amazonas, Manaus, faz. Esteio, km 34 zf3	-2,5	-60
A4	ANSP 21937; ANSP 188291			Guyana, Potaro-Siparuni, Iwokrama Reserve	4,7	-58,73
A5		AMNH 476787; AMNH 476786	ML 59378	Venezuela, Caura.	7,38	-64,53
A6		USNM 622354; USNM 622139	ML 54362	Guyana, Parabara Savannah.	2,12	-59,22
A7		MPEG 66424	"Rebio Maicuru 5nov2008"	Brazil, Pará, Almeirim	0,93	-53,23
A8		ANSP 188289; ANSP 188291; ANSP 188292; ANSP 188290		Guyana, Iwokrama Reserve, Turtle Mountain.	4,42	-58,44
A9		USNM 625110		Guyana, Sipu river.	1,25	-58,57
A10		MNRJ 45807		Brazil, Amapá, Mazagão	0,07	-51,17
A12	ANSP 2445	ANSP 2444; ANSP 2445		"Orinoco"	?	?
A13		FMNH 260181		Surinam, Zuid River.	3,33	- 56,81
A14			ML 59358; 59359; 59369	Venezuela, 0.5 km E of river Rio Grande, El Palmar	7,97	-61,88
A15			ML 59376	"Venezuela", Rio Cuyuni + 10 km (Obs: Coordinates are from Guyana)	6,38	-58,68
A16			ML 134576	Surinam, Sipaliwini, Bakhuis Gebergte	3,66	-56,2
A17			ML 131016	Guyana, Upper Takutu-Upper Essequibo	1,42	-58,95
A18			WA583160	Brazil, Pará, Prainha	-1,79	-53,48
B1	MPEG 74094; MPEG 74096	MPEG 74094; 74096; 74097	"M usta e Mic semitorquatus"	Brazil, Pará, Santarém, RESEX Tapajós-Arapiuns.	-3,09	-55,53
B2	MPEG 62429	MPEG 62429	"Megascops Careiro"	Brazil, Amazonas, Careiro	-4,08	-60,66
B3	MPEG 71378	MPEG 71374; 71375; 71376; 71377; 71378	M usta 6; M usta 1	Brazil, Acre, Jordão	-9,19	-71,85
B4	LSUMZ B9665	LSUMZ 132015	ML 135024	Bolivia, Pando.	-11,33	-67,67

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APPENDIX. (Continued)

Locality code [1]	Tissue [2]	Skin [3]	Song [4]	Locality name	Latitude [5]	Longitude
B5	MPEG 70660; MPEG 70663	MPEG 70660; MPEG 70663; MPEG 70670	"M usta Porto Velho"	Brazil, Rondônia, Porto Velho	-8,95	-63,87
B6	MPEG 70206; MPEG 70205	MPEG 70206; MPEG 70205	"1007 Megascops usta Tanguro"	Brazil, Mato Grosso, Querência	-13,02	-52,39
B7	MZUSP 88058	MZUSP 88058; MPEG 48526	XC 20907	Brazil, Pará, Santana do Araguaia	-9,52	-50,63
B8	LSUMZ B947	LSUMZ 101691	ML 11052	Bolivia, La Paz	-15,5	-68
B9	MPEG 72556; MPEG 72557; MPEG 72619	MPEG 72556; 72557; 72619		Brazil, Amazonas, Tabatinga, north bank of Solimões River	-4,36	-69,76
B10	MPEG 72973	MPEG 72973		Brazil, Amazonas, Atalaia do Norte	-4,52	-71,56
B11	LSUMZ B2912	LSUMZ 23529		Peru, Loreto Department.	-5	-75
B12	MPEG 74006	MPEG 74006		Brazil, Pará, Jacareacanga, west bank of Tapajós river	-5,9	-57,9
B13	MPEG 70987; MPEG 70997	MPEG 70987		Brazil, Amazonas, Humaitá, west bank of Madeira River	-7,51	-63,33
B14	LSUMZ B2829	LSUMZ 23042		Peru, Loreto Department, 1km N Rio Napo	-7,59	-75,93
B15	MPEG 57930	MPEG 57930; MZUSP 38322; MPEG 15284		Brazil, Pará, Serra do Cachimbo	-9,26	-54,93
B16	MPEG 77180			Brazil, Amazonas, São Gabriel da Cachoeira (north of Negro River)	-0,13	-67,02
B17	MPEG 77825			Brazil, Amazonas, São Gabriel da Cachoeira (south of Negro River)	-0,3	-66,82
B18	MZUSP JF297; MZUSP JF754			Brazil, Amazonas, Jufari	-1,2	-61,95
B19	LSUMZ B46287			Peru, San Martin.	-6,73	-76,38
B20	MPEG 67246; MPEG 69086			Brazil, Mato Grosso, Paranaíta	-9,58	-56,71
B21	MPEG 81521			Brazil, Tocantins, Wanderlandia	-6,7	-48,12
B22	KUNHM 944			Peru, Loreto, Rio Corrientes	-2.56	-75.85

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APPENDIX. (Continued)

Locality code [1]	Tissue [2]	Skin [3]	Song [4]	Locality name	Latitude [5]	Longitude
B23		AMNH 270463; AMNH 431818	ML 59397	Venezuela, Rio Cassiquiare	2,01	-67,07
B24		ANSP 184585	ML 84479; ML 78330	Ecuador, prov. Napo	-0,2	-76,5
B25		FMNH 247145	XC35367; ML 30868; ML 33783; ML 34372; ML 29235; ML 29294	Peru, Loreto, Iquitos	-3,4	-73,15
B26		FMNH 397727; AMNH 819830	ML 11496; ML 29469; ML 132280; ML 135176; ML 75206; ML 74930	Peru, Dept. Madre de Dios	-12,67	-71,27
B27		AMNH 270462; AMNH 270460; USNM 328958		Venezuela, Duida Mountains	3,25	-65,4
B28		FMNH 248551; FMNH 248550; FMNH 248552		Colombia, Meta, La Macarena	2,07	-73,96
B29		AMNH 115739; AMNH 115738; ANSP 52222; ANSP 52221; FMNH 102979		Colombia, La Morelia, Caqueta	1,49	-75,72
B30		USNM 325915; USNM 325916		Brazil, Amazonas, Serra Imerí	0,5	-65,25
B31	FMNH 456485	MPEG 62428; MPEG 42462		Brazil, Amazonas, Japurá	-2,05	-67,26
B32		MZUSP 3592		Brazil, Amazonas, Rio Juruá	-2,37	-65,44
B33		MNRJ 4779		Brazil, Amazonas, Pinhel	-2,55	-55,14
B34		MZUSP 20354		Brazil, Amazonas, Lago do Batista	-3,18	-58,15
B35		AMNH 281397; AMNH 281392		Brazil, Amazonas, Autazes, Rosarinho, Rio Madeira	-3,7	-59,13
B36		MPEG 16790		Brazil, Amazonas, Estirão do Equador	-4,52	-71,56
B37		MZUSP 62160		Brazil, Amazonas, Rio Aripuanã	-5,07	-60,24
B38		MZUSP 84594		Brazil, Amazonas, Comunidade São Benedito, ramal Sauré	-6,55	-58,42
B39		MPEG 61994		Brazil, Acre, Porto Walter	-8,34	-72,6
B40		MPEG 63691		Brazil, Acre, Feijó	-8,46	-70,56
B41		MPEG 60463		Brazil, Acre, Floresta Estadual do Aretimary	-9,35	-68,09
B42		MPEG 63692		Brazil, Acre, Porto Acre	-9,58	-67,55

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APPENDIX. (Continued)

Locality code [1]	Tissue [2]	Skin [3]	Song [4]	Locality name	Latitude [5]	Longitude
B43		MPEG 61521		Brazil, Acre, Plácido de Castro	-10,13	-60,33
B44		MPEG 59772		Brazil, Acre, Assis Brasil	-11,06	-70,27
B45		AMNH 406865		Peru, Rio Mazán	-3,28	-73,11
B46		AMNH 238841		Peru, Alto rio Ucayali	-4,05	-73,45
B47		LSUMZ 87281; LSUMZ 84357; LSUMZ 91698; FMNH 277679; LSUMZ 75122		Peru, Dept. Amazonas	-4,35	-78,12
B48		AMNH 406864		Peru, Rio Pisqui	-7,75	-75,02
B49		LSUMZ 156207		Peru, Dept. Ucayali, w. bank Rio Shesha, ca. 65 km ENE Pucallpa	-8,38	-74,53
B50		AMNH 820912; AMNH 820913		Peru, Depto. Huanuco, rio Llulla Pichis.	-9,58	-74,85
B51		FMNH 295081; FMNH 299045		Peru, Dept. Junin	-10,5	-75,07
B52		FMNH 297888; FMNH 297889; FMNH 293363		Peru, Depto. Pasco	-10,6	-75,37
B53		LSUMZ 63961; LSUMZ 51612; LSUMZ 63959; LSUMZ 63960; LSUMZ 34023; LSUMZ 37024; LSUMZ 62155		Peru, Dept. Loreto, Río Curanja, Balta	-11	-71,83
B54		AMNH 820837; AMNH 820810		Peru, Rio Ene (at mouth of R. Quipachiari)	-11,45	-74,15
B55		FMNH 208177; FMNH 222284; FMNH 208178; AMNH 781789		Peru, Cuzco, Marcapata	-13,53	-70,9
B56		ANSP 186787		Ecuador, Prov. Sucumbios	0,33	-76,3
B57		AMNH 708675; AMNH 708677; AMNH 708676; FMNH 102527; AMNH 708674; AMNH 708678; LSUMZ 70499; LSUMZ 50293		Ecuador, Prov. Oriente	-2,06	-76,97
B58		AMNH 255106; AMNH 255102; AMNH 255105; AMNH 255193; AMNH 255104; AMNH 255107		Ecuador, Noca	-2,37	-74,08

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APPENDIX. (Continued)

Locality code [1]	Tissue [2]	Skin [3]	Song [4]	Locality name	Latitude [5]	Longitude
B59		AMNH 172969; AMNH 172971		Ecuador, near River Napo	-3,33	-76,07
B60		LSUMZ 123553; LSUMZ 168771		Bolivia, Depto. Santa Cruz	-16,15	-62,04
B61		LSUMZ 37315		Bolivia, Cochabamba, Prov. Chapare.	-16,3	-65,3
B62		AMNH 818044		Bolivia, Sta. Rosa	-17,74	-61,21
B63		MNRJ 4785		Brazil, Rondônia, Rio Jamari	-8,27	-63,27
B64		MZUSP J1633		Brazil, Rondônia, Expediçao Jirau	-9,2	-64,43
B65		MZUSP J1507		Brazil, Rondônia, Mutum	-9,6	-65,06
B66		MZUSP 65918		Brazil, Rondônia, Pedra Branca	-10,03	-62,07
B67		MPEG 36538		Brazil, Rondônia, Rio Paraíso	-10,72	-62,23
B68		MZUSP 88778; MPEG 69086; MPEG 69086; MPEG 67245; MPEG 67247; MPEG 67246		Brazil, Mato Grosso, Paranaíta	-9,58	-56,71
B69		MPEG 33870		Brazil, Mato Grosso, Matupá, Faz. S. José	-10,17	-54,83
B70		MZUSP 32311; MZUSP 32310		Brazil, Mato Grosso, Xavantina, Rio das Mortes	-11,45	-50,44
B71		MNRJ 45805; MNRJ 30560		Brazil, Mato Grosso, Jacaré	-12	-53,24
B72		MNRJ 30566; MNRJ 30565		Brazil, Mato Grosso, Garapu, Alto Xingu, MT	-13,13	-52,37
B73		MZUSP 81659		Brazil, Mato Grosso, Sapizal	-13,37	-59,01
B74		MZUSP 78048		Brazil, Mato Grosso, Vila Bela da Santíssima Trindade	-15	-59,57
B75		AMNH 34597		Brazil, Mato Grosso, Chapada	-15,26	-55,45
B76		MPEG 34663; MPEG 34664		Brazil, Pará, Conceição do Araguaia	-8,26	-49,26
B77		MZUSP 66536		Brazil, Pará, Urucurituba	-3,32	-55,3
B78		MZUSP 81757		Brazil, Pará, Jacareacanga, east bank of Tapajós river	-9,31	-56,78
B79			ML 59396	Venezuela, Amazonas, left bank Rio Negro, opposite Isla Cigarron	1,75	-67
B80			ML 112831	Brazil, Amazonas, Fazenda Sao Francisco, Terra Verde Lodge	-3,14	-60,47
B81			ML 49255	Ecuador, Morona-Santiago	-3,05	-78,05

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APPENDIX. (Continued)

Locality code [1]	Tissue [2]	Skin [3]	Song [4]	Locality name	Latitude [5]	Longitude
B82			ML 53376	Ecuador, Napo, 235.0 km E of Quito, La Selva-lodge area on Garza Cocha	-5,08	-76,4
B83			XC121151; "M usta Jacar-eacanga"; ML 35599	Brazil, Pará, Itaituba	-5,35	-57,09
B84			ML 89115; ML 89060; ML 126830	Brazil, Mato Grosso, Alta Floresta, Reserva Ecologica Cristalino	-9,49	-55,8
B85			ML 51923	Bolivia, Santa Cruz, Noel Kempff Mercado National Park, Campamento Los Fierros	-14,55	-60,93
B86		AMNH 277583		Brazil, Amazonas, Parintins	-2,36	-56,44
C1	MPEG 71331; 71332	MPEG 71331; 71332	"Indivíduo 4 coletado"; "indivíduo 5"; "Megascops usta Caxiuana Team plot 2"	Brazil, Pará, Melgaço, FLONA Caxiuanã	-1,73	-51,45
C2	MPEG 70678	MPEG 40578; 70674; 70684; 70678; MPEG 53840	XC94645	Brazil, Pará, Belterra, FLONA Tapajós	-3,04	-54,94
C3	MPEG 70632; 70627	MPEG 70632; 70647; 70627	"Megascops usta trilha bacaba 01"; ML 126692; XC22514	Brazil, Pará, Parauapebas, FLONA Tapirapé-Aquiri	-5,77	-50,5
C4	MZUSP 83559	MZUSP 83558; MZUSP 83559; MZUSP 93276		Brazil, Pará, Porto de Moz	-1,75	-52,23
C5	USNM 6991; 7020	USNM 562192; USNM 541330; USNM 562193; MZUSP 64307; MPEG 55335		Brazil, Pará, Altamira	-3,66	-52,37
C6	MPEG 70845; 70846	MPEG 70845; 70846		Brazil, Pará, Itaituba, east bank of Tapajós River	-4,28	-55,91
C7			"Megascops watsonni precious woods"	Brazil, Pará, Portel, Madeireira Precious Woods	-2,98	-50,17
C8			"Megascops watsonii Trairao"	Brazil, Pará, Trairão	-4,58	-55,94
C9			ML 126657	Brazil, Pará, Parauapebas, FLONA Carajas	-5,98	-50,32

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APPENDIX. (Continued)

Locality code [1]	Tissue [2]	Skin [3]	Song [4]	Locality name	Latitude [5]	Longitude
D1	MPEG 70268; 70433; 70434	MPEG 70267; 70268; 70433; 70434; 70435; USNM 513892; MZUSP 1983; MPEG 19786	"Megascops usta individuo 1"; "M usta Mata Pirelli 26.05.2010"; ML 59395	Brazil, Pará, Benevides	-1,43	-48,29
D2	MPEG 76937; 76957			Brazil, Maranhão, REBIO do Gurupi	-3,84	-46,72
D3		MNRJ 4782		Brazil, Pará, Cametá (municipality), Fazenda Vijará	-2,15	-49,3
D4		AMNH 430283		Brazil, Pará, Baião	-2,41	-49,41
D5		MZUSP 77127; MZUSP 77128		Brazil, Pará, Tailândia	-2,6	-48,78
D6			XC84114	Brazil, Pará, Paragominas, Cikel	-3,81	-48,38
D7			"Megascops watsonii Base 04.mp3"	Brazil, Pará, Tucuruí, Base 04, east bank of Tocantins river	-4,28	-49,43
E1	MPEG 70437; 70438	MZUSP 79948; MZUSP 79947; MPEG 70437; MPEG 70438	ML 127980; ML 127911; ML 127829	Brazil, Alagoas, Ibateguara	-9	-35,86
E2			ML 128031	Brazil, Alagoas, Murici	-9,21	-35,87
F1	MZUSP BA226	MZUSP 90983; MPEG 71820; MPEG 71819	XC46306	Brazil, Bahia, Camacan, RPPN Serra Bonita.	-15,38	-39,55
F2	MPEG 64808	MHNCI 4697; MHNCI 4846; MPEG 64808	XC99919	Brazil, Paraná, Curitiba	-25,23	-49,2
F3	KUNHM 157			Paraguay, Concepción, San Luis National Park	-22,67	-57,35
F4		MZUSP 13967		Brazil, Bahia, Rio Gongogi	-14,18	-39,25
F5		MNRJ 43651; MNRJ 4362; MNRJ 44276		Brazil, Rio de Janeiro, Ilha Grande	-23,09	-44,14
F6		MZUSP 49442		Brazil, São Paulo, Ribeirão Fundo	-21,1	-47,48
F7		MNRJ 31121; USNM 608026; MZUSP 60605		Brazil, São Paulo, Faz. Barreiro Rico	-23,17	-47,28
F8		MZUSP 61986		Brazil, São Paulo, Estação Biológica de Boracéia	-23,39	-45,54
F9		MZUSP 47577		Brazil, São Paulo, Onça Parda	-24,19	-47,51
F10		MZUSP 2426		Brazil, São Paulo, Iguape	-24,43	-47,33

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APPENDIX. (Continued)

Locality code [1]	Tissue [2]	Skin [3]	Song [4]	Locality name	Latitude [5]	Longitude
F11		FMNH 356565; MZUSP 68392; MZUSP 61741; MZUSP 61740; MZUSP 66545		Brazil, São Paulo, Icapara	-24,68	-47,42
F12		MHNCI 345		Brazil, Paraná, Vale do Ivaí	-23,18	-53,42
F13		MHNCI 3232		Brazil, Paraná, Foz do Iguaçu	-25,33	-54,35
F14		USNM 576981		Paraguay, Amamby Dept. Cerro Gora Nat. Park	-22,65	-56,19
F15			WA126698	Brazil, Bahia, Porto Seguro, Veracel	-16,38	-39,16
F16			XC85265	Brazil, Bahia, Boa Nova	-14,35	-40,21
F17			ML 113381	Brazil, Espirito Santo, Reserva Florestal de Linhares	-19,08	-39,89
F18			XC7159	Brazil, Rio de Janeiro, PN Itatiaia	-22,38	-44,63
F19			ML 94909	Brazil, São Paulo, Estação Ecológica de Caetetus	-22,41	-49,69
F20			XC91649	Brazil, São Paulo, Ubatuba, Folha Seca	-23,46	-45,17
F21			XC102229	Brazil, São Paulo, São Miguel Arcanjo, Parque do Zizo	-23,95	-48,05
F22			XC25671	Brazil, São Paulo, Miracatú, Sítio do Cervo	-24,38	-47,7
F23			XC75524	Brazil, São Paulo, Parque Estadual Intervales	-24,28	-48,42
F24			ML101565	Paraguay, Caninde, Reserva Natural del Bosque Mbaracayu	-24,12	-55,45

[1] Locality codes are the same presented in Figures 1 and 2. Letters refer to clades A – F recovered in our analyses, followed by sequencial numbers distinguishing each individual locality.

[2] Voucher specimen or tissue number(s) associated with the tissue(s) sequenced. See below for institution acronyms.

[3] Study skin number. See below for institution acronyms.

[4] Vocal sample(s) identification information. Unless associated with a public / open sound archive (see below for institution acronyms), samples are stored in private archives and are referred in quotes according to their original labels. Recordings belonging to private archives are available upon request.

[5] Latitude and longitude data were obtained directly from specimens´ labels or with the aid of the following gazetteers from South American countries inhabited by the *Megascops atricapillus / watsonii* lineages: Lorain & Traylor (1985; 1983); Paynter (1997; 1993; 1992; 1989; 1982); Paynter & Traylor (1991).

[6] Institution codes: Academy of Natural Sciences of Drexel University, Philadelphia, USA (ANSP); American Museum of Natural History, New York, USA (AMNH); Field Museum of Natural History, Chicago, USA (FMNH); University of Kansas Natural History Museum, Lawrence, USA (KUNHM), Louisiana State University Museum of Natural Science, Baton Rouge, USA (LSUMZ); Macaulay Library, Ithaca, USA (ML); Museu de História Natural Capão de Imbuia, Curitiba, Brazil (MHNCI); Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Museu Paraense Emílio Goeldi, Belém, Brazil (MPEG); Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP); Smithsonian Institution National Museum of Natural History, Washington, USA (USNM); Xenocanto, Leiden, The Netherlands (XC); Wikiaves, Brazil (WA).