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# Molecules, ecology, morphology, and songs in concert: how many species is *Arremon torquatus* (Aves: Emberizidae)?

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The acceptance of the generalized or unified concept of species (i.e. that species are segments of population lineages) implies that an important task for systematists is to focus on identifying lineages and on testing hypotheses about the acquisition of properties such as phenotypic diagnosability, reciprocal monophyly, or mechanisms of reproductive isolation. However, delimiting species objectively remains one of the most challenging problems faced by biologists. In the present study, we begin to tackle the thorny issue of species delimitation in a complicated group of Neotropical passerine birds (the *Arremon torquatus* complex, Emberizidae) in which sets of characters vary substantially across space, but do not obviously vary in a concerted fashion. To earlier discussions of species limits in the group, we add a historical perspective offered by a recent molecular phylogeny, present quantitative analyses of morphological and vocal variation, and incorporate ecological niche models as a new tool that aids species delimitation by highlighting cases of ecological distinctiveness and cases where populations appear to be in independent evolutionary trajectories, despite being connected by environments unlikely to represent barriers to gene flow. We demonstrate that at least one pair of taxa (and likely another) currently treated as conspecific are, in fact, distinct lineages that merit species status under essentially any species criterion. However, other pairwise comparisons are not as straightforward owing to nonconcordant patterns of variation in different traits and to the impossibility of distinguishing which characters are causes and which are consequences of reproductive (and evolutionary) isolation. After considering several alternatives, we propose a provisional classification of the complex recognizing eight tentative species-level taxa. Although this classification is likely to change as more detailed work is conducted, it provides a better foundation for studying the biology of these birds and helps to better describe their diversity, which is obscured when all taxa are subsumed into a single species name. The present study highlights several outstanding challenges, both practical and conceptual, for future studies.

ADDITIONAL KEYWORDS: Andes – geographic variation – ecological differentiation – neotropical birds – reproductive isolation – species concepts – species delimitation.

## INTRODUCTION

Two central questions in biology are what species are, and what criteria should be used to recognize them. Much of the heated debate on the so-called 'species problem' has resulted from failing to distinguish these two issues (de Queiroz, 1998; de Queiroz, 2005). A solution to the problem is to accept that the only necessary property of species is that they are segments of lineages at the population level (i.e. a population extended through time; Simpson, 1951), and that the multiple species definitions that have been

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*Corresponding author. E-mail: ccadena@uniandes.edu.co proposed are simply different criteria that can be used to distinguish lineages on the basis of secondary properties arising at different stages of evolutionary differentiation. Accepting this unified concept of species (de Queiroz, 2005) implies that systematists can focus on identifying lineages and on testing hypotheses about where those lineages stand in the process of differentiation by examining whether they have attained properties such as phenotypic diagnosability, reciprocal monophyly, or mechanisms of reproductive isolation. That these properties are not by themselves necessary conditions of the definition of species does not undermine their central importance in evolutionary biology (e.g. reproductive isolation; Coyne & Orr, 2004).

The best approximation to the delimitation of lineages and to testing the emergence of their secondary properties is achieved by integrating information from multiple sources, such as phenotypic, genetic, and ecological variation (Wiens & Penkrot, 2002; Sites & Marshall, 2003; Sites & Marshall, 2004; Yoder *et al.*, 2005). Wiens & Graham (2005) proposed that ecological niche models constructed based on environmental variables that are considered to shape species' geographic ranges can be used as a novel tool to inform species delimitation. Embracing the concept that species are segments of population lineages, they presented a hypothetical example involving two allopatric populations of uncertain status to describe the implications for species limits of different scenarios regarding the relationship between the climatically defined niche of each population and the projection of that niche onto geographic space. First, they proposed that populations could be considered distinct species if their climatic niches are equivalent and are separated geographically by areas outside their climatic niche envelopes that would limit gene flow. Second, they reasoned that if the two populations share similar climatic niche envelopes and their ranges are connected by areas of suitable environmental conditions, the likelihood of dispersal and gene flow would be high, which would not add support to the hypothesis that they represent different species. Finally, they proposed that if the populations have dissimilar niche envelopes, niche conservatism would maintain their geographic separation, supporting the hypothesis that they are distinct species (see also Raxworthy *et al.*, 2007; Rissler & Apodaca, 2007; Stockman & Bond, 2007; Bond & Stockman, 2008).

We agree with Wiens & Graham (2005) in that ecological niche modelling (ENM) can illuminate species delimitation, but the scenarios they described are amenable to alternative interpretations, particularly when ENM is employed in conjunction with other sources of information. Specifically, the scenario in which two populations with similar niches are connected by areas of continuous suitable environments not only fails to imply that these populations represent a single lineage, but indeed provides an ideal scenario to test the hypothesis that the populations are evolutionarily isolated from each other (Rissler & Apodaca, 2007). If allopatric or parapatric populations do not show evidence of intergradation (phenotypic intermediacy or extensive gene flow) despite the opportunity for interbreeding offered by habitat continuity, this scenario may indeed suggest they are distinct lineages evolving in isolation. On the other hand, the occurrence of populations under distinct climatic conditions in allopatry can hardly be considered evidence that they have different environmental tolerances, and thus correspond to different species (Wiens & Graham, 2005: fig. 1d). To make such a claim, one would need to demonstrate that the realized environment (i.e. the combination of environmental conditions that actually exists in a given landscape; Jackson & Overpeck, 2000) is comparable in the areas where each population occurs (Cadena & Loiselle, 2007). Otherwise, such a pattern may simply reflect that the fundamental niche of a single lineage is constrained differentially by varying environmental conditions in different parts of its geographic range (Kearney & Porter, 2004).

We propose that the most important insight that ENM brings to studies on species delimitation is that it can highlight the continuity of habitable areas in space, which in turn allows drawing on various kinds of data to test hypotheses related to the status of lineages in the process of evolutionary differentiation. Thus, we argue that a scenario in which two populations occur in areas where they could be connected by dispersal, but where individuals of each of them consistently occur under distinct climatic conditions, represents a much more compelling case illustrating how niche conservatism may maintain lineages as independent units compared to the example where populations are widely spaced. In the present study, we illustrate these ideas with a multidimensional analysis of species limits in the *Arremon torquatus* complex (Passeriformes, Emberizidae), a widespread group of Neotropical montane birds exhibiting unparalleled patterns of geographic variation and within which species delimitation has been contentious. Combining ENM with data on phylogenetic relationships, genetic differentiation, morphometrics, plumage, vocalizations, and distribution ranges, we provide a new hypothesis of species limits in the group, highlighting cases of clear-cut evolutionary distinctiveness, previously unnoticed patterns of differentiation, and several outstanding challenges, both practical and conceptual, for future studies.

We assume that species comprise segments of population lineages, but focus on assessing the status of differentiation of lineages in terms of secondary properties (*sensu* de Queiroz, 2005), particularly on reproductive isolation. From a theoretical standpoint, the origin of isolating barriers is the cornerstone of research in speciation (Coyne & Orr, 2004). Similarly, as noted by Avise & Wollenberg (1997), reproductive isolation is not just another simple property of lineages, but rather an essential component of the genetics of differentiation that results in the overt discontinuities observed in nature (Rieseberg, Wood & Baack, 2006). From a more pragmatic perspective, although consensus may be emerging regarding the ontological status of species (de Queiroz, 1998; de Queiroz, 2005), in practice, taxonomy still relies on particular contingent properties, among which reproductive isolation is one of the most prominent because it is central to the pervasive biological species criterion (BSC; Mayr, 1942; Mayr, 1963). In particular, the BSC prevails in ornithology, and has been adopted by authoritative classification committees (American Ornithologists' Union, 1998; Helbig *et al.*, 2002; Remsen *et al.*, 2009). Species lists produced by such authorities serve as baselines for most research in avian biology and conservation; thus, their reliance on the BSC influences ornithological science profoundly. Therefore, the development of new approaches that allow applying the BSC more objectively remains an important priority in systematics (Remsen, 2005). As we shall show below, models of species distributions based on niche theory, when combined with other data, can be brought to bear on situations in which the application of the BSC has been most contentious, namely those in which reproductive isolation cannot be assessed directly because members of populations of uncertain status do not occur in strict sympatry.

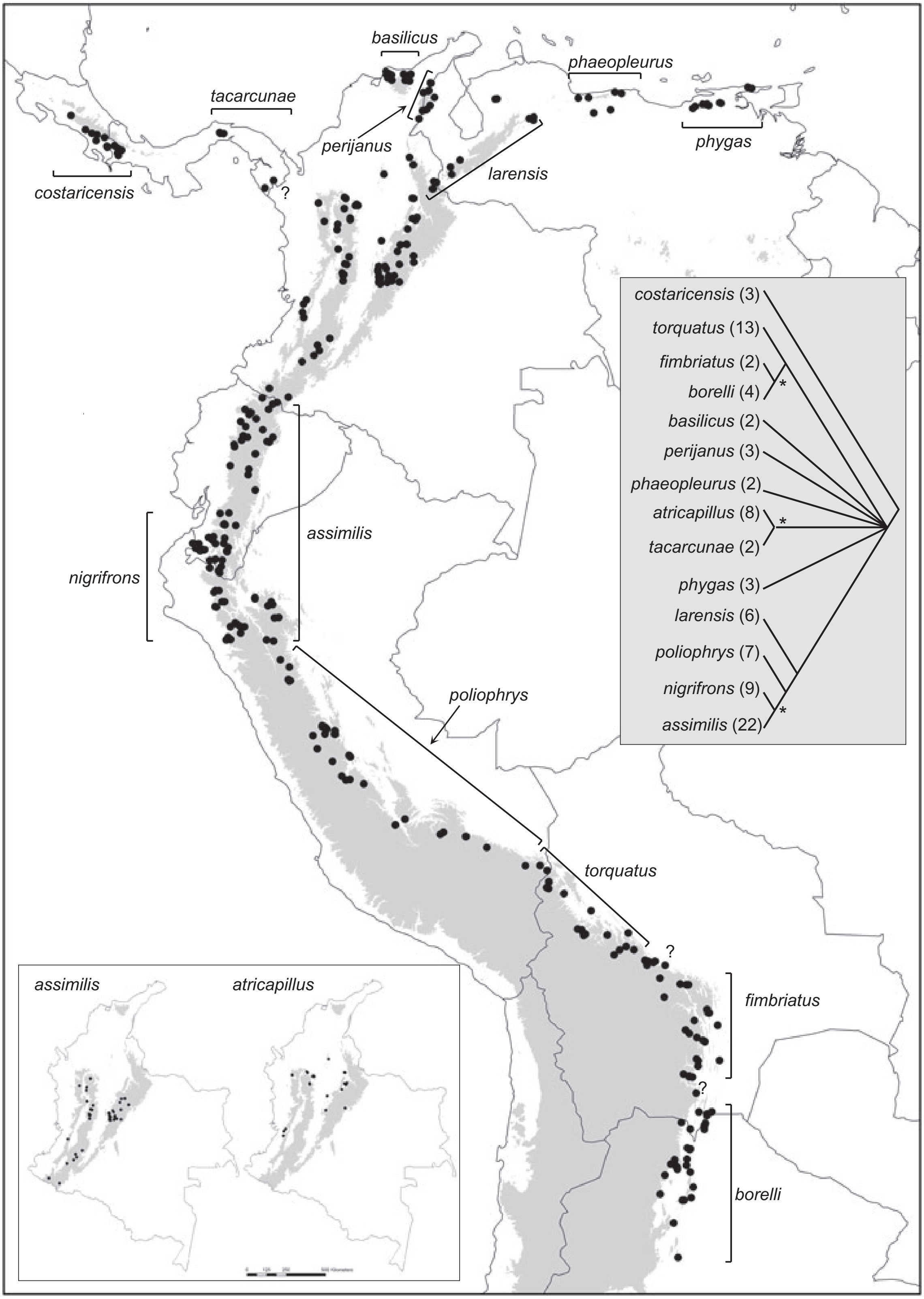
## MATERIAL AND METHODS

### STUDY SYSTEM

The *A. torquatus* complex is a clade comprising 14 taxa currently treated as subspecies (Remsen *et al.*, 2009) that occur in montane areas of the Neotropics, ranging from central Costa Rica south to northern Argentina (Chapman, 1923; Paynter, 1978) (Fig. 1). Although presently considered a single species, several studies have suggested that *A. torquatus* may comprise two and possibly more species, but the ideas of how these are to be circumscribed are conflicting (Paynter, 1978; Remsen & Graves, 1995). Much of the controversy relates to the status of the *atricapillus* group, which occurs in mid-montane areas of the three cordilleras of the Colombian Andes (*atricapillus*), eastern and central Panama (*tacarcunae*), and, according to some, Costa Rica and western Panama (*costaricensis*). Some studies have treated the *atricapillus* group as a distinct species, whereas others consider its members as subspecies of *A. torquatus*; even among those that separate *atricapillus* as a species there is disagreement because some include, and others exclude, *costaricensis*. Part of the confusion has arisen from conflicting perspectives on patterns of geographic variation in the group. The populations of eastern Panama including *tacarcunae* have been said to 'form a nearly perfectly graded series between *costaricensis* of western Panama and Costa Rica, and *atricapillus* of South America' (Wetmore, Pasquier & Olson, 1984). At the same time, however, at least some specimens of *costaricensis* are remarkably similar in plumage to South American members of the *torquatus* group (i.e. *assimilis*), and quite distinct from *atricapillus*, which has led to statements such as 'I cannot appreciate how *tacarcunae* can be considered to be intermediate between *costaricensis* and *atricapillus*. On the contrary, I find *tacarcunae* difficult to distinguish from *atricapillus* and to be well-differentiated from *costaricensis*' (Paynter, 1978). Clearly, making sense of geographic variation in plumage patterns to understand species limits in the *A. torquatus* complex has proved challenging.

Cadena, Klicka & Ricklefs (2007) described phylogenetic relationships and patterns of geographic variation in mitochondrial DNA (mtDNA) sequences in *A. torquatus* based on a total of 78 individuals

**Figure 1.** Geographic distribution of members of the *A. torquatus* complex in Central and South America. Areas above 1500 m elevation are shown in grey. Each dot indicates a site where members of the complex have been collected, tape-recorded, or reliably observed. Brackets indicate the ranges of each taxon. Unbracketed points within Colombia correspond to localities of *assimilis* and *atricapillus*, which are shown separately in the inset for clarity. Question marks indicate areas where the identity of populations is uncertain owing to lack of specimens or to the existence of possible intergrades. In southern Ecuador and northern Peru, records on the West slope of the Andes correspond to taxon *nigrifrons* and those on the east slope to *assimilis.* The inset on the right shows a simplified diagram of phylogenetic relationships among members of the complex as determined from mtDNA sequence data (Cadena *et al.*, 2007; C. D. Cadena, Z. A. Cheviron & W. C. Funk, unpubl. data). Nodes that did not receive significant bootstrap or posterior probability support are collapsed into polytomies; all relationships shown as resolved are strongly supported. Stars indicate nodes connecting pairs of taxa that are not reciprocally monophyletic with respect to each other; the number of individuals per taxon assayed for mtDNA variation is shown in parentheses.


basilicus
tacarcunae
phaeopleurus
perijanus
phygas
costaricensis
?
larensis
costaricensis (3)
torquatus (13)
fimbriatus (2)
borelli (4)
*
basilicus (2)
perijanus (3)
phaeopleurus (2)
atricapillus (8)
*
tacarcunae (2)
phygas (3)
larensis (6)
poliophrys (7)
nigrifrons (9)
*
assimilis (22)
assimilis
nigrifrons
poliophrys
torquatus
?
fimbriatus
?
borelli
assimilis
atricapillus

representing 13 of the 14 named taxa, a data set that has now been expanded with sequences for approximately ten additional individuals, including two of the originally missing taxon (C. D. Cadena, Z. A. Cheviron & W. C. Funk, unpubl. data). Their analyses indicated that *costaricensis* is the sister group of a well-supported clade formed by all other members of the complex, including *atricapillus* and *tacarcunae*, which are each other's closest relatives (Fig. 1). Relationships among major groups within this large clade were not strongly supported, but several distinct clades were recovered, with the outcome that some populations occurring in distant geographic locations are more closely allied than they are to populations occurring in close geographic proximity. For the most part, named taxa within the complex formed reciprocally monophyletic mtDNA clades, and genetic distances among many of these clades were substantial (see below). The patterns of mtDNA variation indicating long-term isolation of several populations and the phenotypic distinctiveness of several of them (Paynter, 1978) suggest that *A. torquatus* comprises more than one species. How such species are circumscribed, however, would vary according to the operational criteria used to delimit them.

The levels of genetic divergence observed among clades of *A. torquatus* may suggest more than one 'biological' species is involved because they exceed those observed between many pairs of reproductively isolated sister species of birds, both in the temperate zone and in the Neotropics (Weir, 2006; Tavares & Baker, 2008), but using only genetic distance and reciprocal monophyly of mtDNA to assess reproductive isolation is fraught with problems as a result of the weak nature of the association between time of divergence and the attainment of reproductive isolation (Price & Bouvier, 2002). However, mtDNA genealogies reflect evolutionary differentiation and provide hypotheses for species limits that can be evaluated with additional data (Templeton, 2001; Yoder *et al.*, 2005; Bond & Stockman, 2008). In the present study, we use the existing mtDNA framework to guide our discussion of species limits based on other sources of information.

We begin by focusing on two pairs of populations (i.e. subspecies in current taxonomy) in the *A. torquatus* complex that represent distinct lineages (*sensu* de Queiroz, 2005) that may have differentiated sufficiently to attain reproductive isolation. One of these pairs consists of *assimilis* and *atricapillus*, both of which occur widely across the Andes of Colombia, but have never been collected or observed at the same geographic location and generally occur at different elevations (Remsen & Graves, 1995) (Fig. 1). The second pair consists of *poliophrys* and nominate *torquatus*; these have linear and presumably non-overlapping ranges along the eastern slope of the Andes of Peru and Bolivia, although it is possible that they may be parapatric and meet in a contact zone in southern Peru (Fig. 1). These pairs of taxa are not each other's closest relatives and appear to form monophyletic mtDNA clades with respect to other taxa in the complex (Fig. 1). However, it is unclear whether this is a result of lack of opportunities for gene flow as a result of spatial disjunction of their ranges or to their status as distinct, potentially overlapping, and reproductively isolated species. Based on patterns observed in these pairs of taxa, we discuss variation in *A. torquatus* as a whole.

## ECOLOGICAL NICHE MODELLING

Ecological Niche Modelling (ENM) refers to the use of environmental (mostly climatic) data recorded at sites where species are known to occur to generate models that characterize their ecological niches in environmental space and to predict potential distribution ranges by projecting these models spatially (Guisan & Thuiller, 2005; Elith *et al.*, 2006). There has been some confusion in the literature over whether these models approximate species' fundamental niches (sensu Hutchinson, 1957), or if they only describe realized niches considering that the known occurrence sites used to construct them have already been influenced by factors that constrain the fundamental niche such as interactions, dispersal limitation, or extinction (Kearney, 2006). We favour the idea that ecological niche models can only be interpreted as characterizations of realized niches in environmental dimensions. Accordingly, cases in which models indicate two populations occur in distinct environments do not imply that each of them is unable to occur under the conditions that characterize localities occupied by the other. Alternatively, in cases in which models indicate two populations occur under the same environmental conditions, it can be concluded that their geographic ranges could potentially overlap, assuming other niche requirements are met. This inference requires assuming that locality data do not include wandering individuals occurring in sink habitats, which strictly speaking are not part of realized niches (Pulliam, 2000). Although we cannot reject this possibility, its effect at the coarse scale of our analyses is likely to be minor.

Our analyses are based on georeferenced primary occurrence data for *A. torquatus* obtained from museum specimens, published reports, and reliable field records. Details on data sources and protocols used to verify the accuracy of georeferences are provided in Cadena & Loiselle (2007).

We characterized ecological niches climatically using a set of 19 climate surfaces on a 30 arc-second resolution grid (i.e. 0.00833 decimal degrees or approximately 1 km on the side) obtained from WORLDCLIM, version 1.2 (Hijmans *et al.*, 2005). These surfaces reflect annual trends (e.g. mean annual temperature), seasonality (e.g. annual range in rainfall), and extreme conditions (e.g. temperature of the coldest month) in variables that are considered to be important in limiting geographic ranges (Hijmans *et al.*, 2005). Prior to constructing models, we reduced the 19 environmental variables to sets of uncorrelated axes of climatic variation using principal components analyses (PCA). For each pair of populations being modelled, we defined a focal region for analysis (i.e. Colombia for *assimilis* and *atricapillus*, and Peru and Bolivia for *poliophrys* and *torquatus*) and sampled environmental variation across the region by recording the values of each bioclimatic variable at 3000–6000 points placed randomly within an elevation range that encompassed all occurrence records of the taxa being modelled plus or minus 200 m. Although the range of *assimilis* extends through Ecuador and into Peru, and that of *atricapillus* into Panama, we focus in Colombia because this is where these taxa may come into contact. After standardizing climatic variables using Z-scores, we subjected the matrices to PCA (PROC FACTOR, SAS, version 9.1; SAS Institute), and selected axes describing nontrivial variation by comparing their eigenvalues to a broken-stick model. Based on the PCA eigenvector coefficients, we generated geographic information system (GIS) layers for each of the selected axes using the raster calculator in ARCMAP, version 9.0 (ESRI). These layers, consisting of grids of equal size to those of the original climate surfaces (i.e. 0.00833 × 0.00833 decimal degrees), were then used for ENM.

To generate niche models and project them onto geographic space we used the DOMAIN algorithm (Carpenter, Gillison & Winter, 1993) implemented in DIVA-GIS, version 5.2 (http://www.diva-gis.org). We chose DOMAIN over other more sophisticated methods developed to model species' distributions that have shown greater predictive accuracy (Elith *et al.*, 2006) because it produces an output that is readily interpretable in the context of ecological niche theory (Hill & Binford, 2002): the algorithm identifies sites that are potentially suitable for occurrence based on multivariate similarity to sites where the target taxa occur. We note, however, that the results reported in the present study are very similar to those obtained with a more complex modelling algorithm (MaxEnt; Phillips, Anderson & Schapire, 2006). In the present study, we consider sites to be suitable if DOMAIN models indicated their similarity to sites of known occurrence was equal or greater than 95%, but our conclusions would not change if the threshold were reduced to 90%.

### MORPHOMETRIC AND PLUMAGE VARIATION

Phenotypic traits such as bill dimensions may readily change in different environments as a result of selection or phenotypic plasticity, implying they are often of limited value as characters to assess the status of populations that occur in geographic isolation. However, patterns of variation in areas of regional sympatry or contact zones can provide insights about interbreeding that can complement inferences made from genetic variation. Accordingly, we examined differentiation in morphometrics between *atricapillus* and *assimilis* and *poliophrys* and *torquatus* based on measurements taken on museum specimens. For the former pair, whose ranges are intermingled in the Colombian Andes, we assessed diagnosability in external morphology using scatter plots to portray variation in bill length, height, and width for a total of 11 specimens of *atricapillus* (seven males and four females) and 21 of *assimilis* (12 males and nine females). If the two taxa intergrade, these measurements should overlap, especially considering that the sample for each taxon and sex includes birds from all three Colombian cordilleras (Appendix 1; see also Supporting information, Fig. S1). By contrast, a simple assessment of variation of this sort would be insufficient to assess the possibility of intergradation between *poliophrys* and *torquatus* owing to their non-overlapping ranges. Our approach to assess intermediacy between them was to conduct discriminant function analyses (PROC DISCRIM and PROC CANDISC in SAS) independently for male and female specimens using six log-transformed morphological measurements (i.e. the three bill measurements mentioned above plus tarsus, tail, and wing length), and to plot discriminant function scores as a function of latitude. Sampling for this analysis included a total of 51 specimens of *poliophrys* (28 males and 23 females), 23 of *torquatus* (12 males and 11 females), and one putative hybrid of unknown sex (see below). If *poliophrys* and *torquatus* intergrade where their ranges abut (Fig. 1), scores obtained for specimens of each taxon should resemble each other more closely in areas closer to the contact zone (i.e. localities at similar latitudes). We note, however, that analyses of morphological variation in this case cannot be conducted over a sufficiently fine spatial scale allowing for definitive conclusions (i.e. involving the putative contact zone; Appendix 1; see also Supporting Information, Fig. S1). Thus, we interpret our results conservatively in combination with other sources of information.

We did not conduct quantitative analyses of plumage coloration. However, we noted whether specimens conformed to patterns of plumage variation described for each subspecies (Chapman, 1923; Paynter, 1978), paying special attention to possible hybrids in areas of geographic contact.

### VOCAL VARIATION

Vocal characters are seldom used in taxonomy of oscine passerines because songs in these birds are learned, and therefore substantial variation often exists within species as a result of processes such as cultural transmission of local dialects (Baptista, 1996; Podos, Huber & Taft, 2004). However, we concur with Remsen (2005) in that the predisposition for learning the song of the parental population rather than other species' songs in oscines (Baptista, 1996) implies that vocalizations are at least under partial genetic control, and are thus useful for delimiting species. Indeed, it has been shown that once elements that are consistent across individuals and geography within species are identified, oscine song can reflect phylogenetic relationships closely (Price & Lanyon, 2002).

To assess the extent of differentiation in vocalizations between *atricapillus* and *assimilis* and *poliophrys* and *torquatus*, we compiled a total of 41 sound recordings, each comprising vocalizations of a different individual. We examined overall sound quality in Adobe Audition 1.5 (Adobe Systems Inc.) and generated spectrograms using SYRINX-PC (http://www.syrinxpc.com; developed by John Burt, University of Washington, Seattle). We analysed only the 24 recordings that conformed to unsolicited songs with undistorted notes that could be unambiguously distinguished from other sounds (Appendix 2). This implied that relatively few recordings were available for any given taxon, particularly for *atricapillus*, for which only three recordings were considered adequate. This limited sample size could be problematic in light of the potential for ample within-species variation in songs in oscines. However, recordings of *atricapillus* were made in three different slopes of two different cordilleras where this species co-occurs with elevational segregation with *assimilis.* Because recordings of *assimilis* from these areas are available, we assume that if these taxa differ vocally in a consistent fashion across slopes, it would be unlikely that differences are artifacts of small sample sizes and poor geographic coverage. Small sample sizes and sparse geographic coverage are more of an issue for the comparison between *poliophrys* and *torquatus*, so we interpret our results for these taxa conservatively.

An individual *A. torquatus* may sing uninterruptedly for a few minutes, repeating series of four to 14 notes (i.e. note complexes; Podos *et al.*, 2004) that vary in pace. Because the arrangement of note complexes may vary through singing, and to minimize the effects of environmental distortion (e.g. reverberation), we quantitatively examined three to five consecutive note complexes per individual recording. For each note complex, we recorded the following 14 acoustic variables: maximum frequency, minimum frequency, bandwidth (frequency range), maximum note bandwidth, minimum note bandwidth, peak frequency (frequency with the highest amplitude), duration, number of notes, note pace (number of notes divided by duration), summed note duration, note proportion (summed note duration divided by duration), maximum note duration, minimum note duration, and mean note duration. We obtained these measurements directly from spectrograms in SYRINX-PC, except for peak frequency, which we measured by generating amplitude spectra using the frequency analysis function of Adobe Audition. Although we took measurements on only three to five note complexes per song, we examined many more qualitatively to verify the consistency of patterns of variation.

To assess differences in vocalizations between pairs of taxa (i.e. *assimilis*–*atricapillus* and *poliophrys*–*torquatus*), we used univariate and multivariate analyses. First, we compared the mean of each individual song variable between taxa using *t*-tests. Second, we determined whether taxa in each pair were vocally diagnosable using discriminant function analyses based on 13 log-transformed acoustic variables (we did not include number of notes because it is the only one variable not related to frequency or time).

To examine the extent of vocal differentiation between the taxa that are the focus of this study in the broader context of variation across the whole *A. torquatus* complex, we compiled recordings of natural songs from as many localities as possible. Unfortunately, the availability of recordings for many populations occurring in distinct geographical areas is limited, which implies that quantitative analyses of the available material would be premature. Thus, in the present study, we chose to only describe the extent of vocal variation we observed across the *A. torquatus* complex by presenting representative spectrograms for different populations.

### GENETIC VARIATION

Phylogenetic relationships and general patterns of geographic variation in mtDNA were presented by Cadena *et al.* (2007) also C. D. Cadena, Z. A. Cheviron & W. C. Funk, unpubl. data, and are summarized schematically in Figure 1 (the localities for which molecular data are available are provided in the

Supporting information, Fig. S1). In the present study, we employ data from these studies to calculate levels of sequence divergence observed between different populations, and discuss the implications of phylogenetic relationships and the extent of genetic differentiation for species limits in relation to variation in other aspects.

## RESULTS

### DIFFERENTIATION BETWEEN *ASSIMILIS* AND *ATRICAPILLUS*

*Ecological differentiation*

Niche models indicate that *assimilis* and *atricapillus* occur in distinct environments. Areas of suitable habitat for *assimilis* occur extensively along the Cordillera Oriental and Cordillera Central of the Colombian Andes, whereas suitable areas are smaller and more sparsely distributed in the Cordillera Occidental, where sites at high elevations are more limited (Fig. 2). The potential distribution of *atricapillus* appears somewhat more patchy, but also extends broadly along the three cordilleras (Fig. 2). However, none of the point localities of *atricapillus* was located in areas where the model predicted presence of *assimilis* or vice versa. Indeed, the DOMAIN algorithm did not classify a single grid cell in Colombia as potentially suitable for both taxa based on climate data.

*Morphometric and plumage variation*

External morphology indicates that *assimilis* and *atricapillus* are 100% diagnosable taxa that exhibit no evidence of intermediacy. Independently of sex and of geographic location, specimens of *atricapillus* have substantially larger bills than specimens of *assimilis:* measurements of bill width, height, and length of each taxon are entirely non-overlapping (Fig. 3). Furthermore, we examined more than two hundred specimens of these taxa combined and did not encounter a single one that could not be assigned to one or the other taxon unambiguously based on the pattern of plumage pigmentation of the head: *atricapillus* has a mostly solid black head with only a few grey feathers in some specimens, whereas *assimilis* exhibits wide grey markings through much of the head.

*Vocal variation*

Vocal variation further confirms the distinctiveness of *assimilis* and *atricapillus* (Fig. 4). The songs of *atricapillus* are higher pitched and are composed of notes emitted at a faster pace than those of *assimilis*, but encompass a much narrower frequency range and are

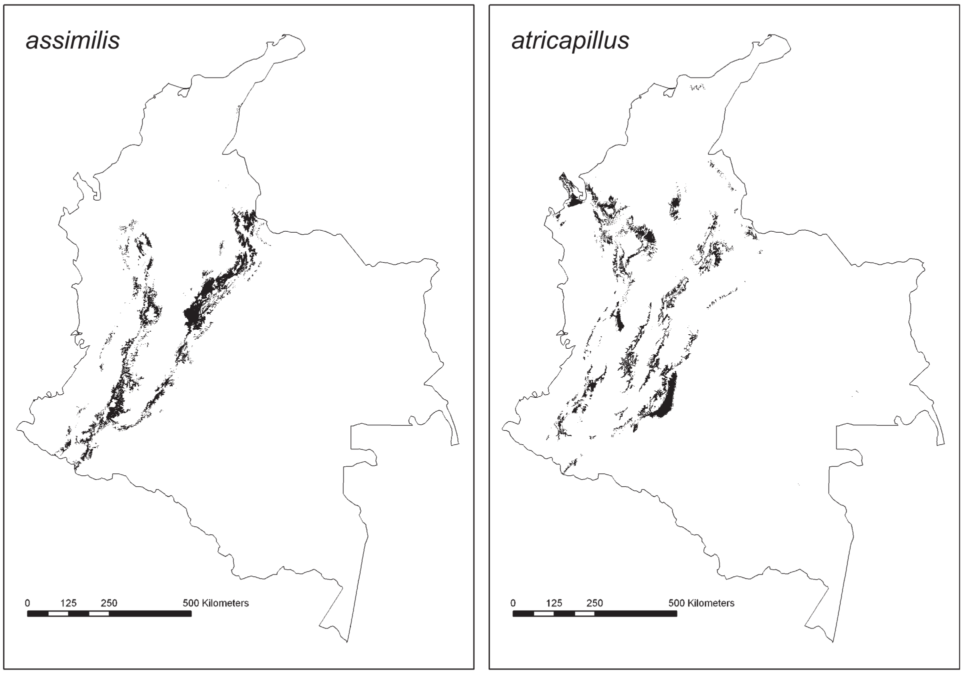


**Figure 2.** Geographic projections on maps of Colombia of ecological niche models constructed using the DOMAIN algorithm for *assimilis* and *atricapillus*. Black areas are those with DOMAIN scores ≥95%. Models were constructed on the basis of three independent climatic axes obtained from principal components analysis of 19 climatic variables. Although climatically suitable areas for both taxa are widespread in some of the same mountain ranges, they do not overlap with each other, indicating their very different ecologies.

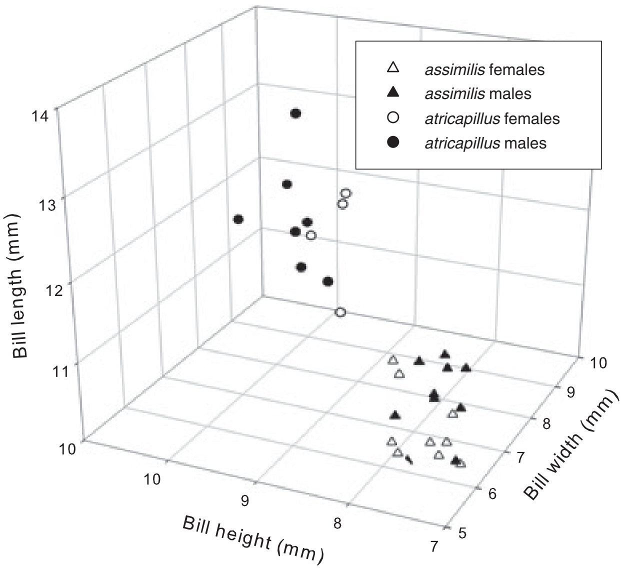


**Figure 3.** Scatter plot showing variation in three bill dimensions measured on Colombian specimens of *assimilis* and *atricapillus*. Taxa are indicated by the shape of the symbols and sex by fill patterns. Samples of specimens of both taxa included material from all three Cordilleras of the Colombian Andes. None of the bill measurements overlap, a strong indication that these taxa are diagnosably distinct and do not intergrade.

less structurally complex, exhibiting lower overall note richness and lacking notes that cover wide frequency ranges over short periods of time, which are typical of the song of *assimilis* (Table 1). Despite limited sample sizes, nine out of the 14 song variables we compared were significantly different between *assimilis* and *atricapillus* (Table 1). The discriminant function analysis indicated that vocalizations of these taxa are diagnosable: all songs were correctly classified to their corresponding taxon.

### *Genetic variation*

As indicated above (Fig. 1), *assimilis* and *atricapillus* are not each other's closest relatives: *assimilis* forms a strongly supported clade with subspecies *nigrifrons* and *poliophrys* from Ecuador and Peru, and *larensis* from northeast Colombia and Venezuela; the affinities of the *atricapillus–tacarcunae* clade are unresolved. Genetic distances between *assimilis* and *atricapillus* are substantial: the minimum observed sequence divergence was 6.3%. By contrast, the highest sequence divergence observed between individuals throughout the whole range of *assimilis* and the closely-allied *nigrifrons* was only 2.0%. The maximum distance observed within the clade formed by *atricapillus* and the closely-allied *tacarcunae* was 2.4%.

## DIFFERENTIATION BETWEEN *POLIOPHRYS* AND *TORQUATUS*

### *Ecological differentiation*

Projecting ecological niche models onto geography shows that although *poliophrys* and *torquatus* do not

**Table 1.** Mean values and standard deviations for 14 acoustic variables measured on spectrograms of songs of *assimilis*, *atricapillus*, *poliophrys*, and *torquatus*

| Song variables | *assimilis* ($N = 12$) | *atricapillus* ($N = 3$) | *t*-test | *poliophrys* ($N = 5$) | *torquatus* ($N = 4$) | *t*-test |
|---|---|---|---|---|---|---|
| Maximum frequency | 9.941 ± 0.54 | 10.200 ± 0.59 | –0.73 | 9.857 ± 0.35 | 10.364 ± 1.02 | –1.06 |
| Minimum frequency | 3.164 ± 0.64 | 6.043 ± 0.39 | –7.30*** | 3.602 ± 0.98 | 5.089 ± 0.46 | –2.77* |
| Bandwidth | 6.777 ± 0.66 | 4.157 ± 0.88 | 5.81*** | 6.255 ± 0.84 | 5.276 ± 1.23 | 1.43 |
| Maximum note bandwidth | 5.269 ± 0.94 | 3.306 ± 0.91 | 1.29** | 3.786 ± 0.74 | 3.821 ± 1.51 | –0.05 |
| Minimum note bandwidth | 0.751 ± 0.44 | 0.574 ± 0.30 | 3.24 | 0.740 ± 0.26 | 0.398 ± 0.25 | 2.01 |
| Peak frequency | 6.675 ± 1.91 | 8.150 ± 0.52 | 0.65** | 6.120 ± 1.24 | 7.920 ± 1.45 | –2.02 |
| Song duration | 4.679 ± 1.55 | 1.738 ± 1.13 | 3.05** | 4.945 ± 1.73 | 4.924 ± 2.05 | 0.02 |
| Number of notes | 8.500 ± 1.75 | 4.667 ± 2.08 | 3.30** | 7.300 ± 1.48 | 8.500 ± 1.73 | –1.12 |
| Note pace | 1.975 ± 0.56 | 2.937 ± 0.78 | –2.49* | 1.577 ± 0.49 | 1.842 ± 0.37 | –0.89 |
| Summed note duration | 1.984 ± 0.53 | 1.130 ± 0.35 | –3.38 | 1.838 ± 0.20 | 1.720 ± 0.54 | 0.46 |
| Note proportion | 0.448 ± 0.12 | 0.741 ± 0.21 | 2.63** | 0.399 ± 0.11 | 0.361 ± 0.03 | 0.65 |
| Maximum note duration | 0.376 ± 0.07 | 0.377 ± 0.05 | –0.77* | 0.389 ± 0.06 | 0.332 ± 0.04 | 1.68 |
| Minimum note duration | 0.109 ± 0.06 | 0.147 ± 0.09 | –0.03 | 0.135 ± 0.07 | 0.092 ± 0.04 | 1.09 |
| Mean note duration | 0.231 ± 0.05 | 0.256 ± 0.07 | –0.96 | 0.257 ± 0.04 | 0.199 ± 0.02 | 2.49* |

Numbers in parentheses are the number of individual recordings on which measurements were taken. For each pair of taxa, results of *t*-tests comparing means are shown, with significance levels being indicated by asterisks.
***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

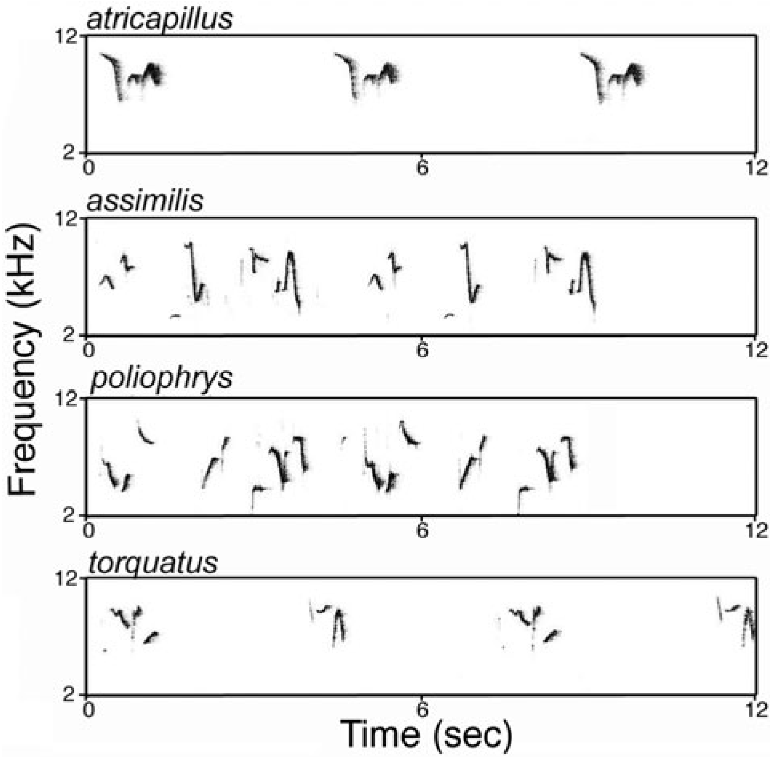


**Figure 4.** Spectrograms showing representative examples of the songs of taxa *atricapillus* (Colombia, Santander, Lebrija, Portugal; recording by J. E. Avendaño, not yet archived), *assimilis* (Colombia, Risaralda, Parque Regional Ucumarí; recording by C. D. Cadena, BSA 6780), *poliophrys* (Peru, La Libertad, E Tayabamba on Trail to Ongón; recording by T. A. Parker III, LNS 17282), and *torquatus* (Bolivia, La Paz, Franz Tamayo, Madidi National Park; recording by B. A. Hennessey, LNS 120885).

have broadly overlapping ranges, their distributions do not appear to be constrained by discontinuities in climatic niche space. Models classified fairly extensive areas of the range of *poliophrys* as climatically suitable for *torquatus* and vice versa (Fig. 5). Of the 34 point localities of *poliophrys*, 16 occurred at sites classified as suitable for *torquatus*, whereas the reverse was true for five of 26 localities. Moreover, much of the intervening area between the southernmost record of *poliophrys* and the northernmost of *torquatus* we employed for modelling was predicted to be suitable for both taxa (compare Figs 1 and 5). However, models identified a large area within the extent of the range of *poliophrys* in southern Peru (i.e. approximately 250–300 km running north-west from near the Bolivia border) where the environments are dissimilar to those under which either taxon is known to occur. This apparent gap in potential distributions corresponds to a remarkably humid area (Killeen *et al.*, 2007).

### *Morphometric and plumage variation*

Discriminant function analyses based on morphological measurements correctly classified 90% of male specimens and 94% of female specimens to their respective taxa. Plotting the discriminant function scores with respect to latitude does not reveal trends that would suggest clinal variation leading to more similar morphology near the area where the ranges of *poliophrys* and *torquatus* may abut (Fig. 6), which suggests intergradation is limited. However, because we did not have access to many specimens from near the potential area of parapatry, the morphological distinctiveness of the two taxa may not be as clear if they intergrade in a narrow contact zone (see below).

Although *torquatus* and *poliophrys* are superficially similar in plumage patterns, the two taxa are diagnosable: *torquatus* has a distinct white superciliary that is grey in *poliophrys*. Unfortunately, there are few available specimens from southern Puno Department (Peru), so material that would allow assessing intermediacy in plumage patterns in areas of possible contact is limited. Several specimens from near the Peru–Bolivia border (Abra Maruncunca) housed at LSUMZ (for museum acronyms, see Acknowledgements) are typical *torquatus* plumage types, and at least one of them has *torquatus* mtDNA (Cadena *et al.*, 2007). Also, an individual captured and photo-

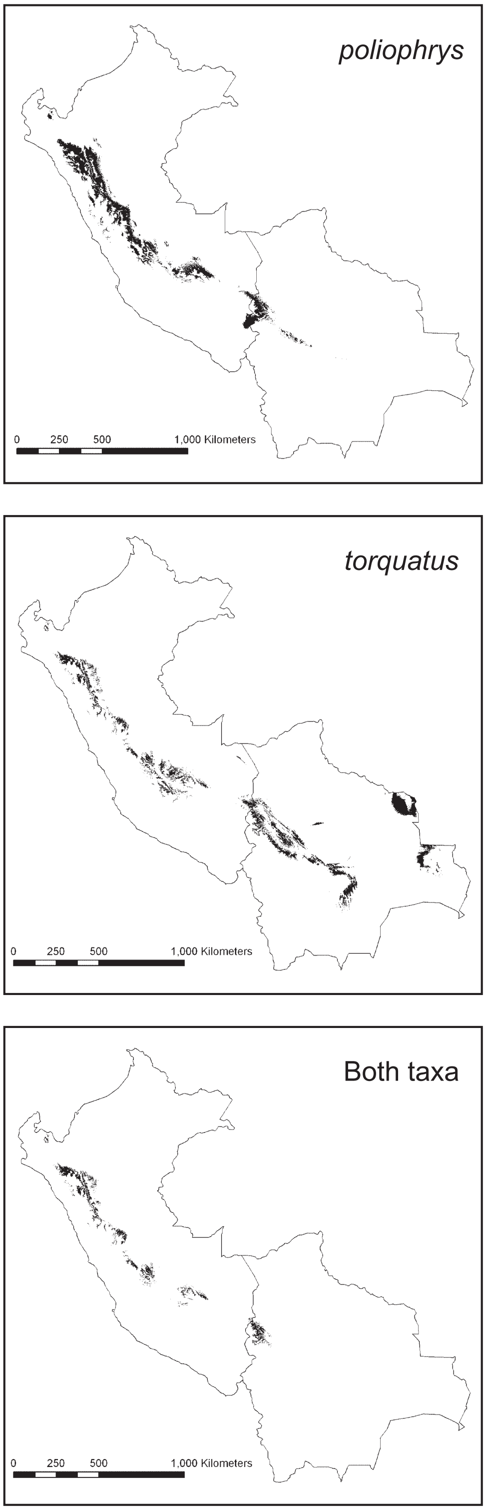


**Figure 5.** Geographic projections on maps of Peru and Bolivia of ecological niche models constructed using the DOMAIN algorithm for *poliophrys* and *torquatus*, and intersection of climatically suitable areas for both taxa. Black areas are those with DOMAIN scores ≥ 95%. Models were constructed on the basis of three independent climatic axes obtained from principal components analysis of 19 climatic variables. Climatically suitable areas for both taxa occur widely, including part of the area separating the southernmost records of *poliophrys* and the northernmost of *torquatus* (for reference, see Fig. 1).

graphed by F. Schmitt near Masiapo (Puno) is referable to *torquatus*. In turn, specimens from easternmost Cusco Department (e.g. YPM 81959) appear to be typical *poliophrys*. Collections from the intervening area are very sparse, but a specimen from Limbani, Carabaya, Puno (AMNH 520399; for geographic location, see Supporting Information, Fig. S1) is intermediate in plumage between *poliophrys* and *torquatus*, showing a mixture of grey and white feathers in the superciliary. It is possible that this bird is a hybrid, but it is not morphometrically intermediate as would be expected if this were the case because the discriminant analysis clearly classified it as *poliophrys* (Fig. 6). Thus, the possibility that this specimen represents an aberrant *poliophrys* cannot be ruled out, although we do not discard the hypothesis that *poliophrys* and *torquatus* may hybridize in a narrow contact zone. Should hybridization occur, its extent and the width of the hybrid zone are unknown. The only other specimen from this area we are aware of (MVZ 126435 from Agualani, near Limbani) is a subadult individual that seems to be a 'pure' *poliophrys*.

It is also worth noting that at least part of the superficial similarity of *poliophrys* and *torquatus* is either plesiomorphic or the result of convergence or parallel evolution of plumage patterns. Analyses reported elsewhere (C. D. Cadena, Z. A. Cheviron & W. C. Funk, unpubl. data) indicate that plumage traits are highly homoplasious in *A. torquatus*, and that characters such as the black pectoral band have been lost or gained repeatedly throughout the history of the group. In this particular case, it is readily apparent that *poliophrys* and *torquatus*, both of which exhibit collars, are closely allied to taxa that lack this trait (e.g. *assimilis* and *borelli*, respectively).

### *Vocal variation*

Vocal variation in *poliophrys* and *torquatus* is consistent with taxon designations. The vocalizations of *poliophrys* have significantly longer and lower-pitched notes than those of *torquatus* (Fig. 4, Table 1). Although many acoustic variables showed some overlap, the variability in vocal traits appeared to be higher in *poliophrys*, and maximum frequency and bandwidth tended to differ between taxa. The discriminant function analysis indicates that *poliophrys* and *torquatus* can be diagnosed

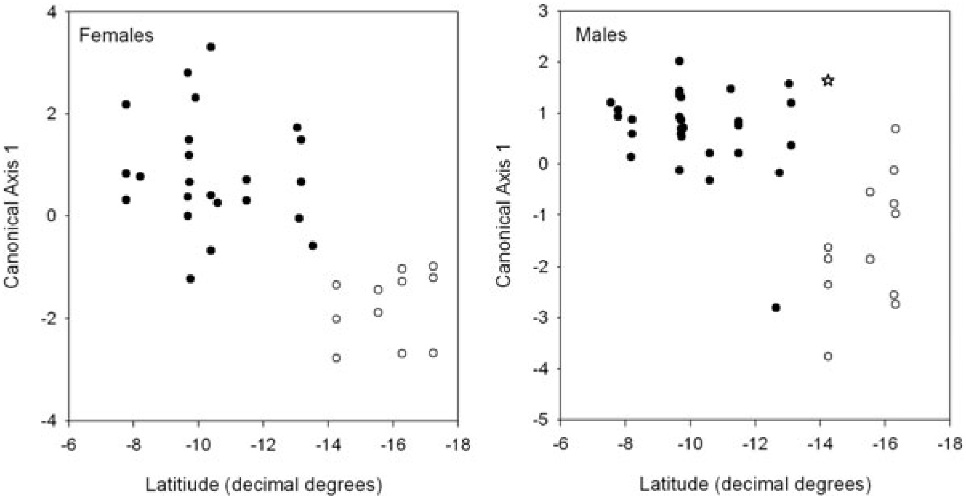


**Figure 6.** Scores obtained from discriminant function analyses based on six morphological measurements taken on female and male specimens of *poliophrys* (black dots) and *torquatus* (white dots) plotted as a function of latitude. The star indicates an individual that was presumed to be a hybrid based on plumage patterns, but appears closest to *poliophrys* morphometrically. Plots indicate most specimens can be readily assigned to their corresponding subspecies and that there are no trends that would indicate specimens are more difficult to distinguish near areas of potential contact (approximately 14° S). One male *poliophrys* that is clearly an outlier is from an isolated population (Cordillera Vilcabamba). There are no additional specimens from this locality so we cannot determine whether this represents errors in measurements or that birds in the area are in fact more similar to *torquatus*.

based on vocalizations: 100% of the songs analysed were correctly classified to their corresponding taxon. Plotting discriminant function scores with respect to latitude does not indicate that songs are more similar in areas where the ranges of both taxa are closer to each other (Fig. 7), which suggests there is no evidence for clinal variation in vocalizations over broad scales. However, the sampling conducted in the present study is not sufficiently detailed to entirely rule out this possibility at finer scales.

*Genetic variation*

Phylogenetic analyses show that *poliophrys* and *torquatus* belong to different major clades within *A. torquatus* (Cadena *et al.*, 2007). Whereas *poliophrys* is sister to a clade formed by *assimilis* and *nigrifrons*, and these three taxa are sister to *larensis*, *torquatus* belongs to a clade of unresolved affinities, within which it is sister to a clade formed by *borelli* and *fimbriatus*. Both *poliophrys* and *torquatus* appear to be reciprocally monophyletic with respect to other taxa, but additional sampling would be desirable to confirm this pattern with more certainty (Cadena *et al.*, 2007). The minimum level of sequence divergence observed between *poliophrys* and *torquatus* is 6.6% (uncorrected *p* distance). The individuals of each taxon assayed for mtDNA variation occurring in closest geographic proximity are separated by approximately 300 km, and are at least 7.5% different in mtDNA sequences. This contrasts with variation within each taxon, which reaches only 2.8% within *poliophrys* and 0.5% within *torquatus* over distances of approximately 900 and 475 km, respectively.

### VOCAL VARIATION ACROSS THE COMPLEX

The available material is too limited to allow quantitative analyses of vocal differentiation among all populations of *A. torquatus*, but the extent of vocal variation in the complex is substantial (Fig. 8). In spite of the variation, there are somewhat distinct groups of taxa with generally similar vocalizations. First, the songs of *assimilis*, *nigrifrons*, *poliophrys*, *basilicus*, *perijanus*, *larensis*, *phaeopleurus*, and *phygas* are rich in note variety, encompass a wide frequency range, and consist of notes that are evenly interspaced. The songs of these taxa are not characterized by distinct phrases, but rather by individual, highly variable notes emitted at regular intervals. Among these taxa, the songs of *phaeopleurus* and *phygas* stand out for having buzzing notes that we have not observed in any other member of the complex, and those of *perijanus* for having a lower diversity of note types. Second, the songs of *borelli*, *fimbriatus*, *torquatus*, *costaricensis*, *atricapillus*, and *tacarcunae* are overall simpler, higher pitched, and are composed of one to three alternated, distinct phrases interspersed with silent periods.

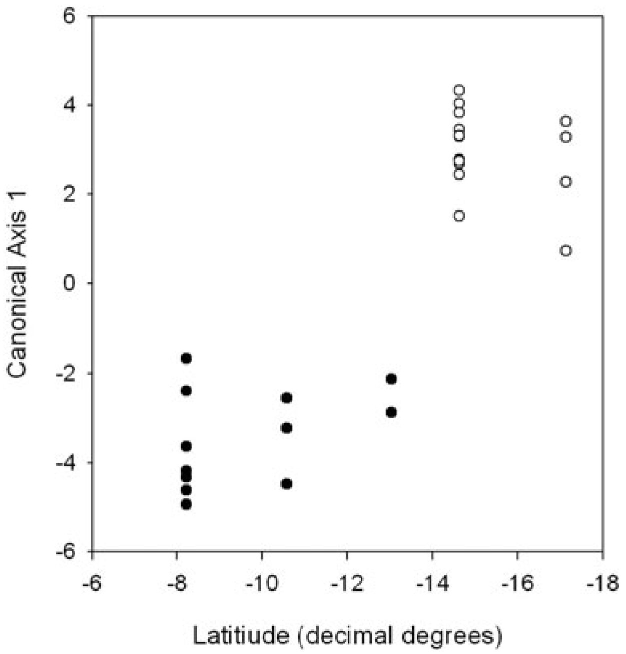


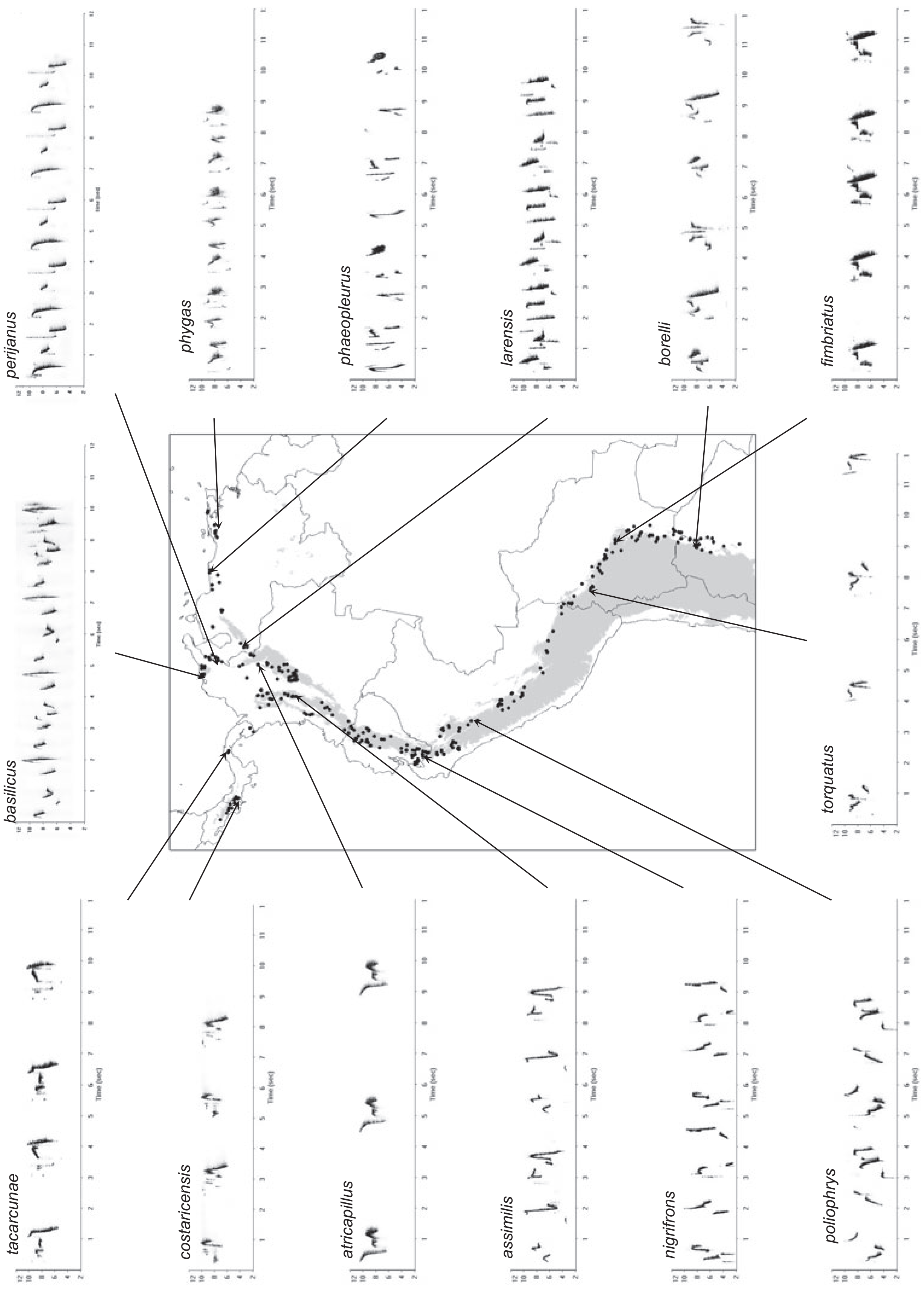
**Figure 7.** Scores obtained from discriminant function analysis based on thirteen acoustic measurements on song spectrograms of *poliophrys* (black dots) and *torquatus* (white dots) plotted as a function of latitude. More than one song is shown for several individuals (total individuals is four *poliophrys* and five *torquatus*), but the pattern and statistical results of the analysis are identical when mean values for songs of each individual are used. Plots indicate all recordings can be readily assigned to their corresponding subspecies, and although sampling is geographically sparse, they do not suggest there are trends that would indicate songs are more difficult to distinguish in areas that approach the potential contact zone more closely.

## DISCUSSION

### ARE *ASSIMILIS* AND *ATRICAPILLUS* REPRODUCTIVELY ISOLATED?

The data obtained in the present study show that *assimilis* and *atricapillus* are diagnosable taxa that show no evidence of intermediacy in external phenotype, have distinct voices, and have been evolving in isolation for a substantial time. Although it had not been previously shown quantitatively, our finding that *assimilis* and *atricapillus* are phenotypically distinct is not novel because this argument has been invoked in studies that advocate *atricapillus* being treated as a different species (Ridgely & Tudor, 1989). The possibility of phenotypic intergradation in western Colombia mentioned by Paynter (1970) can be ruled out based on our analyses. We note, however, that the relevance of the lack of intergradation between *atricapillus* and *assimilis* for addressing their taxonomic status was brought into question by

**Figure 8.** Geographic variation in vocalizations in the *Arremon torquatus* complex. A single spectrogram is shown for each taxon for which recordings are available, with arrows indicating the locality where the recording was made. Sample sizes are insufficient to determine the extent of vocal variation within taxa, but the spectrograms shown seem representative in general terms of vocalizations over the ranges of each taxon (for distributions, see Fig. 1). Note the similarity of songs from the southern extreme of the range (*torquatus*, *fimbriatus*, and *borelli*) to those of northern taxa (*costaricensis*, *atricapillus*, *tacarcunae*). Songs of other populations are much more complex, yet similar in overall structure to one another. Spectrograms show songs from the localities: *costaricensis* (Costa Rica, Puntarenas, Las Cruces Biological Station; J. R. Zook, unarchived), *tacarcunae* (Panama, Cerro Jefe; T. A. Parker III, LNS 25634), *phygas* (Venezuela, Monagas, Cerro Negro; P. Boesman, unarchived), *atricapillus* (Colombia, Santander, Lebrija, Portugal; J. E. Avendaño, unarchived), *phaeopleurus* (Venezuela, Miranda, Oripoto; P. Schwartz, LNS 67488), *assimilis* (Colombia, Risaralda, Parque Regional Ucumarí; C. D. Cadena, BSA 6780), *perijanus* (Colombia, Cesar, Manaure, San Antonio; A. M. Cuervo, xeno-canto 39901), *larensis* (Venezuela, Mérida, Chorotal, Carretera La Azulita-Mérida; D. Ascanio, unarchived), *nigrifrons* (Ecuador, Azuay, upper Yunguilla Valley, J. V. Moore, Krabbe *et al.* 2001), *poliophrys* (Peru, La Libertad, E Tayabamba on Trail to Ongón; T. A. Parker III, LNS 17282), *torquatus* (Bolivia, La Paz, Franz Tamayo, Madidi National Park; B. A. Hennessey, LNS 120885), *fimbriatus* (Bolivia, Santa Cruz, Siberia; T. A. Parker III, LNS 33643), *borelli* (Argentina, Jujuy, Parque Nacional Calilegua; J. Mazar Barnett, Mayer 2000). For references, see Appendix 2.


perijanus
phygas
phaeopleurus
larensis
borelli
fimbriatus
basilicus
torquatus
tacarcunae
costaricensis
atricapillus
assimilis
nigrifrons
poliophrys
Time (sec)

Remsen & Graves (1995), who noted that these taxa have no opportunity to intergrade because they are separated in elevational distribution, with *atricapillus* occurring at lower elevations.

ENM shows that *assimilis* and *atricapillus* indeed occur under distinct environmental conditions, which is a reflection of their different elevational distributions. This matches a scenario in which populations occur under distinct climatic regimes (Wiens & Graham, 2005: fig. 1d). These authors argued that such situations indicate that niche conservatism is likely to maintain the populations in geographic isolation and, thus, that they could be considered different species in a broad sense. We do not disagree with this interpretation (see below), but the way in which environments that appear to be suitable for the occurrence of these taxa are distributed spatially can and should inform the inference of whether populations are in the position to intergrade or not. Although not a single grid cell was classified as being sufficiently similar in climate to sites of known occurrence of both *assimilis* and *atricapillus*, many sites suitable for each of them are in very close geographic proximity (within 1–2 km of each other). In addition, our models are based on climate data from only the past 50 plus years (Hijmans *et al.*, 2005), a minute period of time relative to the time frame over which these taxa have been isolated, which probably exceeds 2–3 million years (Cadena *et al.*, 2007). Thus, in light of the history of climate change in the Colombian Andes over the Quaternary (Hooghiemstra & Van der Hammen, 2004; Hooghiemstra, Wijninga & Cleef, 2006), sites matching climatic conditions suitable for the occurrence of both taxa may have existed in the past, implying that historical opportunities for gene flow have likely been higher than what a static view of present-day climatic conditions would suggest. In sum, we argue that although conditions suitable for the occurrence of both *atricapillus* and *assimilis* may not presently occur in any 1 km$^2$ cell, and that these taxa have not been collected in syntopy, the way in which suitable environments are arrayed implies that for the practical purpose of assessing their taxonomic status, these taxa can be considered sympatric (i.e. they exhibit mosaic sympatry *sensu* Mallet, 2008). This view is supported by patterns of mtDNA variation (i.e. rather limited population genetic structure within *assimilis* and *atricapillus* across their ranges; Cadena *et al.*, 2007), which imply that dispersal over historical time frames has not been limited to small spatial scales. We also do not discard the possibility that these two taxa actually occur in syntopy. The range of *atricapillus* remains little known, and its retiring habits, local distribution, and apparently low abundance can make it difficult to detect, implying that continued fieldwork may result in finding it at sites where *assimilis* is known to occur.

In addition, the gap in elevation separating the ranges of *assimilis* and *atricapillus* described by Remsen & Graves (1995) has been bridged by recent records indicating that *assimilis* ranges down to 1800 m, whereas *atricapillus* extends to 2000 m in the same cordillera (Donegan *et al.*, 2007; O. Laverde, unpubl. data). Therefore, we suggest that that the observation that these forms generally remain segregated by elevation indicates that habitat selection likely plays a role in maintaining them as distinct entities where they occur in the same geographical area. In other words, these taxa do not appear to be ecologically interchangeable (Templeton, 1989; Stockman & Bond, 2007). The significance of patterns of habitat use stands out considering they are consistent over a wide geographical setting: both *atricapillus* and *assimilis* have been recorded in all but one of the six slopes of the Colombian cordilleras (the former is not known from the east slope of the Cordillera Occidental, which may reflect lack of historical exploration; Cuervo *et al.*, 2003). Remsen & Graves (1995) rightly pointed out that the occurrence of *atricapillus* at lower elevations is not unique in the *A. torquatus* complex, and argued that this cannot be considered a sufficient criterion to support its status as a distinct species. Certainly, the elevational range of *atricapillus* does not indicate that it is ecologically distinct from allopatric low elevation taxa, but it does serve as an important ecological mechanism isolating it from *assimilis*, the only taxon with which it is known to coexist at the landscape scale (see also Olson, Goodman & Yoder, 2004). This ecological isolating mechanism likely acts in concert with the marked differentiation in songs we observed; we hypothesize that vocalizations are likely to serve as an important mate recognition mechanism that would impede these taxa from hybridizing where their ranges may come into contact.

In sum, our analyses show unequivocally that *assimilis* and *atricapillus* are two distinct lineages evolving in isolation that have attained multiple secondary properties of species that include diagnosability in plumage, morphometrics, and vocalizations, reciprocal monophyly in mtDNA, and reproductive isolation likely mediated by habitat selection and differentiation in songs. Therefore, we suggest that these taxa be treated as different species in classifications that apply essentially any species criterion, including the BSC.

## ARE *POLIOPHRYS* AND *TORQUATUS* REPRODUCTIVELY ISOLATED?

Ecological niche models indicate environments climatically suitable for the occurrence of *poliophrys* and *torquatus* are largely continuous in the area where their ranges abut, a scenario that corresponds to the one portrayed by Wiens & Graham (2005: fig. 1c). We suggest that the continuity of climatic niche envelopes in space implies that it is unlikely that niche conservatism impedes these taxa from being sympatric, which in turn leads to the prediction that if they are not reproductively isolated, there should be evidence of gene flow and intergradation near their range boundaries. The ability of these taxa to disperse historically over areas exceeding the potential gap in their distributions is clear based on patterns of genetic variation indicating limited to no genetic structure across all of their ranges (Cadena *et al.*, 2007).

Inferences of whether *poliophrys* and *torquatus* may exchange genes to the extent that it is no longer justifiable to maintain they correspond to a single species under the BSC are somewhat tentative because relevant data are not available at the preferred very fine spatial resolution. The best approximation we have are data on morphometric and plumage variation, which suggest these taxa exhibit little to no intergradation, and that the phenotypic transition from one plumage form to the other occurs over a maximum distance of 50 km of mostly continuous habitat. Hybrid zones of some avian taxa considered 'good' species under the BSC are wider than this (Rising, 1983; Brumfield *et al.*, 2001; Carling & Brumfield, 2009), an observation that could be used to support the argument that *poliophrys* and *torquatus* demonstrate 'essential' reproductive isolation (sensu Johnson, Remsen & Cicero, 1999).

The hypothesis that *poliophrys* and *torquatus* are different species under the BSC is not free of caveats, the most significant of which are the 300-km gap in sampling of mtDNA variation that exists in southern Peru and the lack of recordings of songs from areas of possible contact. In the absence of sequence data at the same spatial resolution as the morphological data, we cannot reject the hypothesis that variation may be decoupled, and that the transition from one to another mtDNA phylogroup actually occurs within the range of the *poliophrys* phenotype. Studies on hybrid zones have shown that plumage traits may introgress asymmetrically from one lineage into another and that hybrid zones might shift in position over time, leaving a genetic wake; such processes result in discordance in the geographical position of molecular and phenotypic contact zones (Brumfield *et al.*, 2001; Krosby & Rohwer, 2009). These patterns need not occur at small spatial scales; a contact zone between highly divergent mtDNA clades in *Phaeothlypis* warblers is more than 1000 km away from the area where different plumage forms are known to hybridize (Lovette, 2004). Because the distribution of available song recordings is also sparse, the exact correspondence between plumage, vocal, and mtDNA variation in the area of potential contact cannot be established with complete certainty at this time. We believe that the possibility of molecular, phenotypic, and vocal decoupling is unlikely because differences in all of these traits are rather striking, and detailed analyses in the area might confirm that *poliophrys* and *torquatus* are reproductively isolated taxa. In addition, even if traits do not vary exactly in parallel, it is not clear to us what would be the implications for species delimitation, considering how such cases are treated in other avian taxa. For example, plumage clines in the manakin hybrid zone located in Panama are known be displaced several kilometers with respect to molecular and morphometric clines (Parsons, Olson & Braun, 1993; Brumfield *et al.*, 2001), but there appears to be general consensus about the status of *Manacus vitellinus* and *Manacus candei* as different species (but see Snow, 1979). However, it may be best to err on the side of caution, and maintain the taxonomic status of *poliophrys* and *torquatus* unchanged until the abovementioned hypothesis and also the possibility that there is clinal variation in song and mtDNA in southern Peru are ruled out (Brumfield, 2005; Isler, Isler & Brumfield, 2005).

## HOW MANY REPRODUCTIVELY ISOLATED SPECIES COMPRISE THE *A. TORQUATUS* COMPLEX?

We have demonstrated that *atricapillus* and *assimilis* on one hand, and likely *poliophrys* and *torquatus* on the other, are reproductively isolated species. The remaining challenge is to decide to which of these 'biological' species, if any, should all other members of the *A. torquatus* complex be assigned. In an effort to develop an objective framework for the application of the BSC in ornithology, Remsen (2005) suggested that allopatric populations should be treated as species if their degree of divergence is at or beyond that of taxa known to have reached reproductive isolation (Isler, Isler & Whitney, 1998; Helbig *et al.*, 2002). Accordingly, one could use the degree of differentiation attained by *assimilis* and *atricapillus* (and perhaps by *poliophrys* and *torquatus*) as an approximate yard stick to assess whether other populations may represent reproductively isolated species. We attempt to do so below, but we note at the outset that there is an important caveat to this approach, namely the problem of distinguishing causes and consequences of reproductive isolation. Although two populations reproductively isolated from each other may differ in a suite of characteristics, the significance of any of these by itself as a mechanism preventing interbreeding is unclear; it may well be that one or a few of them confer reproductive isolation, and that others diverge as a consequence of lack of gene flow. This issue is important because when comparing allopatric populations with reference to known pairs of reproductively isolated species, they may be found to be more divergent in some aspects and less divergent in others. Indeed, this is the case in *A. torquatus.*

Genetic divergence between several populations of *A. torquatus* is comparable to, and actually greater than, the divergence observed between *assimilis* and *atricapillus*, reaching values of uncorrected sequence divergence of approximately 9% (e.g. *costaricensis* versus *poliophrys*). Levels of sequence divergence within the sister group of *A. torquatus* (i.e. the traditionally defined genus *Arremon*; Cadena *et al.*, 2007), reach 11% in the same mtDNA region, but several of the reproductively isolated species of *Arremon* recognized by current taxonomy are in the approximate range of 7–9% divergence (J. Klicka, C. D. Cadena and J. L. Pérez-Emán, unpubl. data). We present these comparisons to indicate that based on what has been observed for good species within the complex and in a closely-allied group, many populations of *A. torquatus* have been isolated from each other for periods of time over which mechanisms of reproductive isolation may well have evolved. Although genetic distances are rather poor surrogates of species status under the BSC because the correlation between time of divergence and reproductive isolation is weak, such a correlation does exist (Coyne & Orr, 2004). We note, however, that even if such a correlation were tight, its application to species delimitation would remain ambiguous owing to the continuity of the degree of reproductive isolation in the transition from populations to species (Mallet, 2008).

Ecology has long been thought to play a crucial role in speciation, and the divergence of populations in allopatry to the point where they can no longer interbreed is often believed to be a byproduct of adaptive evolution resulting from varying selective pressures in different environments (Mayr, 1942). Accordingly, much of the residual variation of regressions between genetic distance (i.e. time) and measures of reproductive isolation can be accounted for by ecological differentiation (Funk, Nosil & Etges, 2006). The data obtained in the present study demonstrate that reproductive isolation may correlate with differences in ecology in *A. torquatus* based on the patterns observed in *assimilis* and *atricapillus.* Several sets of populations arguably are just as ecologically distinct as these two, the most obvious being those occurring at high-elevations (e.g. *assimilis*, *poliophrys*) and those occurring in mid-montane areas (e.g. *atricapillus*, *costaricensis*, *larensis*, *phygas*). Other patterns of ecological differentiation may not be as obvious at first glance, but become clear using ENM. For example, Cadena & Loiselle (2007) showed that the sites where *basilicus* occurs in the Sierra Nevada de Santa Marta of northern Colombia are environmentally distinct from those occupied by populations occurring in the Andes of northeast Colombia and in northwest Venezuela (i.e. *perijanus*, *larensis*). Similarly, the environments occupied by populations occurring at relatively high latitudes in Bolivia and Argentina (*fimbriatus*, *borelli*) are markedly distinct from those occupied by other members of the complex (Cadena & Loiselle, 2007).

On the other hand, ecological similarity in the environments connecting populations not known to occur in sympatry but that do not show evidence of intermediacy suggests that intrinsic barriers to gene flow may prevent them from merging into a single species. We have discussed the case of *poliophrys* and *torquatus* in some detail, but there are others. One of them is that of *assimilis* and *poliophrys*, whose ranges closely approach each other along the Peruvian Andes. These two taxa are readily diagnosable based on plumage and form distinct mtDNA clades that differ by a minimum 3.8% sequence divergence (Cadena *et al.*, 2007). The southernmost locality of *assimilis* and the northernmost of *poliophrys* are separated by approximately 70 km over which climatically suitable environments for both taxa are largely continuous (see Fig. 5 for *poliophrys*; data for *assimilis* are not shown). Another case of distinct populations that occupy similar environments in close proximity is that of *atricapillus* and the population occurring in Depto. Norte de Santander, Colombia (these birds have been historically referred to taxon *perijanus*, but mtDNA data indicate they are much more closely allied to *larensis* from the Venezuelan Andes; Cadena *et al.*, 2007). Both taxa occur on the west slope of the Cordillera Oriental of Colombia over a similar elevational range, and have been collected within 120 km of one another on this slope. They are arguably more distinct phenotypically from each other than *assimilis* and *atricapillus*, and they are divergent to roughly the same level in mtDNA and in vocalizations (see below).

Despite the substantial variation in songs that we observed among populations of *A. torquatus* (Fig. 8), patterns of vocal variation are consistent in some ways with phylogenetic relationships. Most of the taxa in the group exhibiting more complex songs form a well-supported clade (i.e. *assimilis*, *nigrifrons*, *poliophrys*, *larensis*), whereas the phylogenetic position of the other taxa with complex songs is uncertain. On the other hand, taxa with simpler songs occurring in Bolivia and Argentina (*torquatus*, *fimbriatus*, and *borelli*) and in Colombia and Panama (*atricapillus* and *tacarcunae*) also form distinct clades. It is noteworthy that vocalizations of populations from distant geographical areas (e.g. *tacarcunae* from Panama and *fimbriatus* from Bolivia) are more similar to each other than any of them are to those of intervening populations (e.g. *assimilis* from Colombia, Ecuador, and Peru). This 'leapfrog' pattern of geographic variation is pervasive in plumage patterns among Andean birds (Remsen, 1984), but, to our knowledge, it has not been reported previously for patterns of vocal variation, except perhaps for ring species (Irwin, Bensch & Price, 2001; Irwin, Thimgan & Irwin, 2008; but see also Ribot *et al.*, 2009). Two plumage traits (i.e. the presence or absence of a black pectoral band and of a white superciliary) also vary in leapfrog fashion in *A. torquatus*, but the two traits do not vary in parallel, leading to a complicated mosaic of geographic variation in plumage (Chapman, 1923; Paynter, 1978). Leapfrog variation in vocal characters is not congruent with variation in either of the two leapfrogging plumage traits (compare Fig. 8 in the present study with fig. 20 in Paynter, 1978).

Comparisons across broad geographic areas are likely to reveal morphometric variation, but whether this indicates anything about the ability of populations to interbreed is at best dubious, so we did not perform comparisons involving allopatric populations. Plumage variation is also best used in sympatry and parapatry to identify intermediate phenotypes that may have resulted from hybridization, but plumage patterns may be important for mate choice and thus be useful indicators of reproductive isolation (Uy, Moyle & Filardi, 2009). Some pairs of populations of *A. torquatus* are arguably more divergent in plumage than *atricapillus* is from *assimilis*. The main difference between these taxa is that *atricapillus* has a solid black head, whereas the head of *assimilis* has conspicuous grey stripes. An example of taxa that could be considered more divergent are *costaricensis* and *torquatus:* the former has a black head with broad grey stripes and an entirely white chest, whereas the latter has a white superciliary and a conspicuous black collar band. However, arguing that these differences could confer reproductive isolation would be premature without knowing the role of plumage signalling in mate selection. At any rate, considering that these populations occur thousands of kilometers apart, this is probably of little relevance.

In sum, our attempt to use the degree of divergence between reproductively isolated species occurring in geographic contact as a guide to making decisions about the status of allopatric populations has not taken us very far. In some aspects (e.g. divergence times, plumage), some populations are more divergent from each other than *assimilis* and *atricapillus*, but in other aspects (e.g. songs, ecology) they may be more similar. Therefore, making any recommendations regarding species status for many pairs of allopatric populations would still involve a substantial degree of subjectivity because it would require giving more importance to some traits over others without knowing which of them are the cause, and which are consequence of reproductive isolation between *assimilis* and *atricapillus*. Indeed, *poliophrys* and *torquatus* appear very likely candidates to be considered different species under the BSC, but these taxa are arguably less different from each other in several aspects than *assimilis* and *atricapillus*, indicating that differentiation in all the aspects these taxa differ is probably not required for populations to reach reproductive isolation.

## PREVIOUSLY PUBLISHED ALTERNATIVES

Because we are unable to provide a novel and satisfactory BSC-based classification, we consider treatments that have been proposed in the literature. The two views of how *A. torquatus* may be split into more than one species are to recognize (1) *atricapillus* and *tacarcunae* or (2) *atricapillus*, *tacarcunae*, and *costaricensis* as comprising members of a different species from the rest of the complex. Adopting any of these options would result in recognizing nonmonophyletic species. Although polyphyletic and paraphyletic species are not inconsistent with the philosophy of the BSC, which emphasizes interbreeding and not common ancestry (Donoghue, 1985; de Queiroz & Donoghue, 1988; Olmstead, 1995), the appropriateness of recognizing nonmonophyletic species is far from generally accepted. One of the most compelling arguments against this practice is that it implies a misrepresentation of the evolutionary units involved in the process of speciation (Cracraft, 1989; Zink & McKitrick, 1995). For the case of *A. torquatus*, even if we ignore this criticism, we see no compelling genetic, phenotypic, ecological, or vocal evidence that would support the recognition of the nonmonophyletic species circumscribed by earlier authors. For example, we find it impossible to contend that taxa as different in all aspects such as *costaricensis* and *poliophrys* belong to a cohesive, collectively evolving group (Rieseberg & Burke, 2001) to the exclusion of *atricapillus* and *tacarcunae.*

## RESOLVING THE CONUNDRUM?

There is still much to learn about patterns of variation in *A. torquatus*, particularly in potential contact zones of parapatrically distributed taxa. Also, increased availability of recordings should improve our ability to distinguish clusters of vocally distinct populations through more detailed analyses. However, achieving a complete picture of patterns of variation may still be inconclusive because some of the difficulties related to using the divergence between the only two sympatric and reproductively isolated taxa available as a yard stick to establish species status for allopatric taxa will remain. Also, it is already clear from our analyses that there will not be strict correspondence between patterns of geographic variation in plumage, morphometrics, song, and ecology; genetic distances; and phylogeny.

Clearly, the *A. torquatus* complex is no exception to the idea that species have fuzzy boundaries (Baum, 1998). However, there is no doubt, under any species criterion, that the hypothesis that *A. torquatus* is one species is untenable, which implies that a revised classification is required. Under established taxonomic practices, advocating taxonomic changes based on our analyses may appear to create turmoil because it would disturb the *status quo*, but would fail to provide an entirely resolved picture of how many reproductively isolated species comprise the *A. torquatus* complex and how are they delimited. Although we appreciate the need for taxonomic stability, we contend that a classification that highlights that we already know some populations are reproductively isolated from each other conveys much more useful information about our understanding of evolution than a classification in which all taxa are lumped because we do not know enough about the potential for interbreeding across all members of the clade. In other words, we would argue that if taxonomy is to really provide a meaningful foundation for studying the biology of these birds, a classification that explicitly states what we do know and what we do not is preferable to a stable one that for all practical purposes amounts to assuming we do not know anything.

In theory, a solution that allows incorporating what we know and what we do not into classifications is having taxonomy be consistent with the emerging view of what species are, rather than with recognizing lineages on the basis of secondary properties that arise at different stages of differentiation (de Queiroz, 2005). *Arremon torquatus* clearly comprises several species, namely segments of lineages at the population level of organization. Some of these are phenetically distinguishable, some statistically diagnosable based on morphology, songs, or ecology, some reciprocally monophyletic, and some reproductively isolated; in other words, under this view, *A. torquatus* is a collection of different kinds of species (Mishler & Donoghue, 1982). In practice, however, existing taxonomic conventions do not readily lend themselves to incorporating all this information into baseline lists used by nonsystematists, which consist only of binomials and trinomials.

To bridge the gap between a classification that emphasizes only reproductive isolation that may turn out to be objectively unworkable, even in the long run, and a novel classification scheme that would be consistent with the ontology of species (*sensu* de Queiroz, 1998) but in practice difficult to implement and communicate, we suggest that the best available alternative is to consider treating the different major clades of *A. torquatus* identified by mtDNA data as different species. We realize there are many reasons why gene trees and organismal trees may be incongruent (Nichols, 2001), that the stochasticity of the lineage sorting process compromises the use of single-locus data to delimit species (Knowles & Carstens, 2007), that reciprocal monophyly of lineages in a single locus need not imply the existence of distinct species (Doyle, 1995; Rosenberg, 2007), and that mtDNA may occasionally be a poor surrogate for differentiation in other traits and loci (Shaw, 2002; Bensch *et al.*, 2006). However, mtDNA is arguably the most appropriate marker to assess differentiation between lineages owing to its small effective population size and thereby shorter coalescence time (Zink & Barrowclough, 2008), and, aside from the resolution of phylogenetic relationships, mtDNA data indicate clearly that *A. torquatus* consists of several discrete lineages of comparable age that are in independent evolutionary trajectories. In addition, these lineages occur in different biogeographic regions, and examining other traits when there is information (e.g. song) reveals that their members have characteristics in common that may be important in maintaining them as cohesive units. Therefore, delineating lineages based on mtDNA data and biogeographic and vocal considerations is consistent with methods of species delimitation proposed by authors that favour an evolutionary species criterion (Wiens & Penkrot, 2002) and has the advantage that the recognition of nonmonophyletic species is avoided. In addition, although we did not perform detailed analyses to test for genetic and ecological interchangeability (Bond & Stockman, 2008), it is clear that the basal mtDNA lineages that we have identified are good candidates for species status based on the cohesion species criterion (Templeton, 1989). Also, although we are aware that some lineages may comprise more than one species (i.e. they certainly include diagnosable and ecologically distinct populations and group nonintergrading taxa whose ranges may be nearly parapatric), we believe it is very unlikely that members of different major lineages will be shown to be conspecific. We also note that although some lineages may appear heterogeneous in plumage patterns and members of different lineages can be difficult to distinguish, similarity in plumage is a poor surrogate of evolutionary relationships in this group (C. D. Cadena, Z. A. Cheviron & W. C. Funk, unpubl. data).

Therefore, we propose a provisional classification that recognizes eight species-level taxa: (1) *A. costa-*

*ricensis* from Costa Rica and western Panama; (2) *A. atricapillus* from central and eastern Panama and the Colombian Andes (includes *atricapillus* and *tacarcunae*); (3) *A. basilicus* from the Sierra Nevada de Santa Marta, northern Colombia; (4) *A. perijanus* from the Serranía del Perijá, northeast Colombia and northwest Venezuela; (5) *A. assimilis* from the Andes of Venezuela, Colombia, Ecuador, and most of Peru (includes *larensis*, *assimilis*, *nigrifrons*, and *poliophrys*); (6) *A. torquatus* from the Andes of extreme southern Peru, Bolivia, and Argentina (includes *torquatus*, *fimbriatus*, and *borelli*); (7) *A. phaeopleurus* from the Cordillera de la Costa, northern Venezuela; and (8) *A. phygas* from the Cordillera de la Costa Oriental, northeast Venezuela. This provisional classification should be further refined through subsequent studies.

## Conclusions

Despite the central importance of species in biology, delimiting them objectively remains one of the most challenging problems faced by systematists. In the present study, we have begun to tackle the thorny issue of species delimitation in a complicated group of Neotropical birds in which sets of characters vary substantially across space, but do not obviously vary in a concerted fashion. To earlier discussions of species limits in the group, we have added a historical perspective offered by a molecular phylogeny, have presented quantitative analyses of morphological and vocal variation, and have incorporated the new tool of ENM to highlight cases of ecological distinctiveness and cases where populations seem to be in independent evolutionary trajectories despite being connected by environments unlikely to represent barriers to gene flow. Although our provisional (and admittedly, not completely satisfactory) classification is likely to change as more detailed work is conducted particularly within some groups (e.g. *A. assimilis*), it helps to better describe the diversity of this clade, which is obscured when all taxa are subsumed into a single species name. We realize that some will prefer to see additional data before accepting in full our proposed classification scheme, but we argue there is strong evidence implying that taxonomic authorities should recognize at least two separate biological species in the *A. torquatus* complex.

Because species are the units most commonly used by biologists working in various fields, there are important implications of this increased understanding of diversity beyond systematics. For example, arguably part of the explanation for the patterns of elevational distribution of *A. torquatus* (*s.l.*) that have long puzzled ecologists and biogeographers (Remsen & Graves, 1995; Cadena, 2007; Cadena & Loiselle, 2007) is that some populations with disparate distributions are referable to different species. An improved understanding of the diversity of this group may also be of importance from a conservation standpoint because some of the newly recognized lineages (e.g., *A. perijanus*) are endemic to small areas, and may become endangered if processes of habitat degradation continue at the present pace (Fjeldså *et al.*, 2005).

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## APPENDIX 1

### SPECIMENS OF *ASSIMILIS*, *ATRICAPILLUS*, *POLIOPHRYS*, AND *TORQUATUS* INCLUDED IN MORPHOMETRIC ANALYSES. FOR GEOGRAPHIC LOCATION OF SPECIMENS SEE SUPPORTING INFORMATION (FIG. S1); FOR ACRONYMS, SEE ACKNOWLEDGEMENTS

***assimilis*** ♀(all from Colombia): FMNH 53761 (Cauca), FMNH 250069 (Cauca), FMNH 292491 (Nariño), FMNH 292492 (Nariño), IAvH 2416 (Cauca), IAvH 11698 (Antioquia), ICN 3218 (Cundinamarca), ICN 3227 (Cauca), ICN 5070 (Cundinamarca); ***assimilis*** ♂ (all from Colombia): FMNH 220629 (Cundinamarca), FMNH 250068 (Nariño), FMNH 292489 (Nariño), IAvH 2337 (Antioquia), IAvH 2470 (Cauca), IAvH 7591 (Cauca), IAvH 11681 (Cundinamarca), IAvH 11696 (Risaralda), ICN 3220 (Cundinamarca), ICN 21671 (Boyacá).

***atricapillus*** ♀(all from Colombia): ICN 16249 (Cundinamarca), ICN 26579 (Valle del Cauca), ICN 27218 (Antioquia), ICN 28442 (Valle del Cauca); ***atricapillus*** ♂ (all from Colombia): IAvH 11697 (Antioquia), ICN 16248 (Cundinamarca), ICN 26302 (Valle del Cauca), ICN 27219 (Antioquia), ICN 28395 (Valle del Cauca), ICN 33290 (Santander), MCZ 158955 (Boyacá).

***poliophrys*** ♀(all from Peru): FMNH 59977 (Huánuco), FMNH 311829 (Cusco), FMNH 282636 (Huánuco), FMNH 283754 (Huánuco), LSUMZ 64704 (Huánuco), LSUMZ 74805 (Huánuco), LSUMZ 74810 (Huánuco), LSUMZ 74815 (Huánuco), LSUMZ 79470 (Cusco), LSUMZ 79475 (Cusco), LSUMZ 79476 (Cusco), LSUMZ 106547 (Pasco), LSUMZ 127832 (Junín), LSUMZ 127834 (Junín), LSUMZ 129073 (Pasco), LSUMZ 129075 (Pasco), LSUMZ 129076 (Pasco), LSUMZ 129077 (Pasco), MUSM 5750 (La Libertad), MUSM 18355 (San Martín), MUSM 18356 (San Martín), MUSM 18362 (San Martín), MUSM 23204 (Cusco); ***poliophrys*** ♂ (all from Peru): AMNH 820439 (Cusco), AMNH 820784 (Ayacucho), FMNH 59978 (Huánuco), FMNH 282635 (Junín), FMNH 283753 (Huánuco), FMNH 299734 (Cusco), LSUMZ 64707 (Huánuco), LSUMZ 72929 (Huánuco), LSUMZ 74806 (Huánuco), LSUMZ 74807 (Huánuco), LSUMZ 74814 (Huánuco), LSUMZ 74816 (Huánuco), LSUMZ 75441 (Huánuco), LSUMZ 79469 (Cusco), LSUMZ 79471 (Cusco), LSUMZ 81076 (Huánuco), LSUMZ 93190 (La Libertad), LSUMZ 104556 (San Martín), LSUMZ 106541 (Pasco), LSUMZ 106542 (Pasco), LSUMZ 127831 (Junín), LSUMZ 127833 (Junín), LSUMZ 127835 (Junín), LSUMZ 129072 (Pasco), MUSM 4790 (La Libertad), MUSM 4812 (La Libertad), MUSM 18358 (San Martín), MUSM 18359 (San Martín).

***torquatus*** ♀: FMNH 183212 (Bolivia, Cochabamba), FMNH 183213 (Bolivia, Cochabamba), FMNH 183214 (Bolivia, Cochabamba), LSUMZ 90968 (Bolivia, La Paz), LSUMZ 90969 (Bolivia, La Paz), LSUMZ 90970 (Bolivia, La Paz), LSUMZ 96813 (Bolivia, La Paz), LSUMZ 96814 (Bolivia, La Paz), LSUMZ 98703 (Peru, Puno), LSUMZ 98705 (Peru, Puno), LSUMZ 98706 (Peru, Puno); ***torquatus*** ♂: LSUMZ 90966 (Bolivia, La Paz), LSUMZ 90967 (Bolivia, La Paz), LSUMZ 90971 (Bolivia, La Paz), LSUMZ 96815 (Bolivia, La Paz), LSUMZ 96816 (Bolivia, La Paz), LSUMZ 98702 (Peru, Puno), LSUMZ 98704 (Peru, Puno), LSUMZ 98707 (Peru, Puno), LSUMZ 98709 (Peru, Puno), LSUMZ 102941 (Bolivia, La Paz), LSUMZ 102942 (Bolivia, La Paz), LSUMZ 102943 (Bolivia, La Paz).

## APPENDIX 2

RECORDINGS OF SONGS OF *ASSIMILIS*, *ATRICAPILLUS*, *POLIOPHRYS*, AND *TORQUATUS* USED IN QUANTITATIVE ANALYSES. ACRONYMS FOR SOUND ARCHIVES (BSA, BANCO DE SONIDOS ANIMALES, INSTITUTO ALEXANDER VON HUMBOLDT, VILLA DE LEYVA, COLOMBIA; LNS, LIBRARY OF NATURAL SOUNDS, CORNELL LABORATORY OF ORNITHOLOGY, ITHACA, NY, USA)

| Taxon | Source | Recordist | Locality | Latitude | Longitude |
|---|---|---|---|---|---|
| *assimilis* | BSA 6324 | C. D. Cadena | Colombia, Cundinamarca, Parque Natural Chicaque | 4.6087 | –74.3066 |
| *assimilis* | BSA 6338 | C. D. Cadena | Colombia, Cundinamarca, Bojacá, Finca Macanal | 4.6625 | –74.3458 |
| *assimilis* | BSA 6773 | C. D. Cadena | Colombia, Risaralda, Parque Regional Ucumarí | 4.7214 | –75.4685 |
| *assimilis* | BSA 6778 | C. D. Cadena | Colombia, Risaralda, Parque Regional Ucumarí | 4.7214 | –75.4685 |
| *assimilis* | BSA 6780 | C. D. Cadena | Colombia, Risaralda, Parque Regional Ucumarí | 4.7345 | –75.4621 |
| *assimilis* | BSA 6804 | C. D. Cadena | Colombia, Risaralda, Parque Regional Ucumarí | 4.7088 | –75.4901 |
| *assimilis* | BSA 7697 | S. Córdoba – M. Alvarez | Colombia, Caldas, Aranzazu, Vereda El Laurel | 5.2225 | –75.4883 |
| *assimilis* | Krabbe *et al.* (2001) CD 4, #5 | N. Krabbe | Ecuador, Chimborazo, Orregán | –1.6500 | –78.5000 |
| *assimilis* | Krabbe and Nilsson (2003), #14 | J. Nilsson | Ecuador, Napo, Pass of Cordillera Guacamayos | –0.6125 | –77.8292 |
| *assimilis* | Krabbe and Nilsson (2003), #17 | N. Krabbe | Ecuador, Imbabura, Apuela Road | 0.3458 | –78.4375 |

APPENDIX 2 *Continued*

| Taxon | Source | Recordist | Locality | Latitude | Longitude |
|---|---|---|---|---|---|
| *assimilis* | Krabbe and Nilsson (2003), #18 | N. Krabbe | Ecuador, Napo, 3-5 km below Oyacachi | –0.2125 | –78.0375 |
| *assimilis* | Krabbe *et al.* (2001) CD 4, #1 | J. V. Moore | Ecuador, Pichincha | –0.1000 | –78.2833 |
| *atricapillus* | Not archived or published | J. Avendaño | Colombia, Santander, Lebrija, Portugal | 7.1625 | –73.2792 |
| *atricapillus* | BSA 6815 | C. D. Cadena | Colombia, Antioquia, Don Matías, Estación Pradera | 6.5292 | –75.2625 |
| *atricapillus* | Not archived or published | O. Laverde | Colombia, Santander, San José de Suaita | 6.1875 | –73.4292 |
| *poliophrys* | LNS 17258 | T. A. Parker III | Peru, La Libertad, E Tayabamba, on trail to Ongón | –8.22083 | –77.1958 |
| *poliophrys* | LNS 17282 | T. A. Parker III | Peru, La Libertad, E Tayabamba, on trail to Ongón | –8.22083 | –77.1958 |
| *poliophrys* | LNS 35950 | T. S. Schulenberg | Peru, Pasco, Oxapampa, Cumbre de Ollón | –10.5792 | –75.2958 |
| *poliophrys* | LNS 36006 | T. S. Schulenberg | Peru, Pasco, Oxapampa, Cumbre de Ollón | –10.5792 | –75.2958 |
| *poliophrys* | LNS 24051 | T. A. Parker III | Peru, Cusco, Canchaillo, below (N) Abra Málaga | –13.1167 | –72.3667 |
| *torquatus* | Mayer (2000), #2 | S. Herzog | Bolivia, Cochabamba – Villa Tunari Rd, Carrasco NP | –17.1375 | –65.5792 |
| *torquatus* | LNS 120885 | A. B. Hennessey | Bolivia, La Paz, Torcillo-Sarayoj; Madidi NP | –14.5958 | –68.9458 |
| *torquatus* | LNS 120922 | A. B. Hennessey | Bolivia, La Paz, Torcillo-Sarayoj; Madidi NP | –14.5958 | –68.9458 |
| *torquatus* | LNS 121717 | A. B. Hennessey | Bolivia, La Paz, Tokoaque; Madidi NP | –14.5958 | –68.9458 |

## SUPPORTING INFORMATION

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

**Figure S1.** Maps showing the locations from which molecular data have been collected for all members of the *Arremon torquatus* complex (A) and from which morphometric (B) and vocal (C) measurements were taken for analyses involving *assimilis*, *atricapillus*, *poliophrys*, and *torquatus*. The star in (B) indicates the geographic location of the putative *poliophrys* × *torquatus* hybrid from Peru described in the text.