Cryptic diversity in a widespread North American songbird: Phylogeography of the Brown Creeper (*Certhia americana*)

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ABSTRACT

The identification of species via morphological characteristics has traditionally left cryptic species undescribed in taxa under selection for morphological conservation (or a lack of selection for morphological change). Treecreepers (Genus: *Certhia*) have a conserved morphological appearance, making it difficult to ascertain relationships in the genus based on morphology alone. Recent genetic and song structure studies of Eurasian Treecreepers identified cryptic species within Old World *Certhia* that were previously undescribed using morphological characteristics. Here, we use mtDNA to investigate cryptic diversity and patterns of diversification in the Brown Creeper (*Certhia americana*), the single described *Certhia* species in the Americas. Phylogenetic analyses identified six well-supported geographically-structured clades; the basal divergence separates a northern and a southern lineage in the Brown Creeper, likely cryptic species previously characterized as many subspecies. Sympatry is prevalent between clades in western North America, where possible contact zones warrant further investigation. Allopatry appears to be the primary driver of deep phylogeographic structure within the Brown Creeper; however, within clade diversity is highly correlated with the life history traits of the populations that comprise the geographically structured phylogroups.

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1. Introduction

Cryptic species, two or more distinct species classified under one species name, have traditionally been a taxonomic problem due to the classification of species primarily using morphological characteristics. The introduction of molecular techniques, such as PCR and increasingly inexpensive DNA sequencing, has led to widespread use of DNA sequence divergence for detecting and differentiating morphologically similar species. A review by Bickford et al. (2007) found that along with the advent of molecular techniques, the description of cryptic species has increased exponentially over the past two decades, including cryptic species from most types of organisms and habitats.

Mayr (1963) wrote that cryptic species 'are apparently rarest in organisms such as birds that are most dependent on vision in the role of epigamic characters.' However, many bird species are morphologically conserved, without elaborate epigamic coloration. In these taxa, species boundaries may also be influenced by population densities, behaviors and non-visual mating cues, such as mating calls. For example, Podos (2010) found evidence that

discrimination via acoustic cues is serving as a behavioral mechanism for assortative mating and sympatric evolutionary divergence in one of Darwin's finches (*Geospiza fortis*). This pattern has been identified in species delimitation of Tyrannidae flycatchers and *Phylloscopus* leaf-warblers, where song and/or DNA are better indicators of species delimitation than morphology alone (Chaves et al., 2008; Olsson et al., 2005; Rheindt et al., 2008).

A morphologically conserved avian lineage known to contain cryptic species is the widespread genus *Certhia*. *Certhia* treecreepers have an overall drab appearance, with brown and white streaked plumage, curved tweezer-like bills and stiff tails that are used as props while ascending trees. The lack of distinctive morphological characteristics between *Certhia* species makes it difficult to ascertain relationships within the genus (Tietze and Martens, 2009). This suggests that *Certhia* species are under selection for morphological stasis (or a lack of selection for morphological change), and that these cryptic species are only easily differentiated with non-morphological characteristics.

Eurasian treecreepers traditionally consisted of six morphologically similar species, with similar niches and several living in sympatry in areas of Europe and the Himalayas. A recent study of the genus that analyzed mitochondrial DNA (mtDNA) and song structure (Tietze et al., 2006) affirmed the delimitation of these six species with the additional identification of two morphologically

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cryptic populations exhibiting strong genetic differentiation which were subsequently raised to species status (*Certhia hodgsoni* and *Certhia manipurensis*). These two new species were differentiated via genetics, song structure and geography, rather than morphology, suggesting the possibility of cryptic diversity elsewhere within the genus *Certhia*.

The Brown Creeper (*Certhia americana*) is widespread throughout North America in mature, old-growth coniferous and mixed coniferous–deciduous forests and is the sole representative of the family Certhiidae in North America. Currently considered one biological species (AOU, 1983), the Brown Creeper exhibits clinal variation in coloration and size with up to 13 recognized subspecies (Unitt and Rea, 1997; Webster, 1986; see Fig. 1). The variation among subspecies is generally slight, with overlap among populations in wing-chord, bill, and tail length (Webster, 1986), causing most subspecies to be identified by geographic region. Even in museum specimen measurements, only 66.9% of individuals can be assigned to the appropriate subspecies (Tietze and Martens, 2009). However, the largest differences in morphology (overall body size and darkness of coloration) occur between northern and southern subspecies where northern subspecies are larger and lighter than southern subspecies.

Throughout much of its range, the Brown Creeper is largely sedentary or a local migrant (Phillips et al., 1964; Stupka, 1963), while eastern and northern subspecies (*alascensis*, *americana* and *nigrescens*) are strongly migratory (Webster, 1986). Creepers exhibit short natal dispersal (Davis, 1978), high fidelity to breeding sites (*Certhia familiaris*; Cramp and Perrins, 1993), and have been shown to form local dialects over very short distances (Baptista and Krebs, 2000), suggesting that Brown Creeper populations may be prone to geographic and genetic structuring. Previous studies of Eurasian treecreepers and the life history of the Brown Creeper indicate

the potential for cryptic diversity within the latter; however, no large-scale genetic or song studies have looked at the diversity within the Brown Creeper as a whole. To explore intraspecific variation in the Brown Creeper, we investigated its mtDNA phylogeographic structure throughout its North and Central American distribution. The goals of this study are: (1) Construct a robust intraspecific mtDNA phylogeny for the Brown Creeper, which will help infer population relationships and investigate cryptic diversity. (2) Examine the patterns of diversification in this species in relation to the regional North American forests and fauna (e.g. *Sitta carolinensis*; Spellman and Klicka, 2007). (3) Examine finer scale population structure within clades. Because of the life history of the Brown Creeper and the formation of local dialects of song, populations may be genetically structured even at a local scale.

2. Methods

2.1. Sampling and laboratory procedures

Tissue samples of 341 Brown Creeper individuals were obtained from across the species' range during the breeding season, including 50 localities that represent all but one (*C.a. stewarti*, resident of Queen Charlotte Island, British Columbia, Canada) of the recognized subspecies (Unitt and Rea, 1997; Webster, 1986; Fig. 1, Table 1). Seven samples of the Old World forms *Certhia brachydactyla* and *C. familiaris* were used as outgroup taxa for phylogenetic analyses.

Total genomic DNA was extracted from all tissue samples using a QIAGEN DNeasy tissue extraction kit following the manufacturer's protocol. The complete sequence of the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene was obtained using polymerase chain reaction (PCR) amplification with the primers L5215 (Hackett, 1996) and H6313 (Johnson and Sorenson, 1998). PCR amplification of all sequences was carried out in 12.5 μL reactions and included an initial denaturation period of 10 min at 95 °C, with 40 subsequent cycles of 94 °C for 30 s, 54 °C for 45 s, and 72 °C for 1 min. This was followed by a 10 min extension at 72 °C and 4 °C soak. PCR products were purified using Exosap-IT (USB Corporation) and sequenced using 20 μL ABI BigDye (Applied Biosystems) sequencing reactions. Sequencing reactions were purified using a magnetic bead clean-up (Agencourt Biosciences) followed by sequencing on an ABI 3130 Genetic Analyzer. Complementary sequences were aligned using Sequencher 4.8 (GeneCodes).

2.2. Phylogenetic analyses

Maximum likelihood (ML) and Bayesian phylogenies were constructed to identify major clades and evaluate relationships among haplotypes. With the outgroup sequences removed, TOPALi v2.5 (Milne et al., 2009) was used to select the model of sequence evolution that best fit the sequence data (TrN + G; AIC = 10344.79). Maximum likelihood analyses were performed using a PhyML analysis (Guindon and Gascuel, 2003) in TOPALi v2.5 (Milne et al., 2009) with 1000 bootstrap replicates. Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Two independent runs of three Markov chains were run for 10 million generations and sampled every 100 generations. In both independent runs, the chains stabilized after 2,000,000 generations; thus, the first 25,000 trees were discarded and a consensus topology was obtained from the last 75,001 trees in the chain.

2.3. Estimates of mtDNA divergence time

BEAST v1.4.8 (Drummond et al., 2006; Drummond and Rambaut, 2007) was used with a relaxed lognormal molecular clock to
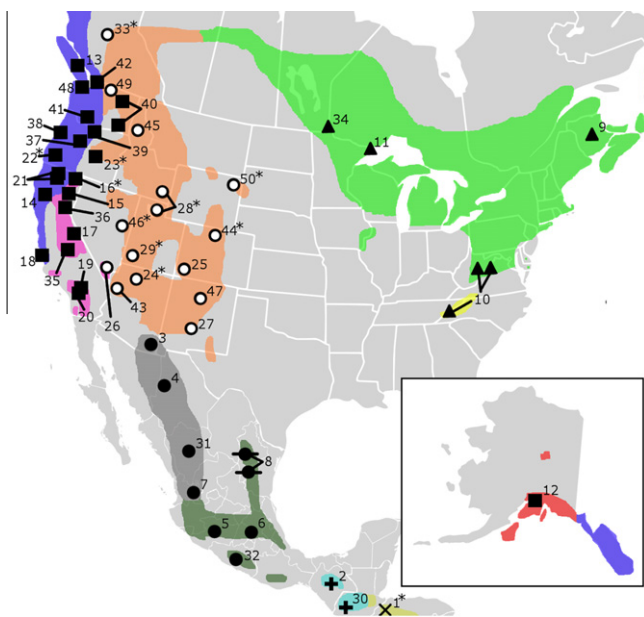
Fig. 1. Breeding distribution of subspecies and sampling localities of the 341 samples of Brown Creeper. Numbers correspond to the population labels in Tables 1 and 3. Symbols indicating position represent the assigned clade in the phylogeny (Fig. 2). Populations with asterisk (∗) indicate populations with individual haplotypes assigned to multiple clades (see Table 1). Different colors correspond to approximate breeding distribution of subspecies: (1) *alascensis* (red); (2) *occidentalis* (violet); (3) *zelotes* (magenta); (4) *montana* (orange); (5) *americana* (lime green); (6) *nigrescens* (yellow); (7) *albescens* (dark gray); (8) *alticola* (forest green); (9) *pernigra* (cyan); (10) *extima* (gold). *C.a. montana* range includes ssp. *leucosticta* and *C.a. occidentalis* includes ssp. *phillipsi*; in both instances ranges overlap. ssp. *nigrescens* overlaps with *americana* in eastern US (not shown). *C.a. stewarti*, resident of Queen Charlotte Island off of British Columbia, is not shown.