

The Phylogeny of New World Chickadees in the family Paridae

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Biology

Introduction

Paridae, the tits and chickadees, are a widespread family of Passerine birds, ranging throughout the Holarctic as well as the Old World tropics (Gosler and Clement, 2007). Tits and chickadees share a number of features across the family; all species are seed eaters or insectivorous, and all nest in cavities (Gosler and Clement, 2007). They are found in a wide range of environments and some species are year-round residents at high latitudes such as the Boreal Chickadee in northern Canada in the Azure Tit in Scandanavia (Gosler and Clement, 2007). Due to their abundance, boldness, and adaptability to man-altered environments, avian research has frequently focused on members of Paridae such as the Great Tit and Blue Tit. The Great Tit's propensity to nest in manmade boxes has made it a model for studying breeding biology, including differences in male reproductive success (Lambrechts and Dhondt, 1986), variation in timing of breeding (Verhulst et al., 1995), inbreeding (Greenwood et al., 1978), natural fitness variation (McCleary et al., 2004), nest ectoparasites (Christe et al., 1996), and differential contributions from sexes in feeding young (Sanz et al., 2000). The Blue Tit was one of the first passerines discovered to have ultraviolet colors in its plumage (Hunt et al., 1998). At Villanova University, a hybrid zone between the Black-capped Chickadee and Carolina Chickadee has been extensively studied as a model for avian tension zones and selectivity against hybrids (Curry, 2005). Here, the propensity for chickadees to nest in manmade boxes allows for the ease of study.

Despite the familiarity of Paridae with the public and in ornithology, phylogenetics of Paridae have not always been so clear. There are 59 species in Paridae and the phylogeny of the family has changed over the past half century. This could be due to the morphological simplicity of the family; few species diverge from the morph of a small energetic bird with a stubby neck, proportionally long tail, and a round head. As a result, for much of the existence of Paridae, over 50 species were in the single genus, *Parus* (Snow, 1967). Two aberrant tit species were never placed in *Parus*: the large-bodied and crested Sultan Tit is placed in *Melanochlora*, while the warbler-like Yellow-browed Tit is placed, appropriately, in *Sylviparus* ("warbler tit") (Snow, 1967). Additionally, two species

formerly in other families have been placed in Paridae. The Fire-capped Tit was previously placed with the Penduline Tits in Remizidae and has been moved to Paridae (Gosler and Clement, 2007). In a substantial taxonomic move, the Hume's Ground-tit was placed in Paridae after being incorrectly placed in Corvidae, with jays and crows (James et al., 2003). Hume's Ground-tit is aberrant with a curved-bill, and nests in a burrow as opposed to a cavity (James et al., 2003). All four of these aberrant species are found in the Himalayas, suggesting this region may be the origin of Paridae (Johansson et al., 2013).

Subgenera were proposed to break up *Parus* in 1996 by Harrap and Quinn. In 2005 Frank Gill, formerly of the Academy of Natural Sciences of Philadelphia, elevated five of these suggestions to genera (Gill et al., 2005). Important to these changes was removing all New World taxa from *Parus*, with the seven chickadees placed in *Poecile* and the five crested titmice placed in *Baeolophus* (Gill et al., 2005). The two crested Old World species were also placed in their own genus, *Lophophanes* (Gill et al., 2005). The small blue European tits, including the Blue Tit and the morphologically similar Azure Tit, were removed from *Parus* to form *Cyanistes* (Salzburger et al., 2002). This taxonomic distinction separated Blue Tit from Great Tit, with size as a major morphological distinction. The Tufted Titmouse was split into the northern Tufted Titmouse and the southern Black-crested Titmouse (AOU checklist, 2002), and the western Plain Titmouse was broken up into the coastal Oak Titmouse and the more interior Juniper Titmouse (AOU checklist, 1996). In the future, it is possible that *Parus* will continue to be split apart to only include the Great Tit and its sister taxa, the Green-backed Tit. Authors suggest that the African tit species, still placed in *Parus* should have their own genus, *Melaniparus* (Johansson et al., 2013).

I examined two datasets for my project. One is the most recent complete phylogeny for all of Paridae and the other is the more recent phylogeny only focusing on the seven American *Poecile* chickadee species. The complete phylogeny of Paridae ("A complete multilocus species phylogeny of the tits and chickadees (Aves: Paridae)", Johansson et al. 2013) was performed using two nuclear genes, myoglobin intron 2 and ornithine decarboxylase introns 6 and 7 and exon 7, and a mitochondrial locus, NADH 2. The three sequences were concatenated and aligned. The tree was created using maximum likelihood analysis with RaxML and a Bayesian Interference analysis using Mr. Bayes 3.1.1. All species in Paridae are included, and the outgroup is the sister family to Paridae, with four species in the Penduline Tit family Remizidae.

Many interesting conclusions can be drawn from this tree (phylogeny not shown). First, *Lophophanes* contains two taxa, the Grey-crested Tit (*L. dichrous*), native to the Himalayas and central China, and the Crested Tit (*L. semilarvatus*), one of two crested passerines found in Europe. *Lophophanes* falls sister to *Poecile*,

including the American chickadees, and *Baeolophus*, the American titmice. *Lophophanes*' position near *Baeolophus* suggests a common crested ancestor, rather than *Lophophanes* gaining a crest independently. Another conclusion is that *Baeolophus* is not sister to chickadees in *Poecile*. This suggests that Paridae colonized the Americas twice, producing titmice and chickadees separately.

My reanalyzed DNA results will focus on the seven New World chickadee species in the genus *Poecile*: the Mountain Chickadee (*P. gambeli*), Mexican chickadee (*P. sclateri*), Boreal Chickadee (*P. hudsonicus*), Chestnut-backed Chickadee (*P. rufescens*), Carolina Chickadee (*P. carolinensis*), Black-capped Chickadee (*P. atricapillus*), and the Siberian Tit (*P. cinctus*). The position of the New World chickadees in the 2013 complete Paridae phylogeny paper is surprising from the perspective of a student in the Curry lab. At Villanova, we study hybridization between the Black-capped and Carolina Chickadees in eastern Pennsylvania. However, in the 2013 Paridae phylogeny, the Black-capped Chickadee comes out as a sister taxa to the Mountain Chickadee, with high (.95) bootstrap support. Despite short branch lengths, it is surprising that Black-capped Chickadee hybridizes with a species that is not its closest relative. A search of the literature shows that Black-capped Chickadee also hybridizes with Mountain Chickadee in regions of range overlap (Grava et al. 2012), as well as hybridizing with Boreal Chickadee (*P. hudsonicus*) (Lait et al. 2012). The hybridization with Boreal Chickadee is surprising: in the 2013 Paridae phylogeny, only Chestnut-backed Chickadee is shown to be more distant to Black-capped Chickadee of the New World chickadees.

The phylogeny of New World chickadees in 2013, the most recent phylogenetic analysis of chickadees, used a different method to create their tree ("The influence of sampling design on species tree inference: a new relationship for the New World chickadees (Aves: *Poecile*)", Harris et al. 2013). Rather than obtaining gene sequences, 80 primer pairs were designed to amplify regions of the genome across chickadees. 40 of these amplified across all seven chickadees and were used in phylogenetic analyses. In addition, the NADH-2 and NADH-3 mitochondrial loci were used in the creation of the tree. The gene trees were analyzed using RAxML to create a species tree, and additional analysis were also run: MRBayes, BEST, *BEAST, and STELLS; all yielding the same species tree with high bootstrap values (.76 is their weakest value for BEST analysis, second weakest is 94 for RAxML analysis). The 2013 Paridae phylogeny tree similarly incorporated introns and exons, thus both studies used coding and noncoding data; however, the 2013 chickadee phylogeny paper targets random sequences of DNA where function, if any, is unknown.

The chickadee phylogeny created from the 2013 chickadee paper is quite different from the 2013 Paridae phylogeny (Figure 1). The only relationship that is

the same between both papers is the phylogeny of the “brown crowned” chickadees. In the seven New World species, Boreal Chickadee, Chestnut-backed Chickadee, and Siberian Tit have brown crowns. Both phylogenies have these three species as monophyletic. The 2013 New World chickadee phylogeny shows Mexican Chickadee as sister to Mountain Chickadee, and, as would be expected from our research at Villanova, Black-capped Chickadee is sister to Carolina Chickadee. However, in the 2013 Paridae phylogeny, Mexican Chickadee is the outgroup of the brown crowned chickadees, and Mountain Chickadee is sister to Black-capped Chickadee, with Carolina Chickadee as a third taxonomic group of chickadees. Therefore, the 2013 chickadee phylogeny paper separates brown and black crowned chickadees, whereas the 2013 Paridae phylogeny places Mexican Chickadee, a black crowned species, with the brown crowned species, and also has different relationships within the black crowned species. These two sets of trees contrast each other, with morphological and behavioral observations favoring the 2013 chickadee tree. A European species, the Marsh Tit (*Parus palustris*), is used as the outgroup in the New World chickadee study, and the same nuclear regions were amplified in the Marsh Tit as with the chickadee species.

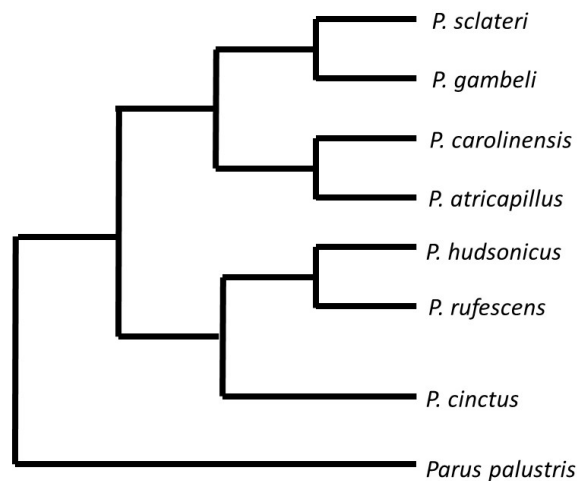


Figure 1. Phylogenetic relationships of the seven *Poecile* species and *Parus palustris*. Adapted from Harris et al. 2013.

Methods

To determine which chickadee phylogeny is the most accurate, I performed my own analysis on the seven chickadee species with Marsh Tit as the outgroup. I obtained the sequence data from GenBank used in both studies, including the 40 chickadee-insert sequences used by the 2013 chickadee phylogeny paper (the 40 of 80 found across all 8 taxa), and the three genes used in the 2013 Paridae phylogeny

paper, myoglobin, ornithine decarboxylase, and NADH-2, as well as two gene sequences for all 8 species that were not previously used in the above mentioned phylogenetic analyses, cytochrome c oxidase 1, and cytochrome B. I took these sequences and used Geneious to create one concatenated sequence. I then used the ClustalW website to align the concatenated sequences between the 8 taxa. After checking the alignment in Geneious to make sure that sequences looked similar, I used the CIPRES website to create a maximum likelihood tree using RAxML.

Results

The resulting tree is illustrated below (Figure 2):

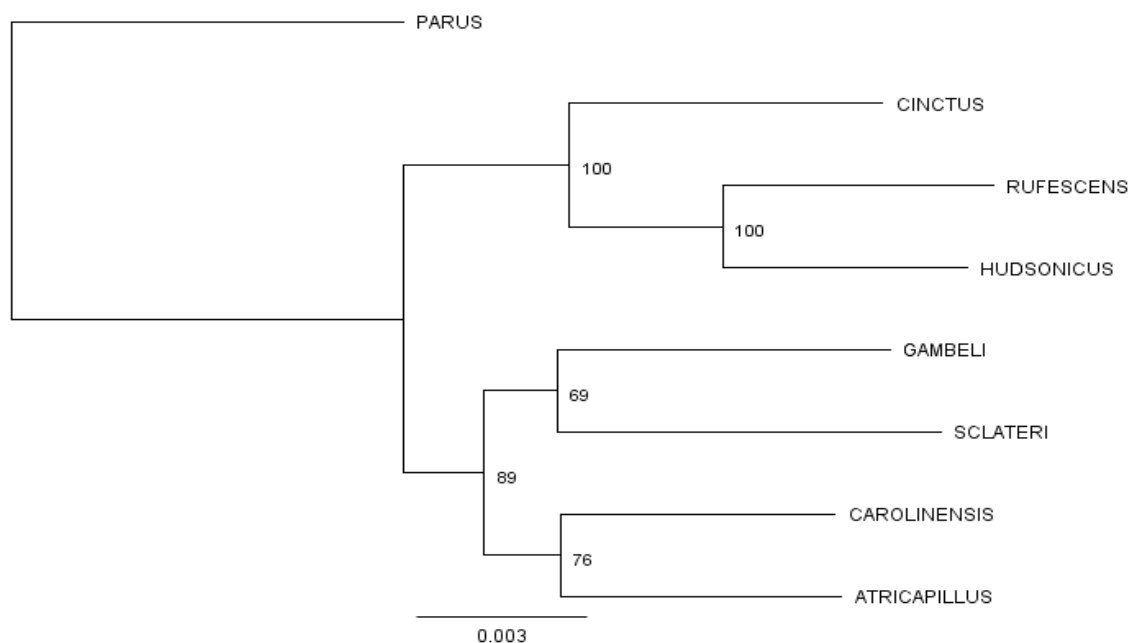


Figure 2. Phylogenetic tree of seven *Poecile* species generated by my novel dataset.

The phylogeny created by this analysis agrees with the 2013 Harris et al. chickadee phylogeny. The brown-crowned species are monophyletic, with Siberian Tit (*Poecile cinctus*) as an outgroup of the sister taxa Chestnut-backed (*P. rufescens*) and Boreal Chickadee (*P. hudsonicus*). Additionally, as with the 2013 chickadee phylogeny paper, the two western species, Mexican Chickadee (*P. sclateri*) and Mountain Chickadee (*P. gambeli*) mapped to be sister taxa. The two species of chickadee that hybridize in eastern Pennsylvania also appear as sister taxa, as Black-capped Chickadee (*P. atricapillus*) and Carolina Chickadee (*P. carolinesis*) map out together. Bootstrap support values were strong for my tree, with my lowest value supporting *P. gambeli* and *P. sclateri* as sister taxa (value of 69). The

second lowest is between *P. carolinensis* and *P. atricapillus* at a value of 76. The outgroup, labelled as “PARUS” is *Parus palustris*, the Marsh Tit.

Discussion

I believe that I obtained a phylogenetic tree similar to the 2013 chickadee phylogeny paper because I concatenated my sequences and because 40 of the 45 sequences that went into the concatenation were the anonymous loci from the 2013 chickadee phylogeny. Many of these sequences were much shorter than the 5 genes added, with cytochrome b being the longest of all individual sequences, but their quantity outnumbered the genes included. In retrospect, it may have been a good idea to not include all 40 anonymous loci to have a relatively similar contribution from both datasets, considering that I was using a concatenated tree that weighs sequences equally. As a result, my tree largely looks like the chickadee phylogeny paper’s tree with slightly lower bootstrap values, presumably due to the influence of the 5 gene sequences.

The phylogeny I generated supports the 2013 chickadee phylogeny paper and supports morphological and behavioral data on chickadees. The brown crowned species and black crowned species aligned together in the tree, supporting morphological features in chickadees. Additionally, the mapping of *P. atricapillus* and *P. carolinensis* as sister taxa reflects the widespread hybridization that occurs between the two species in their narrow zone of contact stretching from New Jersey to Kansas. *P. atricapillus* and *P. carolinensis* are morphologically identical and but are readily distinguished by song, further supporting their placement as sister taxa in my tree. However, due to the distance between *P. atricapillus* and *P. hudsonicus*, my tree does not support the hybridization observed between *P. atricapillus* and *P. hudsonicus* without potential fitness consequences due to genetic incompatibilities (Lait et al., 2012). This interbreeding should be explored further, as the two species represent a unique genetic distance from each other for hybridization. However, it should be noted that a genetic distance of this magnitude does not preclude avian species from occasionally hybridizing, as hybridization between genera is an observed phenomenon in the New World Sparrow family, Emberizidae (Jung et al., 2014).

Chickadees and European tits are familiar to both the general public and to scientific researchers. Due to their abundance, propensity to feed from manmade feeders, and ability to nest in cavities, they allow researchers to easily collect behavioral, morphological, or genetic data. In this way, some species of chickadees and tits function as models for researching topics such as hybridization or response to climate change, and help researchers understand how Passerines and birds in general behave and evolve (Curry, 2005). Uncovering accurate phylogenetic

relationships between the species of this charismatic and well-studied group of birds will help researchers understand the evolutionary timing in the appearance of behavioral or morphological features that influence selection pressures and geographic distribution. The phylogenetic tree provides an evolutionary context for future studies of such traits.

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