

WESTERN BIRDS



Volume 44, Number 2, 2013

MORPHOLOGICAL AND MOLECULAR EVIDENCE CONFIRM THE FIRST DEFINITIVE EASTERN WHITE-BREADED NUTHATCH (*SITTA C. CAROLINENSIS*) FOR NEW MEXICO

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ABSTRACT: We report the first confirmed eastern White-breasted Nuthatch (*Sitta carolinensis carolinensis*) from New Mexico. The bird was collected on 15 October 2011, at Boone's Draw, Roosevelt County. Morphological and plumage characters matched those of the eastern White-breasted Nuthatch, the bill being markedly shorter and the back paler than those of the subspecies resident in New Mexico, *S. c. nelsoni*, and other western subspecies. The DNA sequence of the mitochondrial gene for NADH dehydrogenase subunit 2 (ND2) was an exact match to published sequences from Michigan and Pennsylvania and was phylogenetically nested within a monophyletic and deeply divergent eastern clade.

The White-breasted Nuthatch (*Sitta carolinensis*) is a common and geographically variable resident of mature deciduous, mixed deciduous, and coniferous forests through most of North America. Grubb and Pravosudov (2008) recognized seven subspecies that constitute eastern (*S. c. carolinensis*), interior western (*S. c. tenuissima*, *S. c. nelsoni*, *S. c. mexicana*, and *S. c. lagunae*), and Pacific (*S. c. aculeata* and *S. c. alexandrae*) groups of populations. The seven subspecies are based on differences in bill size and shape and plumage coloration, but at least some of the variation in these characters is clinal. The eastern subspecies averages the shortest in bill length and palest in back color, while interior western and Pacific populations have longer bills and darker mantles (Phillips 1986, Pyle 1997, Sibley 2000, Wood 1992). There are three distinct vocal groups, based on typical call notes, that are concordant with boundaries between subspecies: (1) eastern United States and Canada (nasal *yenk*); (2) Great Basin, Rocky Mountains and Mexico (a rapid *yijijijijiji* or *yiji-yiji-yiji-yiji*), and (3) Pacific slope (high-pitched, drawn-out *aaarn*) (Gaines 1988, Sibley 2000, Dunn and Alderfer 2011). Recent multi-locus phylogenetic analyses of the White-breasted

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Nuthatch uncovered four geographically distinct clades, with boundaries that are also consistent with vocal groups and subspecies: (1) eastern, (2) eastern Sierra Nevada and northern Rockies, (3) Rocky Mountain, Great Basin, and Mexico, and (4) Pacific (Spellman and Klicka 2007, Walstrom et al. 2012). Clades two and three are sister groups that together constitute vocal group 2. The deep phylogenetic structure and overall concordance between patterns of geographic variation in measurements, vocalizations, plumage, mitochondrial DNA, and nuclear DNA suggest that there may be more than one species of White-breasted Nuthatch.

In New Mexico, the White-breasted Nuthatch is a common resident in practically all wooded areas of the state. It descends to lower elevations and withdraws from high peaks during the winter (Ligon 1961), but little else is known about its seasonal movements. The resident subspecies in New Mexico is the interior west *S. c. nelsoni* (AOU 1957, Phillips 1986), which has been placed in the Rocky Mountain, Great Basin, and Mexico phylogenetic clade (Spellman and Klicka 2007). Only one previous specimen of the White-breasted Nuthatch from New Mexico (University of Arizona [UAZ] 5057) has been identified as any subspecies other than *S. c. nelsoni*. That specimen, collected in Socorro County by Gale Monson on 23 December 1941, was identified by Alden H. Miller as *S. c. tenuissima* (J. P. Hubbard pers. comm.), a subspecies constituting the eastern Sierra Nevada clade (Spellman and Klicka 2007) and for which the type locality is in the Panamint Mountains, Inyo County, California. Birders have reported White-breasted Nuthatches of the eastern subspecies in Curry and Sandoval counties on the basis of calls, but they were unable to obtain recordings or conclusive photos (W. H. Howe and C. R. Rustay pers. comm.). This phenotypically distinctive and genetically divergent eastern subspecies of the White-breasted Nuthatch has therefore not yet been fully documented in New Mexico (S. O. Williams pers. comm.).

Here we describe a female eastern White-breasted Nuthatch collected on 15 October 2011 from the eastern plains of New Mexico, representing the first definitive state record of *S. c. carolinensis*. Morphological and molecular data independently support assignment of the specimen to the eastern clade.

METHODS

At 08:10 on 15 October 2011, Baumann, Nicholas D. Pederson, and Cole J. Wolf mist-netted a White-breasted Nuthatch at Boone's Draw, Roosevelt County (34° 06' 41" N, 103° 31' 44" W), a first record of this species at this locality over three previous seasons of netting. We collected the bird (New Mexico Department of Game and Fish permit 3217; U.S. Fish and Wildlife Service permit MB094297-1) because measurements taken in the field and compared to Pyle (1997) suggested tentatively that it was of the eastern subspecies. We took the specimen to the Museum of Southwestern Biology (MSB) at the University of New Mexico, where Andrew B. Johnson prepared it as a study skin MSB 37575 and frozen tissue sample (NK174933). We compared the plumage patterns and bill measurements of MSB 37575 to those of specimens at the MSB, UAZ, and University of Kansas Museum of Natural History.



Figure 1. Comparison of the pale bluish gray mantle of MSB 37575 (middle) and an eastern White-breasted Nuthatch (MSB 21144, left), with the dark bluish gray mantle of *S. c. nelsoni* (MSB 26967, right).

Molecular Methods

Following the manufacturer's protocol, we extracted DNA from frozen muscle tissue of MSB 37575 with a Qiagen DNEasy kit. We amplified the gene for mitochondrial NADH dehydrogenase subunit 2 (ND2) by using the primers H6313 and L5219 (Sorensen et al. 1999) in a 15- μ L reaction with 1 μ L of DNA and the following reagents: 0.5 mM of each primer, 2 mM of each dNTP, 0.75 units AmpliTaq Gold (Life Technologies), 1.5 μ L of MgCl₂, and 1.5 μ L of Taq Gold buffer. Amplification reactions were done according to the following protocol: 95 °C for 8 min, (95 °C for 45 sec, 50 °C for 30 sec, 72 °C for 45 sec) \times 35 cycles, 72 °C for 10 min. We visualized products of the polymerase chain reaction on a 1% agarose gel, then cleaned with Exo-Sap-It (USB Corporation). Using BigDye 3.1 chemistry (Life Technologies), we sequenced the products with external primers and read them with an ABI 3130 automated sequencer. We assembled the sequences and inspected them manually with Sequencher 4.7 (GeneCodes). We used the software packages Muscle (Edgar 2004) and Phylml (Guindon and Gascuel 2003), respectively, to align the sequence with previously published ND2 sequences and analyze it phylogenetically. We used the program Mega (Kumar et al. 2008) to calculate average uncorrected pairwise distances between MSB 37575 and the four major clades identified by Spelman and Klicka (2007).



Figure 2. Comparison of the pale salmon wash on the flanks and buffy wash on the belly and upper breast of MSB 37575 (middle) and an eastern White-breasted Nuthatch (MSB 21144, left) with the bluish gray flanks of *S. c. nelsoni* (MSB 26967, right).

RESULTS

Plumage

The forecrown of MSB 37575 is dark blue, while the hindcrown and nape are black, contrasting with the white face and sides of the neck. The black of the nape expands out toward the anterior edge of the mantle. The mantle is pale bluish gray, notably lighter than the dark bluish gray of *S. c. nelsoni* (Figure 1). The tertials of MSB 37575 are also pale bluish gray with black inner webs, while the tertials of *S. c. nelsoni* are darker blue and less contrasting than those of MSB 37575 (Figure 1). The greater coverts are pale blue with large black centers and white edging on the distal ends of the feathers. The primaries are brownish with paler tips. The rectrices, except the pale blue central pair, are black with large white corners. The throat, upper breast, and

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lower belly are white with the belly and lower breast tinged buffy. The flanks of MSB 37575 are white with a pale salmon wash, while the flanks of *S. c. nelsoni* are bluish gray (Figure 2). The undertail coverts are white with limited rufous edging on some feathers.

Measurements

The exposed culmen of MSB 37575 measures 17.1 mm, which fits with our measurements for 13 female eastern White-breasted Nuthatches (mean 17.3 mm, SD 1.0; Table 1). It is below the range of our measurements of 15 female specimens of *S. c. nelsoni* from New Mexico and Arizona (range 17.5–19.8 mm; Table 1).

Molecular Results

We successfully amplified and sequenced a 968-base-pair fragment of the ND2 gene. Chromatograms of the sequence were clear and unambiguous, without double peaks or internal stop codons that would indicate the erroneous amplification of a nuclear pseudogene. The complete sequence is available at Genbank (accession no. JQ965152). Comparison with published sequences of the White-breasted Nuthatch confirmed that MSB 37575 is part of the eastern clade with 100% bootstrap support. Over the area sequenced, the ND2 gene from MSB 37575 is a 100% match with published haplotypes of *S. c. carolinensis* from Michigan and Pennsylvania (haplotypes E11, E19, and E23 in Spellman and Klicka 2007). The average pairwise difference between the New Mexico specimen and sequences from other eastern specimens was 0.3% (Table 2). Average divergences between the New Mexico specimen and the other clades were substantially higher: 3.8% with the Pacific clade, 6.3% with the Rocky Mountain, Great Basin, and Mexico clade, and 6.9% with the eastern Sierra Nevada clade (Table 2).

DISCUSSION

MSB 37575 can safely be identified as an eastern White-breasted Nuthatch on the basis of measurements, plumage color, and mitochondrial DNA sequence. The bill measurements are within the expected range of the eastern subspecies but below the range of *S. c. nelsoni*, resident in New Mexico, on the basis of measurements from a series of specimens. The most noticeable plumage features distinguishing eastern and western populations are the mantle color, flank color, and the degree of contrast on the tertials, and these differences are consistent between the sexes. In *S. c. carolinensis* and MSB 37575, the mantle and tertials are pale bluish gray, making the black inner webs of the tertials contrast sharply, while the mantle of western populations is dark bluish gray and the tertials are less contrasting (Figure 1). *S. c. carolinensis* and MSB 37575 show whitish flanks with a pale salmon wash, while *S. c. nelsoni* has bluish gray flanks (Figure 2). These plumage distinctions can be used in the field, with care, by birders or banders for subspecific identification and are described in several widely used identification guides (Pyle 1997, Sibley 2000, Dunn and Alderfer 2011).

Molecular data corroborate the morphological analysis. The mitochondrial

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Table 1 Measurements of Exposed Culmen (mm) of MSB 37575, *Sitta c. carolinensis*, and *S. c. nelsoni*, by Sex

Subspecies or specimen	Sex	n	Exposed culmen (mean, mm)	SD	Range
MSB 37575	F	—	17.1	—	—
<i>S. c. carolinensis</i> ^a	F	13	17.3	1.0	15.0–18.5
<i>S. c. nelsoni</i> ^b	F	15	18.3	0.6	17.5–19.8
<i>S. c. carolinensis</i> ^a	M	16	17.6	0.9	15.9–18.7
<i>S. c. nelsoni</i> ^b	M	15	19.5	0.7	18.1–20.5

^aSpecimens from Kansas in the University of Kansas Museum of Natural History

^bSpecimens from New Mexico and Arizona in the Museum of Southwestern Biology.

DNA sequence matches published sequences that are phylogenetically nested within the deeply divergent eastern clade, with 100% bootstrap support. Because the geographic sampling of published sequences is thorough, the only reasonable explanation for these data is that MSB 37575 originated within the range of *S. c. carolinensis*. The approximate western limit of *S. c. carolinensis* extends from central Texas north through central Oklahoma, central Kansas, northeastern Colorado, western Nebraska, and the Dakotas to southern Manitoba (AOU 1957, 1998, Grubb and Pravosudov 2008, Sullivan et al. 2009, Such and Such 2012;). As northern populations of *S. c. carolinensis* move irruptively (Heintzelman and MacClay 1971), primarily in fall (Phillips et al. 1964, Phillips 1986), the occurrence of this subspecies in New Mexico should be expected, especially given the closeness of breeding populations and the subspecies' apparent regularity in eastern Colorado during migration (Leukering et al. 2012). It is likely that many observed seasonal movements represent young of the year dispersing farther in higher numbers in late summer and fall (Grubb and Pravosudov 2008). MSB 37575 was in its first year on the basis of 30% skull ossification and the presence of a bursa of Fabricius.

Previous reports suggest that other eastern White-breasted Nuthatches have occurred in New Mexico. C. R. Rustay (pers. comm.) heard a White-

Table 2 Mean Levels of Percent Divergence (p) in the Mitochondrial Gene ND2 between the MSB 37575 and the Four Major Clades of *Sitta carolinensis*

	(1)	(2)	(3)	(4)
(1) MSB 37575				
(2) Eastern clade	0.003			
(3) Pacific clade	0.038	0.038		
(4) Rocky Mountain, Great Basin, and Mexico clade	0.063	0.061	0.073	
(5) Eastern Sierra Nevada clade	0.069	0.068	0.080	0.017

^aAs defined by Spellman and Klicka 2007.

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breasted Nuthatch giving “*yenk*” calls like those of *S. c. carolinensis* at Hillcrest Park on 18 December 2010 in Clovis. Similarly, White-breasted Nuthatches have been heard giving vocalizations reminiscent of the eastern subspecies in the Corrales bosque (~35° 14' N, 106° 35' W), Sandoval County, on several occasions between December and March (Williams and Howe 2011). As of December 31 2006, the Melrose migrant trap (~34° 26' N, 103° 48' W), Roosevelt County, had four records of the White-breasted Nuthatch on dates ranging from 1 August to 21 September (Parmeter 2007), but none of these individuals was identified to subspecies. Additionally, Wyoming has a single report of a vocal eastern White-breasted Nuthatch from Rawhide Wildlife Habitat Management Unit, Goshen County, in April (Faulkner 2010).

Four subspecies of White-breasted Nuthatch have been described from the eastern United States: *S. c. carolinensis*, *S. c. cookei*, *S. c. atkinsi*, and *S. c. litorea* (Oberholser 1917, AOU 1957, Phillips 1986, Dickinson 2003). An analysis of the eastern White-breasted Nuthatches by Wood (1992), however, found much of the variation to be clinal, with no appreciable difference in back coloration. For this reason, he proposed that all eastern White-breasted Nuthatches should be considered one subspecies, *S. c. carolinensis*. Spellman and Klicka (2007) subsequently found that all of the eastern populations are monophyletic by mtDNA, with little or no geographic structure. The eastern clade of the White-breasted Nuthatch may warrant species status on the basis of its deep level of molecular divergence, reciprocal monophyly, and morphological and vocal distinctness (Spellman and Klicka 2007, Walstrom et al. 2011).

In conclusion, MSB 37575 represents the first confirmed eastern White-breasted Nuthatch for the state of New Mexico. Its occurrence there has been reported on several occasions, and it likely occurs with some regularity on the eastern edge of the state. White-breasted Nuthatches found in New Mexico should be scrutinized for potential vagrants of the eastern or other subspecies, particularly during fall migration and when found away from areas of known breeding. Identification should be based on a combination of characters, potentially including vocalizations, back and flank color, tertial pattern, bill length, and mitochondrial DNA.

ACKNOWLEDGMENTS

We thank Andrew B. Johnson for curation of MSB 37575. We thank Nicholas D. Pederson and Cole J. Wolf for their assistance in the field. We thank Natalie A. Wright and Mark B. Robbins for help obtaining measurements from the University of Kansas Museum of Natural History. The University of Arizona (George Bradley and Alex Badyaev) provided a loan of specimens. We thank John P. Hubbard for his assistance on previous records of the White-breasted Nuthatch in New Mexico. William H. Howe and Christopher R. Rustay provided valuable information about previous experience with probable eastern White-breasted Nuthatches in New Mexico. We thank Gregg Moore for allowing access to his property for our field work. We thank Doug Faulkner, Kimball Garrett, and Philip Unitt for perceptive comments on the manuscript.

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Accepted 21 February 2013