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Affinities of Three Vagrant Cave Swallows from Eastern North America

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ABSTRACT.—We analyzed the mitochondrial cytochrome *b* gene of three vagrant Cave Swallow (*Petrochelidon fulva*) specimens from Illinois, New York, and New Jersey and compared them to published sequences from across the breeding range of the species. All three specimens were assigned to the southwestern United States/Mexico subspecies (*P. f. pallida* group) on the basis of plumage coloration. Molecular results reveal that all three birds possess unique and novel mitochondrial haplotypes that are closely related to haplotypes from known *P. f. pallida* individuals. None of the three haplotypes from the vagrant individuals is within the monophyletic clade of haplotypes that corresponds to the Caribbean subspecies (*P. f. fulva*). *Received 27 January 2011. Accepted 8 July 2011.*

The expansion in breeding and wintering range of the Cave Swallow (*Petrochelidon fulva)* has coincided with a dramatic increase in vagrant birds far to the east and north of their normal range, particularly in autumn. Several have been found dead and these specimens deposited in museum collections. The origins of these vagrants are not always clear due to difficulties with identification. Identifying these vagrants with certainty using genetic methods can help unravel the poorly understood relationship between vagrancy and breeding range expansion. Genetic methods have been previously used to identify vagrant birds to species (e.g., Thorup et al. [2009] identified two *Phylloscopus* warblers and Witt et al. [2010] identified a *Brachyramphus* murrelet), but this is the first attempt to do so at the population level.

The Cave Swallow, according to mitochondrial DNA (mtDNA) data (Kirchman et al. 2000), consists of two diagnosable forms, one breeding in the Greater Antilles and Florida (*P. f. fulva* group) and the other breeding in the southwestern United States and Mexico (*P. f. pallida* group; West [2005]; nomenclature follows AOU [2000]). Most specimens are diagnosable via plumage coloration patterns, but field identification of the two forms is extremely difficult.

Previous specimen records of vagrant Cave Swallows in eastern North America have primarily been identified as *P. f. pallida,* including autumn specimens from New York, New Jersey, Ontario, South Carolina, Virginia, and Ohio (McNair and Post 1999, Dinsmore and Farnsworth 2006, Spahn and Tetlow 2006, O'Brien 2007, Post 2008). There are winter specimens from South

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Carolina of *P. f. pallida* and an autumn specimen from Missouri in 1977 that was identified by measurements and plumage as *P. f. fulva* (Easterla 2008). There are also recent spring records from eastern North America (e.g., Massachusetts [Szantyr 2010]; and Ontario [Wormington 2010]).

A Cave Swallow collided with a window at McCormick Place (41° 51.308′ N, 87° 36.770′ W ± 8 m) on 10 November 2008 along the Chicago, Illinois lakefront and was found dead by MHH and deposited at the Field Museum of Natural History (FMNH). The bird was prepared as a study skin (FMNH 461103) and a tissue sample was taken (MCP08-625). Multiple Cave Swallows were found dead at the Cape May Congress Hall, Cape May, New Jersey (38° 55.856′ N, 74° 55.469′ W ± 100 m) on 27 November 2007 (O'Brien 2007). One of these birds was deposited at the University of New Mexico's Museum of Southwestern Biology (MSB 29350), prepared as a study skin with partial skeleton (ABJ 2455), and a tissue sample was taken (NK170651). Our objectives, based on these specimens, were to (1) identify the specimens to subspecies based on morphology and genetics, and (2) consider the identifications in terms of expanding populations and vagrancy.

METHODS

We sequenced a portion of the mtDNA cytochrome *b* (cyt *b*) gene of each specimen to independently assess their identification and geographic origins with respect to the mtDNA data set previously published by Kirchman et al. (2000). We also included data recently published (Dor et al. 2010) from a vagrant Cave Swallow salvaged in New York and, to increase our sample size of breeding birds, we sequenced one additional Cave Swallow from Valverde County, Texas (MSB 18680).

We extracted total genomic DNA from two vagrant (FMNH 461103 and MSB 29350) and one breeding (MSB 18680) Cave Swallow using the DNeasy tissue extraction kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocols. We used primers L14841 (all 3 samples; Kocher et al. 1989), H16065 (FMNH sample; Helm-Bychowski and Cracraft 1993), and H4a (MSB samples; Harshman 1996) to amplify and directly sequence a portion of the mtDNA cyt *b* gene. We followed Patel et al. (2011) for thermal cycling, visualization, and sequencing protocols for the FMNH sample. Cytochrome *b* for the MSB samples was amplified in 15 µl reactions using 2 µl of the DNA extract and the following reagents: 0.15 µl of Taq Gold polymerase (ABI, Mountain View, CA, USA), 200 µM of each dNTP, 1.5 mM $MgCl_2$, and 0.5 µM of each primer. Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) thermal-cyclers were used to conduct the following PCR protocol: 95° for 8 min, (95° for 30 sec, 50° for 30 sec, 72° for 60 sec) × 35 cycles, and 72° for 10 min. PCR products were visualized on 1% agarose gel and cleaned using ExoSAP-IT (USB, Cleveland, OH, USA). Sequencing reactions with external primers used BigDye 3.1 chemistry (Applied Biosystems, Foster City, CA, USA) and were visualized using an ABI 3130 automated sequencer. We assembled the sequences and inspected chromatograms manually using Sequencher 4.7 (at MSB) and 4.10.1 (at FMNH; Gene Codes Corp., Ann Arbor, MI, USA).

We aligned 921 bp of these sequences (Genbank accession #s JN227534–JN227536) with sequences deposited in Genbank by Kirchman et al. (2000) (accession #s AF182379–182391) and Dor et al. (2010) (accession # GU460285) using Sequencher 4.10.1 (Gene Codes Corp., Ann Arbor, MI, USA). Sequences from Kirchman et al. (2000) were taken from breeding colonies throughout the Cave Swallow's breeding range; the Dor et al. (2010) specimen was an autumn vagrant found dead on 19 November 2005 in Tompkins County, New York (Cornell University Museum of Vertebrates 51713).

We generated a 95% statistical parsimony haplotype network using TCS Version 1.21 (Clement et al. 2000). We used PAUP* (Version 4.0b10; Swofford 2003) to construct a maximum parsimony tree using a heuristic search with TBR branch swapping and 100 random addition replicates. Support for nodes was estimated by 1,000 bootstrap replicates with one random addition per replicate. PAUP* was also used to calculate uncorrected *p*-distances. We conducted a Bayesian analysis using MrBayes 3 (Ronquist and Huelsenbeck 2003) and used a general-time-reversible model of sequence evolution incorporating parameters for invariable sites and gamma rate heterogeneity. All parameters were estimated as part of the analysis and we conducted two parallel runs, each with four Markov chains and for 5 million generations. We sampled the Markov chains every 500 generations and used these 10,000 parameter point estimates minus the burn-in (500 generations) to create a 50% majority rule

consensus tree, and to calculate the Bayesian posterior probabilities to assess nodal support.

Morphological identification of the Illinois and New Jersey specimens was based on comparisons with specimens in the bird collections of FMNH and MSB and through comparisons with published references. Wing chord measurements were taken with a standard wing rule and tail measurements were taken with a clear ruler. These measurements were compared to published measurements in Phillips (1986), West (1995), and Pyle (1997).

RESULTS

Both the Illinois and New Jersey Cave Swallows show a pale buffy throat, relatively pale cinnamon rump and forehead, and lack extensive rufous on the flanks that distinguish *P. f. pallida* from *P. f. fulva*. However, the New Jersey specimen has several fresh, sheathed feathers growing in on the rump that are strikingly darker (chestnut) than the existing, pale orange-cinnamon rump feathers (photograph at http://arctos.database.museum/guid/MSB:Bird:29350). This coloration is due to wear and underscores the difficulty of subspecies identification by plumage alone. The Illinois specimen has a wing measurement of 104 mm and tail measurement of 44 mm. The New Jersey specimen has highly asymmetrical wing measurements (right wing 107 mm, left wing 102 mm) and a tail measurement of 49 mm.

The Illinois and New Jersey specimens were both females with 100% skull ossification and ovaries measuring 4 × 2 mm (finely granular), and 2 × 3 mm, respectively. Both are hatch-year birds, as indicated by suspended wing molt, with primaries 5–9 (IL) and primaries 4–9 (NJ) and the corresponding primary coverts relatively worn and pale, and primaries 1–4 (IL) and primaries 1–3 (NJ) fresh and dark.

The Illinois, New Jersey, and New York Cave Swallow haplotypes are each unique and different from one another and from the breeding specimens of *P. f. pallida* from Tom Green County, Texas. The Illinois specimen is one base pair different from both Texas haplotypes (uncorrected *p*-distance of 0.1%; Table 1). The New Jersey specimen is three to five base pairs different from the Texas haplotypes (0.3–0.5%), and the New York specimen is one to four base pairs different from the Texas haplotypes (0.1–0.4%). The shortest number of steps between any *P. f. pallida* and a member of the *P. f. fulva* clade is five (Valverde County, Texas to Cuba; Fig. 1). All three vagrants, based on uncorrected cyt *b* *p*-distances, are genetically closer to previously published breeding *P. f. pallida* than to breeding *P. f. fulva* (Table 1).

TABLE 1. Uncorrected *p*-distances for the Illinois, New Jersey, and New York Cave Swallow specimens highlighting (bold) the relatively lower uncorrected *p*-distances between the vagrant specimens and breeding *P. f. pallida* group individuals than between the vagrants and *P. f. fulva* group.

	n	IL	NJ	NY
IL	1			
NJ	1	0.004		
NY	1	0.003	0.005	
[*P. f. pallida*][a]	**5**	**0.002**	**0.005**	**0.004**
YUC	2	0.003	0.007	0.007
TX3	1	0.001	0.003	0.002
TX1/2	2	0.001	0.005	0.004
[*P. f. fulva*][a]	**7**	**0.009**	**0.011**	**0.010**
CU	1	0.007	0.009	0.008
FL1	1	0.012	0.014	0.013
FL2	1	0.009	0.011	0.010
PR/JA	4	0.008	0.010	0.009

[a] These are the averages of all pairwise comparisons between breeding birds sampled by Kirchman et al. (2000) and each of the three vagrant specimens.

The Bayesian analysis supports the monophyly of the *P. f. fulva* group (Bayesian posterior probability = 0.97; Fig. 2), to the exclusion of the three vagrants. Bootstrap support for the *P. f. fulva* group is relatively weak (57%) because of the small number of informative characters among these recently diverged haplotypes. Neither analytical method provides strong statistical support for the monophyly of *P. f. pallida*. Thus, the exact position of the vagrant samples with respect to Caribbean and Texas/Mexico birds is unclear.

DISCUSSION

Both the Illinois and New Jersey Cave Swallows, based on plumage characteristics, can be assigned to the *P. f. pallida* group, although the potential for color changes due to plumage-wear makes this identification tentative. Wing and tail measurements of the Illinois specimen, as well as wing measurements of the New Jersey specimen were in the area of overlap between the two subspecies groups, but the tail measurement of the specimen from New Jersey was outside the range of *P. f. fulva* but within the range of *P. f. pallida*,

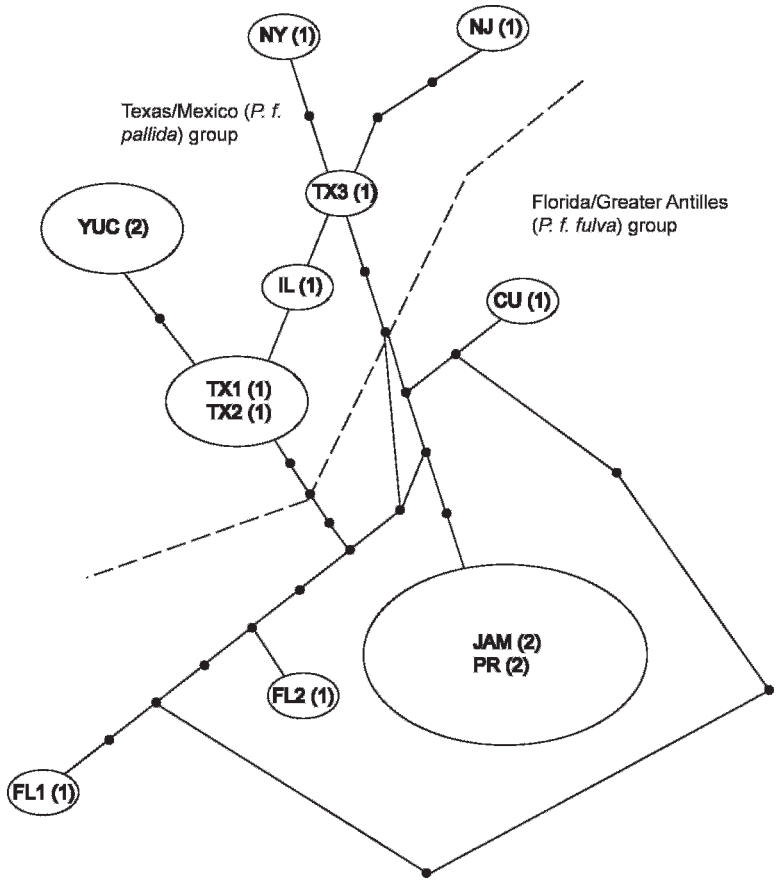

FIG. 1. Haplotype network of all *P. fulva* samples in the study showing two clusters, one for each subspecies group. Each line represents a single mutational step with solid circles indicating unsampled haplotypes. The dashed line indicates the division between *P. fulva* subspecies groups. The size of each circle is proportional to the total number of samples with the corresponding haplotype and the number in parentheses indicates the number of samples carrying that haplotype from a given location.

based on the measurements presented by West (1995).

Kirchman et al. (2000), using mtDNA cyt *b* data, found strong bootstrap support (79% for each clade in a maximum parsimony analysis) for the reciprocal monophyly of *P. f. pallida* and *P. f. fulva*. The addition of the sequences from the Illinois, New Jersey, and New York vagrants caused an unexpected breakdown of reciprocal monophyly (Fig. 2). However, the haplotype network shows clear affinities of all three vagrants to *P. f. pallida* (Fig. 1), despite all three vagrants as well as the newly-added Texas specimen having mtDNA haplotypes that differ from those published by Kirchman et al. (2000). That these three individuals carried previously unsampled haplotypes suggests they may have originated from populations other than those sampled by Kirchman et al. (2000), although it is possible that sampling more individuals may have revealed these haplotypes.

A combination of plumage, genetic distances, haplotype network, and the Bayesian support for the *P. f. fulva* group are consistent with the vagrant Cave Swallow specimens having originated from the *P. f. pallida* populations of the southwestern USA or Mexico. This evidence reaffirms the putative link between rapid population expansion and the spate of vagrancy in this species over the past two decades.

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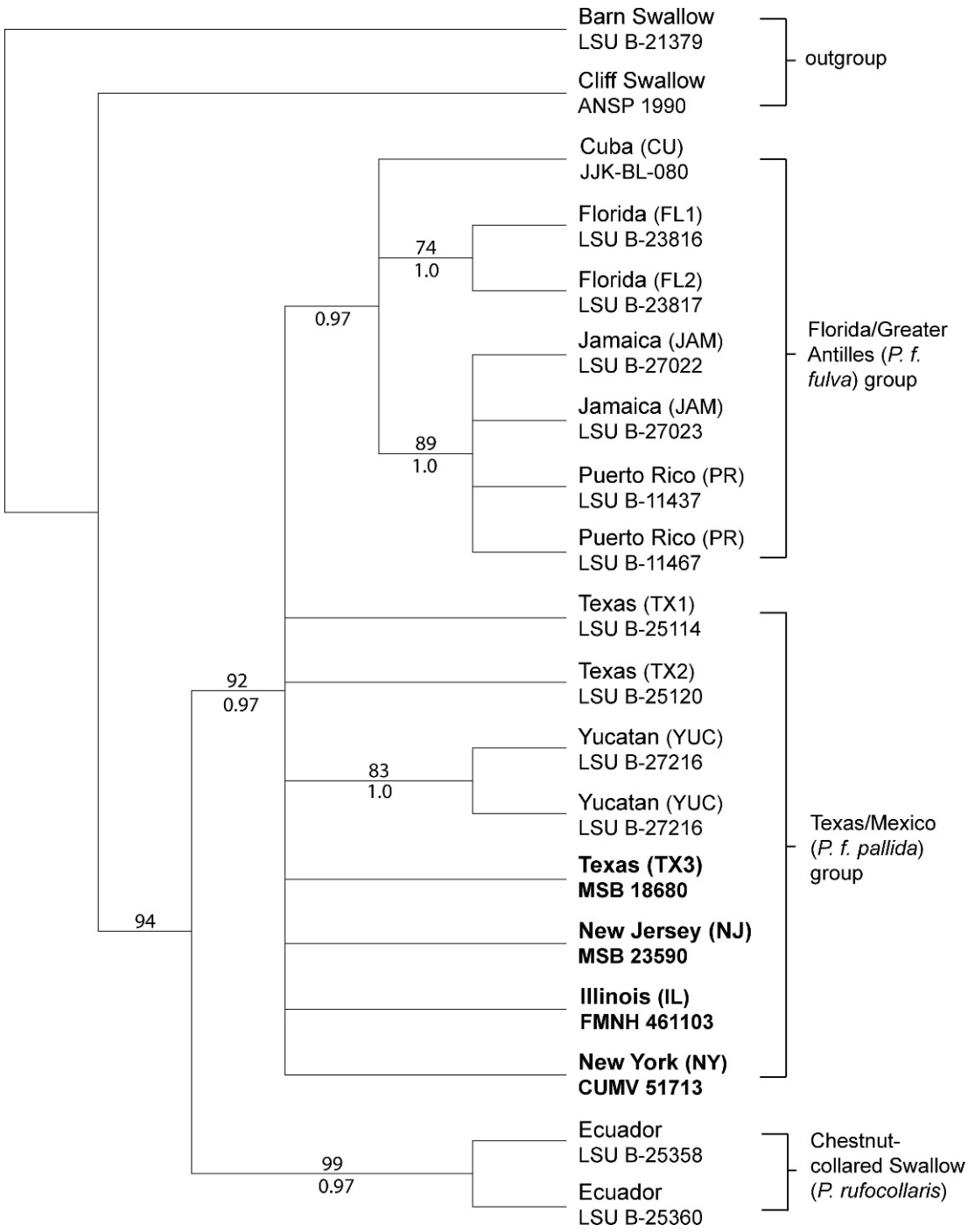

FIG. 2. Maximum parsimony bootstrap tree of all *P. fulva* samples examined. Bootstrap values >70% are shown above the nodes and Bayesian posterior probabilities >0.95 are shown below the nodes. Samples in bold indicate sequences new to this study. Sample numbers are tissue numbers from Kirchman et al. (2000) or specimen numbers (new sequences). ANSP = Academy of Natural Sciences, Philadelphia; CUMV = Cornell University Museum of Vertebrates; FMNH = Field Museum of Natural History, Chicago; MSB = Museum of Southwestern Biology, University of New Mexico; LSU =

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Louisiana State University Museum of Natural Science; JJK = Jeremy J. Kirchman. The outgroup included Barn (*Hirundo rustica*) and Cliff (*Petrochelidon pyrrhonota*) swallows.