



RESEARCH ARTICLE

Continent-wide patterns of divergence in acoustic and morphological traits in the House Wren species complex

J. Roberto Sosa-López* and Daniel J. Mennill

Department of Biological Sciences, University of Windsor, Windsor, Ontario, Canada

* Corresponding author: jrobertososa@gmail.com

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ABSTRACT

Phenotypic traits are important for assessing differences between populations, especially in groups with poorly resolved taxonomy. One such group, the House Wren complex, presents extensive taxonomic controversy and is thought to comprise many independent evolutionary units. Although the songs and morphological features of House Wrens (*Troglodytes aedon*) show extensive variation, differences between populations have not been quantified. We assessed variation in acoustic and morphometric traits within this complex and compared patterns of variation with currently recognized subspecies boundaries. First, we compared songs and morphology among eight recognized subspecies (*T. a. aedon*, *T. a. parkmanii*, *T. a. cahooni*, *T. a. brunneicollis*, *T. a. nitidus*, *T. a. musculus*, *T. a. beani*, and *T. a. rufescens*), controlling for significant effects of latitude. Second, we used variation in male song, a trait with an important role in mate choice and male–male competition, to assess divergence among subspecies. We compared variation among subspecies to variation across seven currently recognized *Troglodytes* species (*T. hiemalis*, *T. pacificus*, *T. tanneri*, *T. sissonii*, *T. cobbi*, *T. rufociliatus*, and *T. ochraceus*). Our results, based on broad sampling of songs ($n = 786$) and morphological traits ($n = 401$) from 609 locations throughout the Americas, show that most of the subspecies examined diverge in song, morphology, or both. In addition, the acoustic differences between subspecies are similar to, and in some instances greater than, the divergence between pairs of currently recognized species. Our results suggest that at least four allopatric subspecies—*T. a. nitidus*, *T. a. musculus*, *T. a. beani*, and *T. a. rufescens*—are likely different species, and we identify many other vocally and morphologically differentiated subspecies that may, upon further detailed genetic analysis, result in new species.

Keywords: Acoustic variation, geographic variation, House Wren, latitudinal variation, morphological divergence, song divergence, species limits, *Troglodytes*, vocalization

Patrones de divergencia a nivel continental en características acústicas y morfológicas en el complejo de especies de *Troglodytes aedon*

RESUMEN

Las características fenotípicas son importantes para evaluar las diferencias entre poblaciones, especialmente en grupos para los cuales la taxonomía no está bien determinada. El complejo de *Troglodytes aedon* es uno de estos grupos, ya que su taxonomía es muy controvertida y se cree que consta de varias unidades evolutivas independientes. Aunque los cantos y las características morfológicas de *Troglodytes aedon* muestran gran variación, las diferencias entre poblaciones no han sido cuantificadas. En este estudio, evaluamos la variación en características acústicas y morfológicas dentro del complejo, y comparamos patrones de variación con los límites que se reconocen actualmente entre subspecies. Primero, comparamos cantos y morfología entre ocho subspecies reconocidas de *Troglodytes aedon* (*Troglodytes a. aedon*, *T. a. parkmanii*, *T. a. cahooni*, *T. a. brunneicollis*, *T. a. nitidus*, *T. a. musculus*, *T. a. beani*, *T. a. rufescens*), controlando por los efectos de latitud. Segundo, usamos la variación en el canto del macho—una característica con un papel importante en la elección de parejas y competencia entre machos—para evaluar la divergencia entre subspecies de *Troglodytes aedon*. Comparamos variación entre subspecies con variación entre siete especies reconocidas de *Troglodytes* (*T. hiemalis*, *T. pacificus*, *T. tanneri*, *T. sissonii*, *T. cobbi*, *T. rufociliatus*, *T. ochraceus*). Nuestros resultados, basados en un muestreo amplio de cantos ($n = 786$) y características morfológicas ($n = 401$) de 609 localidades a lo largo de las Américas, muestra que la mayoría de las subspecies examinadas divergen en canto, morfología, o los dos. Además, mostramos que las diferencias acústicas entre subspecies son similares a, o en algunos casos mayores que, la divergencia entre pares de especies reconocidas actualmente. Nuestro estudio sugiere que por lo menos cuatro subspecies alopatricas—*T. a. nitidus*, *T. a. musculus*, *T. a. beani*, y *T. a. rufescens*—probablemente son especies distintas, e identifica muchas otras subspecies que muestran diferenciación vocal y morfológica, lo cual podría resultar en nuevas especies tras análisis genéticos detallados.

Palabras clave: Variación acústica, variación geográfica, *Troglodytes aedon*, variación latitudinal, divergencia morfológica, divergencia en canto, límites de especies, *Troglodytes*, vocalización

INTRODUCTION

Biologists are faced with the difficult task of estimating biological biodiversity. Current inferences of species diversity in many groups are likely underestimated (Wilson 2003). For instance, the number of avian lineages in the tropics is thought to be greater than is currently recognized (Milá et al. 2012). Furthermore, recent findings of new bird species (e.g., Lara et al. 2012, Seeholzer et al. 2012, Hosner et al. 2013), along with revisions to the taxonomic status of many other species (e.g., Chesser et al. 2012, 2013), clearly indicate that further research in this field is required (Brumfield 2012).

One of the principal challenges for biologists when assessing diversity is to draw boundaries between species. This challenge is overcome by documenting phenotypic and genetic variation of organisms across geographic regions (Nyairi 2007). The use of informative traits is crucial for delimiting species boundaries. Avian acoustic signals are important because they play a direct role in mate choice, male–male competition, and species recognition in many taxa (Catchpole and Slater 2008, Wilkins et al. 2013), thereby acting as premating isolation barriers. It is not surprising, therefore, that research focusing on song as an important phenotype has produced significant insight into avian taxonomy (e.g., Toews and Irwin 2008, Alström et al. 2011, Campagna et al. 2012, Lara et al. 2012, Sosa-López et al. 2013).

With well-known historical taxonomic problems and a distribution that includes most parts of the Americas (Figure 1), the House Wren complex stands out as an ideal group for exploring vocal geographic variation and its taxonomic implications. The American Ornithologists' Union (AOU) currently recognizes 30 subspecies of House Wren (*Troglodytes aedon*) within this complex (AOU 1998); however, the number of subspecies varies among taxonomic authorities (e.g., Brewer 2001, Navarro-Sigüenza and Peterson 2004, Kroodsma and Brewer 2005, Clements et al. 2012, Gill and Donsker 2013). Several authorities agree that all subspecies can be clustered into five main groups on the basis of slight morphological and geographical differences (e.g., AOU 1998, Clements et al. 2012). (1) The “*aedon* group” includes two subspecies: *T. a. aedon* in southeastern Canada and the eastern United States, and *T. a. parkmanii* from southwestern Canada and the central and western United States to Baja California, Mexico. (2) The “*brunneicollis* group” includes three subspecies: *T. a. cahooni* from the mountains of southern Arizona south to central Mexico, *T. a. brunneicollis* in the mountains of northeastern Mexico, south of the Sierra Madre del Sur of Oaxaca, and *T. a. nitidus* in the mountains of Zempoaltepec, Oaxaca. (3) The “*musculus* group” includes 20 subspecies, populating most areas from central Mexico

south to Tierra del Fuego, with some subspecies restricted to islands. (4) The “*martinicensis* group” includes six subspecies, each restricted to its own island in the Lesser Antilles: *T. a. guadeloupensis* in Guadeloupe, *T. a. martinicensis* in Martinique (probably extinct), *T. a. mesoleucus* in St. Lucia, *T. a. musicus* in St. Vincent and Grenada, and *T. a. rufescens* in Dominica. (5) The “*beani* group” includes only the subspecies *T. a. beani* and is restricted to Cozumel Island in the Yucatan Peninsula, Mexico. Some taxonomic authorities treat most of these groups as full species. For example, Howell and Webb (1995) recognize the *brunneicollis* group and the *beani* group as full species, and both Navarro-Sigüenza and Peterson (2004) and Kroodsma and Brewer (2005) recognize the *aedon* group, *brunneicollis* group, *musculus* group, and *beani* group as full species. Clearly, there is little agreement on whether the major groups within this complex should be considered different species.

A series of recent genetic studies suggests that the *aedon* group, *brunneicollis* group, and *musculus* group have independent evolutionary trajectories (Brumfield and Capparella 1996, Rice et al. 1999, Martínez Gómez et al. 2005; also see Mann et al. 2006, Campagna et al. 2012). For instance, Brumfield and Capparella (1996) provided genetic data suggesting three or more distinct lineages—the *aedon* group, *brunneicollis* group, and *musculus* group—and placed the *brunneicollis* group and the *aedon* group in the same clade, with the *musculus* group as the sister taxon. Rice et al. (1999) and Martínez Gómez et al. (2005) also support three distinct lineages, but they placed the *brunneicollis* group as the sister taxon. These studies used different subspecies of the *brunneicollis* group in their analysis; Brumfield and Capparella (1996) used *T. a. cahooni*, whereas Rice et al. (1999) and Martínez Gómez et al. (2005) used *T. a. nitidus*. Interestingly, *T. a. cahooni* is thought to be sympatric with *T. a. aedon* in southern Arizona, while *T. a. nitidus* has an allopatric distribution, isolated in the mountains of Zempoaltepec, Oaxaca (Kroodsma and Brewer 2005). In another study, Campagna et al. (2012) suggested the existence of significant genetic differences within the *aedon* group, between *T. a. aedon* (eastern Canada) and *T. a. parkmanii* (western Canada), placing *T. a. aedon* and subspecies of the *musculus* group in the same clade, and *T. a. parkmanii* as the sister taxon. There is a lack of consensus on whether the five currently recognized groups represent independent lineages, obscuring the taxonomy of this species complex.

Groups of *Troglodytes* that are restricted to islands (i.e. the *beani* group and *martinicensis* group) have received less attention than their mainland counterparts. Several authors have suggested that the island taxa within this complex are likely to be distinct species, based on their isolated distribution and on morphological differences in size as well as color (Navarro-Sigüenza and Peterson 2004,



FIGURE 1. Map of North and South America, showing the recording locations for the songs of eight subspecies of *Troglodytes aedon* (open circles) and seven comparison species (filled symbols). The dark gray area shows the breeding-season distribution of *T. aedon*. Dashed lines indicate approximate boundaries between continental House Wren subspecies. Spectrograms at right depict one example of a male song from each of the eight subspecies analyzed. Spectrograms at left depict one example of a male song from each of the seven species that were analyzed for comparison. For all spectrograms, the x-axis tick marks show increments of 0.5 s (note that the time axis varies between spectrograms, to maximize display area), and y-axis tick marks show increments of 1 kHz from 1 kHz to 13 kHz.

Kroodsma and Brewer 2005). Recent research on the Falkland Islands suggests that this is true for Cobb's Wrens (*T. cobbi*; Campagna et al. 2012, Chesser et al. 2013, Remsen et al. 2013).

Together, these previous investigations reveal complicated relationships within the House Wren complex and suggest the existence of several species, even within some of the five main groups. The vocalizations of House Wrens are known to exhibit substantial variation among these subspecies, but no study to date has empirically quantified the extent of bioacoustic variation (Johnson 1998). Similarly, morphometric variables are also suspected to vary in the House Wren complex, but no study has yet quantified this variation (Brewer 2001). A lack of behavioral and morphological data, and poor knowledge of genetic relationships, limits our ability to answer critical questions about the taxonomy of this group.

Here, we assess geographic variation in acoustic and morphological traits across subspecies in the House Wren complex. Our first objective was to assess whether differences in phenotypic traits correspond to recognized subspecies. Our motivation was to validate House Wren subspecific divisions using both fine-structural characteristics of male song and morphology. Our second objective was to understand the extent of song diversification among subspecies in the complex and provide a relative measure of song diversification to help improve the taxonomic classification of this group. To this end, we assessed the bioacoustic differences between subspecies of House Wrens that have an ambiguous taxonomic status and compared the magnitude of these differences to that seen between currently recognized *Troglodytes* species.

METHODS

Subspecies analyzed. We classified all recordings and morphological samples by subspecies using the taxonomy proposed by Kroodsma and Brewer (2005). We obtained acoustic recordings and morphological samples for eight subspecies: *T. a. aedon*, *T. a. parkmanii*, *T. a. cahooni*, *T. a. brunneicollis*, *T. a. nitidus*, *T. a. musculus*, *T. a. beani*, and *T. a. rufescens*. We pooled all our acoustic and morphological data corresponding to the *musculus* group under the category of *T. a. musculus* because we lacked data on subspecies boundaries within this South American group. We also pooled two possible subspecies recordings (but not morphological data) obtained from the Lesser Antilles into *T. a. rufescens* because we did not have information on the island where these recordings were collected (for recording details, see Supplemental Material Table S1). This lack of geographic resolution in the Lesser Antilles is not ideal, but we considered it important to include these recordings in our analysis because remarkably few recordings exist for these birds, and their taxonomic

status is of great importance from a conservation perspective.

The distributions of some taxa within the complex are thought to overlap. For example, *T. a. parkmanii* and *T. a. cahooni* overlap in southern Arizona (AOU 1998, Kroodsma and Brewer 2005). For these groups, we used only recordings acquired during the breeding season, to avoid confusing northern migrants with resident southern birds. To further avoid any mismatch in subspecies identification, recordings made above 1,600 m elevation were considered to be from *T. a. cahooni*, whereas recordings made below this elevation were considered to be from *T. a. parkmanii* (Brewer 2001).

Acoustic analysis. Our sampling approach involved directly collecting recordings during field expeditions and gathering existing recordings from 16 natural-sound libraries and private collections (for details, see Supplemental Material Tables S1, S2). We collected recordings directly using three sets of equipment: a Marantz PMD660 digital recorder with a Sennheiser MHK67 shotgun microphone (recordings collected in WAV format; 44.1 kHz, 16 bits); a Marantz PMD660 digital recorder with a Telinga parabola with a Sennheiser ME62/K6 omnidirectional microphone (recordings collected in WAV format; 44.1 kHz, 16 bits); or a Nagra Ares-BB+ digital recorder with a Telinga parabola with a Stereo Pro 6 Telinga microphone (recordings collected in WAV format; 48 kHz, 16 bits).

We carefully scrutinized the recordings provided by libraries and private collections to prevent inclusion of more than one recording from the same individual. There were three instances in which we excluded recordings from our analysis: when multiple recordings clearly came from the same individual, when the identity of the bird in the recording was unclear and recordings were collected <1 hr apart, and when recordings were made on the same day but did not specify the recording time.

The recordings used in the analysis contained between 1 and 60 songs from the same individual. We randomly selected one song from each recording by generating a sequence of random numbers and matching the numbers with the number of the song in the recording. Each selected song was extracted and saved in a separate sound file with ≥ 0.5 s of silence at the beginning and the end. We created a spectrogram for every song using a 1,024-point fast Fourier transform, with 93.75% overlap, Blackman window, 22-Hz frequency resolution, and 2.9-ms temporal resolution. We applied a 1-kHz high-pass filter and measured all fine-structural characteristics of the songs using AviSoft-SASLab Pro version 5.2.04 (R. Sprech, Berlin, Germany).

We conducted measurements using the “automatic parameter measurements” tool in Avisoft-SASLab Pro, thereby minimizing human subjectivity in collecting

acoustic measurements. We detected the start and end of each element in the song by using a separation threshold of -25 dB in relation to the maximum amplitude of the element; we distinguished separate elements when the amplitude dropped below the -25 dB threshold for ≥ 5 ms. Frequency variables were calculated using a threshold setting of -20 dB in relation to the song's peak amplitude (see Supplemental Material Figures S1A, S1B), including all the peaks that exceeded the threshold. Measurements based on the power spectrum, such as entropy (see below), were derived from the average spectrum across an entire element. Songs of *Troglodytes* start with a series of low-amplitude introductory elements, and the threshold of automatic detection could not always detect these very quiet elements. In these cases, we selected the onset of the introductory section manually by looking at the first element on the spectrogram.

We quantified a total of 15 fine-structural features (depicted in Supplemental Material Figure S1). The first set of measurements was conducted at the level of the individual element. (1) Element length (s): the average duration of each element within the song. (2) Inter-element interval (s): the average length of the silent space between elements, calculated as the average time from the end of the preceding element to the start of the current element for all the elements across the entire song. (3) Mean maximum frequency (kHz): the average maximum frequency of all elements within the song. (4) Mean bandwidth (kHz): the average bandwidth of every element within the song, calculated as the difference between the lowest (minimum) and the highest (maximum) frequency for each element. (5) Mean peak frequency (kHz): the average peak frequency of all elements within the song (peak frequency was determined as the frequency with the highest amplitude in the power spectrum for each element). (6) Mean entropy: the average entropy measurement for each element within the song. (Note that the entropy parameter is a measure of the randomness of the sound, with values ranging from 0 to 1; pure-tone elements have values close to 0, and noisy sounds have values close to 1.) The second set of measurements was conducted at the level of the song. (7) Song length (s): the duration from the beginning of the first element to the end of the last element in the song. (8) Number of elements: total number of elements detected within the song. (9) Number of trills: We defined "trill" as a section of the song composed of a series of identical syllables repeated three or more times in a row (syllables can be composed of one or more elements, i.e. one or more continuous tracings on a sound spectrogram, as in Catchpole and Slater 2008). (10) Minimum frequency (kHz): the lowest frequency with amplitude delimited by the threshold from the power spectrum of each element, and across the entire song. (11) Maximum frequency (kHz): the highest frequency with

amplitude delimited by the threshold from the power spectrum of each element, and across the entire song. (12) Peak frequency shifts per second: the number of times that the frequency peak switched between a value above and below 5.0 kHz, from one element to the next, sequentially across the entire song. We chose a threshold of 5.0 kHz because this was the midpoint between the mean minimum and mean maximum frequencies; we counted the number of switches and divided them by the song length. This is similar to the variable "transitions per second" used by Toews and Irwin (2008) and Campagna et al. (2012). The third set of measurements was conducted to describe the variation in frequency and entropy between elements in a song. (13) Standard deviation (SD) in maximum frequency (kHz), (14) SD in bandwidth (kHz), and (15) SD in entropy.

Statistical analysis of acoustic data. To reduce the number of variables for analysis, and to avoid multicollinearity among variables in our analysis, we performed a principal component analysis (PCA), with varimax rotation, on the acoustic measurements outlined above. The analysis resulted in five principal component factors with eigenvalues >1 that together explained 74.8% of the total variation in the original 15 acoustic variables. The first factor was strongly associated with maximum frequency, SD in maximum frequency, SD in bandwidth, and SD in entropy; the second factor was strongly associated with mean maximum frequency, mean peak frequency, and peak frequency shifts per second; the third factor was strongly associated with song length, number of elements, and number of trills; the fourth factor was strongly associated with mean bandwidth and mean entropy; and the fifth factor was strongly associated with minimum frequency, element length, and inter-element interval (Table 1).

We then performed one-way analyses of covariance (ANCOVAs) to test whether subspecies differed from each other in the fine-structural characteristics of their songs (summarized by the five principal component factors), while controlling for variation in latitude (as a proxy for distance). In each analysis, we included one of the five principal component factors (Table 1) as the dependent variable. We used subspecies (i.e. *T. a. aedon*, *T. a. parkmanii*, *T. a. cahooni*, *T. a. brunneicollis*, *T. a. nitidus*, *T. a. musculus*, *T. a. beani*, or *T. a. rufescens*) as a fixed factor in the model, and latitude was entered as covariate. We then performed post hoc tests between pairs of subspecies using sequential Bonferroni-adjusted correction for multiple comparisons ($\alpha < 0.05$; Holm 1979, Rice 1989). To improve normality, the second and fifth principal component factors were log transformed prior to analysis (Quinn and Keough 2002).

Morphological analysis. We gathered morphological data during field expeditions, and from specimens

TABLE 1. Loadings of the first five principal component factors summarizing 15 acoustic variables measured from 573 individuals of different subspecies of *Troglodytes aedon*. Eigenvalues and the percentage of variation explained are presented for each component, and variables with the strongest loading are in bold.

	Factor 1 ^a	Factor 2 ^a	Factor 3 ^a	Factor 4 ^a	Factor 5 ^a
Eigenvalues	3.17	2.75	2.02	1.81	1.45
Variance explained (%)	21.10	18.30	13.50	12.10	9.60
Factor loadings					
Element length (s)	-0.16	0.33	0.06	-0.34	0.42
Inter-element interval (s)	0.02	-0.17	-0.18	-0.04	0.80
Mean maximum frequency (kHz)	0.28	0.82	0.03	0.42	0.03
Mean bandwidth (kHz)	0.30	0.35	-0.03	0.77	0.23
Mean peak frequency (kHz)	0.12	0.90	0.07	0.07	-0.04
Mean entropy	0.18	0.14	0.03	0.83	-0.13
Song length (s)	0.12	0.13	0.79	0.09	0.39
Number of elements	0.08	0.19	0.89	-0.01	-0.16
Number of trills	0.02	-0.07	0.72	-0.05	-0.18
Minimum frequency (kHz)	-0.05	0.42	-0.06	-0.33	-0.53
Maximum frequency (kHz)	0.76	0.42	0.16	0.16	-0.03
Peak frequency shifts per second	0.11	0.74	0.12	0.10	-0.20
SD in maximum frequency (kHz)	0.92	0.21	0.04	0.07	0.02
SD in bandwidth (kHz)	0.91	0.08	0.02	0.24	0.13
SD in entropy	0.81	-0.08	0.07	0.12	-0.12

^a Principal component analysis was based on the correlation matrix. Components with eigenvalues >1 were extracted. Factor scores were calculated using the regression method. The hypothesis that the correlation matrix contained only zero correlations was rejected (Bartlett's test: $\chi^2 = 6,066.9$, $df = 105$, $P < 0.001$).

preserved in three museum collections: the American Museum of Natural History in New York, the Field Museum of Natural History in Chicago, and Museo de Zoología "Alfonso L. Herrera" in Mexico City (see Supplemental Material Tables S3, S4). Following Pyle (1997), we measured seven morphological characters: wing chord and tail length at 1 mm accuracy, and tarsus length, exposed culmen length, culmen length, bill depth, and bill width at 0.1 mm accuracy. We then applied the same series of statistical analyses that were used for the acoustic analysis. First, we reduced the number of variables using a PCA with varimax rotation. The analysis resulted in two principal component factors with eigenvalues >1 that together explained 68.2% of the total variation in the seven morphological variables. The first factor was strongly associated with tarsus length, exposed culmen length, culmen length, bill depth, and bill width; and the second factor was strongly associated with wing chord and tail length (Table 2).

We then performed one-way ANCOVAs to test whether subspecies differed from each other in morphological characters, while controlling for variation in latitude. In each analysis, we included one of the two principal components as the dependent variable (Table 2). We used subspecies (i.e. *T. a. aedon*, *T. a. parkmanii*, *T. a. cahooni*, *T. a. brunneicollis*, *T. a. nitidus*, *T. a. musculus*, *T. a. beani*, or *T. a. rufescens*) as fixed factor in the model, and latitude was entered as covariate. We then performed a post hoc

test between pairs of subspecies using sequential Bonferroni correction for multiple comparisons.

For both the song and morphology ANCOVAs, residuals were normally distributed and all other assumptions were satisfied (Quinn and Keough 2002), except for homogeneity of regression slopes in the fifth acoustic factor and in the first morphological factor. The robustness of ANCOVA

TABLE 2. Loadings of the first two principal component factors summarizing seven morphological variables measured from 401 skins of different subspecies of *Troglodytes aedon*. Eigenvalues and the percentage of variation explained also are presented for each component. Factors with strong contributions to each principal component score are in bold.

	Factor 1 ^a	Factor 2 ^a
Eigenvalues	3.40	1.37
Variance explained (%)	48.60	19.60
Factor loadings		
Wing chord	0.40	0.75
Tail length	-0.24	0.81
Tarsus length	0.70	0.27
Exposed culmen length	0.86	0.12
Culmen length	0.86	0.11
Bill depth	0.76	-0.11
Bill width	0.78	-0.18

^a Principal component analysis was based on the correlation matrix. Components with eigenvalues >1 were extracted. Factor scores were calculated using the regression method. The hypothesis that the correlation matrix contained only zero correlations was rejected (Bartlett's test: $\chi^2 = 1,316.5$, $df = 21$, $P < 0.001$).

TABLE 3. Loadings of the first four principal component factors summarizing 15 acoustic variables measured from 786 *Troglodytes* songs, including subspecies of *Troglodytes aedon* and current recognized *Troglodytes* species. Eigenvalues and the percentage of variation explained also are presented for each component. Bold font indicates factors with strong contributions to each principal component score.

	Factor 1 ^a	Factor 2 ^a	Factor 3 ^a	Factor 4 ^a
Eigenvalues	4.21	3.47	2.89	1.15
Variance explained (%)	28.10	23.10	19.20	7.70
Factor loadings				
Element length (s)	0.83	-0.29	0.28	0.01
Inter-element interval (s)	-0.11	-0.04	-0.18	0.88
Mean maximum frequency (kHz)	0.04	0.24	0.94	0.05
Mean bandwidth (kHz)	-0.60	0.48	0.32	0.31
Mean peak frequency (kHz)	0.38	-0.06	0.84	-0.09
Mean entropy	-0.63	0.43	0.16	0.17
Song length (s)	0.87	-0.11	0.29	0.15
Number of elements	0.87	-0.11	0.29	-0.11
Number of trills	0.75	0.03	0.04	-0.14
Minimum frequency (kHz)	0.55	-0.26	0.33	-0.32
Maximum frequency (kHz)	0.03	0.77	0.44	-0.09
Peak frequency shifts per second	0.16	0.04	0.70	-0.34
SD in maximum frequency (kHz)	-0.08	0.91	0.09	-0.04
SD in bandwidth (kHz)	-0.32	0.89	0.00	0.11
SD in entropy	-0.23	0.80	-0.17	-0.02

^a Principal component analysis was based on the correlation matrix. Components with eigenvalues >1 were extracted. Factor scores were calculated using the regression method. The hypothesis that the correlation matrix contained only zero correlations was rejected (Bartlett's test: $\chi^2 = 12,329.8$, $df = 105$, $P < 0.001$).

to deviation of homogeneity of regression slopes increases with sample size (Hamilton 1976), and owing to our large sample size, we consider our analysis robust to the violation of this assumption for these two factors.

Acoustic divergence. We performed an additional PCA on all acoustic measurements, this time including the same eight House Wren subspecies in our original analysis, but adding measurements of the songs of seven recognized species: Cobb's Wren (*T. cobbi*), Clarion Wren (*T. tanneri*), Socorro Wren (*T. sissonii*), Rufous-browed Wren (*T. rufocillatus*), Ochraceous Wren (*T. ochraceus*), Winter Wren (*T. hiemalis*), and Pacific Wren (*T. pacificus*). The analysis resulted in four principal component factors with eigenvalues >1 that together explained 78.5% of the total variation in the original 15 acoustic variables. The first factor was strongly associated with song length, minimum frequency, element length, number of elements, number of trills, mean bandwidth, and mean entropy; the second factor was strongly associated with maximum frequency, SD in maximum frequency, SD in bandwidth, and SD in entropy; the third factor was strongly associated with mean maximum frequency, mean peak frequency, and peak frequency shifts per second; and the fourth factor was strongly associated with inter-element interval (Table 3).

We calculated acoustic divergence scores as the pairwise distance between principal component factors for different wren taxa. To account for both the distance between group means as well as within-group variance, we used Cohen's *d* scores in this analysis, providing a more accurate estimate

of divergence (Toews and Irwin 2008). We calculated Cohen's *d* as the difference between the two groups' mean principal component factor scores divided by the pooled SD (Cohen 1992). We calculated these acoustic divergence scores between each of the eight House Wren subspecies and all other House Wren subspecies (e.g., *T. a. aedon* vs. all other House Wren subspecies pooled). For comparison, we also calculated acoustic divergence scores between pairs of recognized *Troglodytes* species (e.g., *T. sissonii* vs. *T. tanneri*), selecting pairs of species that are known to be closely related: *T. ochraceus* vs. *T. rufocillatus* (Martínez Gómez et al. 2005); *T. pacificus* vs. *T. hiemalis* (Toews and Irwin 2008); *T. cobbi* vs. *T. musculus* (Campagna et al. 2012); and *T. sissonii* vs. *T. tanneri* (two species restricted to adjacent islands off Mexico's Baja coast). We conducted this comparison for each of the four principal component factors that summarize variation in acoustic features. All statistical analyses used PASW Statistics version 18.0 (Chicago, Illinois, USA).

RESULTS

We measured geographic variation in the songs and morphology of House Wrens across 609 sites that comprised most of the geographic distribution of this species complex in North and South America. We gathered a total of 1,065 recordings from different sources and selected 786 recordings for analysis from different individuals (Figure 1; Supplemental Material Tables S1,

TABLE 4. Summary of ANCOVA results for differences between subspecies of *Troglodytes aedon* in both acoustic ($n = 573$) and morphological ($n = 401$) traits, using latitude as a covariate.

Dependent variables	Model	<i>F</i>	df	<i>P</i>	η^2	R^2 adj.
Acoustic analysis						
Factor 1	Overall model	4.6	8	<0.001	0.06	0.04
	Subspecies	4.8	7	<0.001	0.05	
	Latitude	0.7	1	0.3	0.001	
Factor 2	Overall model	55.9	8	<0.001	0.4	0.43
	Subspecies	35.9	7	<0.001	0.3	
	Latitude	0.3	1	0.5	0.001	
Factor 3	Overall model	16.7	8	<0.001	0.1	0.18
	Subspecies	11.6	7	<0.001	0.1	
	Latitude	8.7	1	0.003	0.01	
Factor 4	Overall model	5.0	8	<0.001	0.07	0.05
	Subspecies	6.1	7	<0.001	0.07	
	Latitude	0.005	1	0.9	<0.001	
Factor 5	Overall model	30.8	8	<0.001	0.3	0.29
	Subspecies	30.8	7	<0.001	0.2	
	Latitude	31.1	1	<0.001	0.05	
Morphological analysis						
Factor 1	Overall model	69.5	8	<0.001	0.5	0.57
	Subspecies	76.4	7	<0.001	0.5	
	Latitude	182.3	1	<0.001	0.3	
Factor 2	Overall model	16.4	8	<0.001	0.2	0.23
	Subspecies	10.8	7	<0.001	0.1	
	Latitude	112.3	1	<0.001	0.2	

S2). Of the 786 recordings, 573 correspond to recordings of eight subspecies within the House Wren complex included in the present study: *T. a. aedon* ($n = 54$), *T. a. parkmanii* ($n = 103$), *T. a. cahooni* ($n = 45$), *T. a. brunneicollis* ($n = 14$), *T. a. nitidus* ($n = 24$), *T. a. musculus* ($n = 281$), *T. a. beani* ($n = 40$), and *T. a. rufescens* ($n = 12$). The remaining 213 recordings correspond to the seven recognized species in the House Wren complex that are included here: *T. cobbi* ($n = 12$), *T. rufociliatus* ($n = 26$), *T. sissonii* ($n = 30$), *T. tanneri* ($n = 41$), *T. ochraceus* ($n = 3$), *T. hiemalis* ($n = 65$), and *T. pacificus* ($n = 36$).

For the morphological analysis, we gathered morphometric data from 401 *Troglodytes* skins, all from adult male specimens, corresponding to *T. a. aedon* ($n = 19$), *T. a. beani* ($n = 12$), *T. a. brunneicollis* ($n = 10$), *T. a. cahooni* ($n = 32$), *T. a. musculus* ($n = 279$), *T. a. nitidus* ($n = 11$), *T. a. parkmanii* ($n = 29$), and *T. a. rufescens* ($n = 9$) (Supplemental Material Tables S3, S4).

Song analysis. Song differed significantly among the currently recognized subspecies in all five principal component factors (Table 4). Descriptively, we found that both *T. a. parkmanii*, in the United States and Canada, and *T. a. beani*, on Cozumel Island, have songs with higher scores for the first principal component factor, related to higher maximum frequencies, and larger variation in maximum frequencies, bandwidth, and entropy (Figure 2). North American subspecies (*T. a. aedon*, *T. a. parkmanii*, *T. a. cahooni*, *T. a. brunneicollis*, and *T. a. nitidus*) and *T. a. rufescens*, in Dominica, have songs with higher scores

for the second principal component factor, related to higher mean maximum and peak frequencies, and higher numbers of shifts in peak frequency (Figure 2A). *Troglodytes a. aedon*, in the United States and Canada, and *T. a. rufescens*, in Dominica, have songs with higher scores for the third principal component factor, related to longer song lengths and higher numbers of elements and trills (Figure 2B). *Troglodytes a. rufescens*, in Dominica, has songs with higher scores for the fourth principal component factor, related to higher mean bandwidth and mean entropy (Figure 2C). *T. a. brunneicollis* and *T. a. nitidus*, in Mexico, *T. a. musculus*, in South America, *T. a. beani*, in Cozumel Island, and *T. a. rufescens*, in Dominica, have songs with highest scores for the fifth principal component factor, related to longer element duration and inter-element interval, and lower minimum frequencies (Figure 2D).

Variation in two of five acoustic principal component factors had a significant association with latitude (Table 4). Values of the third factor, related to song length and number of elements and trills, decreased significantly with latitude, from north to south (Figure 3A). Values of the fifth factor, related to element duration, inter-element interval, and minimum frequency, increased significantly with latitude, reaching the highest values in Central America and then decreasing toward South America (Figure 3B).

Post hoc tests between subspecies following the ANCOVA on acoustic traits showed that *T. a. aedon*, *T.*

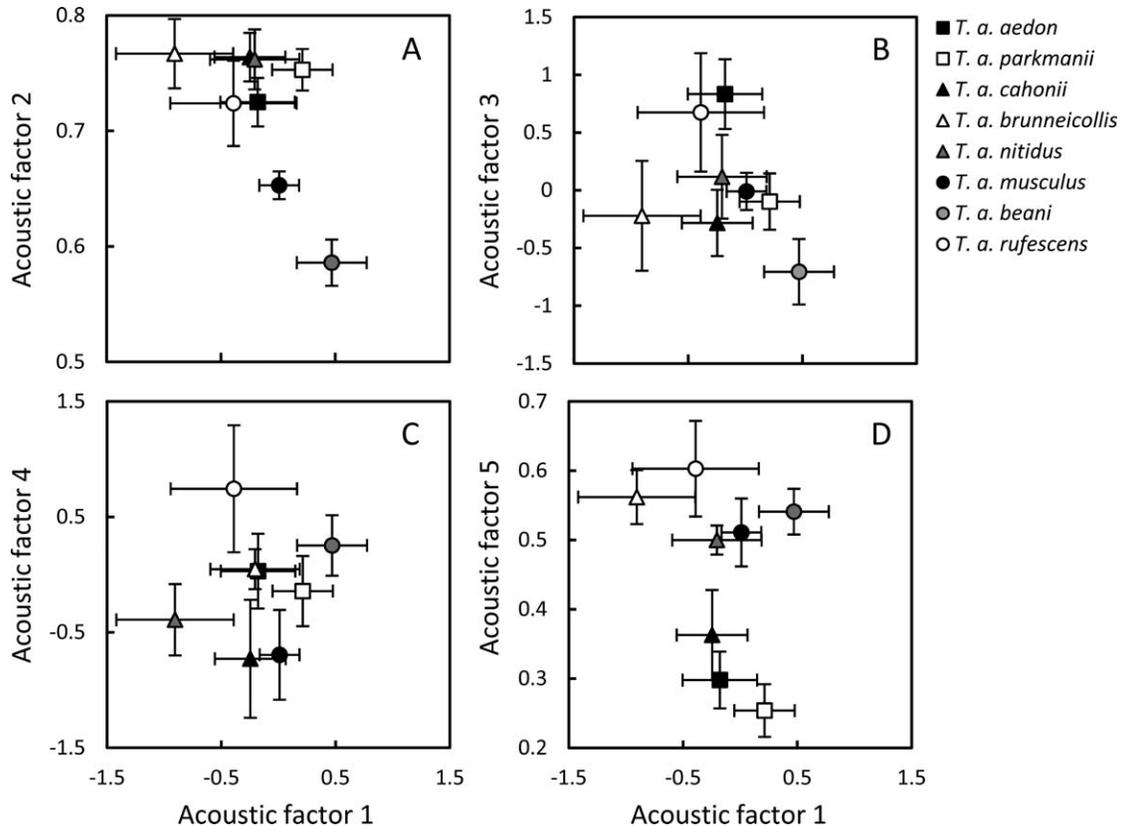


FIGURE 2. Acoustic variation between subspecies of *Troglodytes aedon* described by principal component factors that summarize variation in acoustic features of male songs. The first principal component factor is plotted against the second (A), third (B), fourth (C), and fifth (D). Points correspond to adjusted means after controlling for latitude. Bars indicate 95% confidence intervals.

a. parkmanii, *T. a. cahoonii*, *T. a. beani*, *T. a. musculus*, and *T. a. rufescens* were significantly different from each other and from all other subspecies, whereas differences in song between *T. a. nitidus* and *T. a. brunneicollis* were nonsignificant (Table 5).

Morphological analysis. Morphological traits differed significantly between subspecies for the two principal component factors (Table 4). Descriptively, we found that *T. a. beani*, from Cozumel Island, has higher scores for the first principal component factor, related to longer tarsus length and beak characteristics, than other subspecies (Figure 4). North American subspecies (*T. a. aedon*, *T. a. parkmanii*, *T. a. cahoonii*, *T. a. brunneicollis*, and *T. a. nitidus*) and *T. a. beani*, from Cozumel Island, have higher scores for the second principal component factor, related to longer wings and tails, compared to *T. a. musculus* and *T. a. rufescens* (Figure 4).

Variation in both morphological principal component factors showed a significant association with latitude (Table 4). Values of the first factor, related to tarsus length and beak morphology, increased significantly with latitude, reaching the highest values in Central America and then decreasing toward South America (Figure 3C). Values of

the second factor, related to wing and tail size, decreased significantly with latitude from north to south (Figure 3D).

The post hoc tests following the ANCOVA on morphological traits showed that *T. a. parkmanii* and *T. a. beani* were significantly different from each other and all other subspecies in all pairwise comparisons, whereas morphological differences between *T. a. aedon* and *T. a. cahoonii*, *T. a. aedon* and *T. a. brunneicollis*, *T. a. cahoonii* and *T. a. brunneicollis*, *T. a. nitidus* and *T. a. musculus*, *T. a. nitidus* and *T. a. rufescens*, and *T. a. musculus* and *T. a. rufescens* were significant for some comparisons and nonsignificant for others (Table 5).

Acoustic divergence. Average divergence scores between the songs of each subspecies and all other subspecies of House Wrens were substantial. We calculated an acoustic divergence score of 0.4 for factor 1 (range: 0.2–0.6); 0.5 for factor 2 (range: 0.3–1.0); 0.9 for factor 3 (range: 0.6–2.2); and 1.1 for factor 4 (range: 0.7–1.4) (Figure 5). Average divergence scores between pairs of closely related species were similar for factor 1 (0.4, range: 0.2–0.7), factor 2 (0.6, range: 0.1–1.1), factor 3 (1.9, range: 1.2–2.9), and factor 4 (0.5, range: 0.2–1.1), based on pairwise comparisons of *T. cobbi* vs. *T. a. musculus*, *T. tanneri* vs. *T. sissonii*,

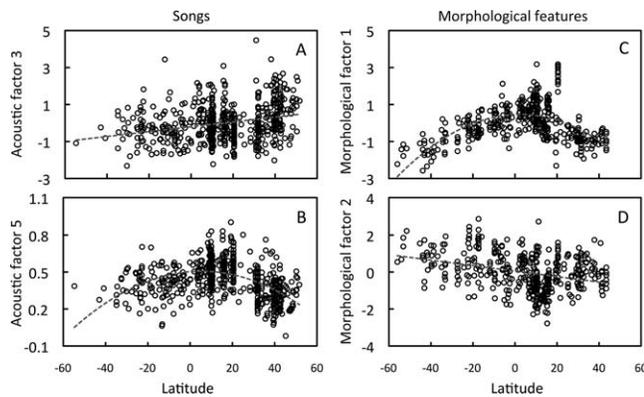


FIGURE 3. Songs and morphological features vary with latitude in subspecies of *Troglodytes aedon*. (A) Values of the third acoustic principal component factor, summarizing variation in song length, number of elements, and number of trills, decreases from north to south (linear regression: adjusted $R^2 = 0.07$, $F_{1, 567} = 46.5$, $P < 0.001$). (B) Values of the fifth acoustic principal component factor, summarizing variation in element length, inter-element interval, and minimum frequency (cubic regression: adjusted $R^2 = 0.2$, $F_{3, 556} = 68.8$, $P < 0.001$). (C) Values of the first morphological principal component factor, summarizing variation in tarsus length, exposed culmen length, culmen length, bill depth, and bill width (cubic regression: adjusted $R^2 = 0.3$, $F_{3, 397} = 83.5$, $P < 0.001$), follow a similar pattern, with an increase in values from north to south, reaching the maximum values in Central America and then decreasing toward South America. (D) Values of the first morphological principal component factor, summarizing wing chord and tail length, decrease from north to south (linear regression: adjusted $R^2 = 0.1$, $F_{1, 399} = 47.7$, $P < 0.001$).

T. ochraceus vs. *T. rufocillatus*, and *T. hiemalis* vs. *T. pacificus*. Our results suggest that acoustic divergence between pairs of subspecies of House Wrens was on the same order as acoustic divergence between recognized species for factor 1 (summarizing variation in element length, mean bandwidth, mean entropy, song length, number of elements and trills, and minimum frequency), factor 2 (summarizing variation in maximum frequency, variation in maximum frequency, variation bandwidth, and variation in entropy), and factor 4 (summarizing variation in inter-element interval). By contrast, divergence within subspecies was less pronounced for factor 3 (summarizing variation in mean maximum frequency, mean peak frequency, and peak frequency shifts per second) in our subspecies-level comparisons than in species-level comparisons (Figure 5; for full Cohen's d values for effect sizes of acoustic divergence, see Supplemental Material Table S5).

DISCUSSION

On the basis of acoustic data from 768 individuals from 373 locations throughout the Western Hemisphere, as well

as morphological data from 401 individuals from 236 locations, we quantified acoustic and morphological variation among subspecies of the House Wren complex. Our results reveal marked differences between all subspecies of House Wrens after controlling for latitude (as a proxy for distance), showing that it is possible to distinguish between them using acoustic traits, morphological traits, or both. Our acoustic analysis showed that vocal divergence between many subspecies was comparable to, or stronger than, the vocal divergence between pairs of currently recognized *Troglodytes* species. Together, these results suggest that at least four allopatric subspecies—*T. a. nitidus* (“Zempoaltepec Wrens”), *T. a. musculus* (“Southern House Wrens”), *T. a. beani* (“Cozumel Wrens”), and *T. a. rufescens* (“Dominica House Wrens”)—may merit species status. In addition, our results shed light into the patterns of acoustic and morphological variation within the House Wren complex and have important implications for the taxonomy of this complex.

We found that most of the pairs of allopatric subspecies included in our analysis (e.g., *T. a. aedon* and *T. a. beani*) have distinctive features to their songs. Divergent acoustic traits in allopatric populations have been reported to occur in many other bird species, with isolation as the most parsimonious explanation for this pattern (e.g., Vázquez-Miranda et al. 2009, González et al. 2011, Campagna et al. 2012, Alexandre et al. 2013, Sosa-López et al. 2013). For example, Cobb's Wrens (a close relative of House Wrens restricted to the Falkland Islands) are acoustically and genetically different from their continental counterparts (i.e. *T. a. musculus*; Campagna et al. 2012), as well as being morphologically different (Woods 1993). Moreover, experimental studies using playback have confirmed that divergent acoustic signals elicit different behavioral reactions in allopatric populations (e.g., de Kort and ten Cate 2001, Kirschel et al. 2009, Danner et al. 2011), supporting the idea that songs play a role in reproductive isolation.

Our morphological analysis shows that, despite general similarities, there are also significant differences between most pairs of allopatric subspecies (e.g., *T. a. nitidus* and *T. a. beani*). The general tendency of latitudinal increase in body size combined with a decrease in beak size in some subspecies of House Wrens (lowland subspecies *T. a. aedon* and *T. a. parkmanii*, vs. highland subspecies *T. a. cahooni*, *T. a. brunneicollis*, and *T. a. nitidus*; Figure 3) suggests that selection may drive morphological divergence along latitudinal gradients (McCormack and Smith 2008, Milá et al. 2010). Conversely, large beaks, such as those observed in *T. a. beani* on Cozumel Island, may be the result of relaxed competition for resources (Scott et al. 2003), a factor thought to drive divergence in beak size in island bird species (Boag and Grant 1984, Alexandre et al. 2013). Other factors, such as drift, however, are also known

TABLE 5. Results of post hoc pairwise comparisons of divergence between subspecies of *Troglodytes aedon*. Pairs of subspecies were compared for both acoustic and morphological divergence, and the principal component factors that showed significant differences following sequential Bonferroni correction are shown.

Subspecies	Acoustic differences?	Morphological differences?	Acoustic factors	Morphological factors
Sympatric pairs of taxa				
<i>T. a. aedon</i> vs. <i>T. a. parkmanii</i>	Yes	Yes	Factor 3	Factors 1, 2
<i>T. a. parkmanii</i> vs. <i>T. a. cahooni</i>	Yes	Yes	Factors 4, 5	Factor 1
<i>T. a. cahooni</i> vs. <i>T. a. brunneicollis</i>	Yes	No	Factor 5	
Allopatric pairs of taxa				
<i>T. a. aedon</i> vs. <i>T. a. cahooni</i>	Yes	No	Factor 3	
<i>T. a. aedon</i> vs. <i>T. a. brunneicollis</i>	Yes	No	Factor 5	
<i>T. a. aedon</i> vs. <i>T. a. nitidus</i>	Yes	Yes	Factor 5	Factor 1
<i>T. a. aedon</i> vs. <i>T. a. musculus</i>	Yes	Yes	Factors 2, 3, 5	Factor 1
<i>T. a. aedon</i> vs. <i>T. a. beani</i>	Yes	Yes	Factors 2, 3, 5	Factor 1
<i>T. a. aedon</i> vs. <i>T. a. rufescens</i>	Yes	Yes	Factor 5	Factor 1
<i>T. a. parkmanii</i> vs. <i>T. a. brunneicollis</i>	Yes	Yes	Factors 1, 4, 5	Factor 1
<i>T. a. parkmanii</i> vs. <i>T. a. nitidus</i>	Yes	Yes	Factors 4, 5	Factors 1, 2
<i>T. a. parkmanii</i> vs. <i>T. a. musculus</i>	Yes	Yes	Factors 2, 5	Factors 1, 2
<i>T. a. parkmanii</i> vs. <i>T. a. beani</i>	Yes	Yes	Factors 2, 3, 5	Factor 1
<i>T. a. parkmanii</i> vs. <i>T. a. rufescens</i>	Yes	Yes	Factor 5	Factors 1, 2
<i>T. a. cahooni</i> vs. <i>T. a. nitidus</i>	Yes	Yes	Factor 5	Factor 1
<i>T. a. cahooni</i> vs. <i>T. a. musculus</i>	Yes	Yes	Factor 2, 5	Factors 1, 2
<i>T. a. cahooni</i> vs. <i>T. a. beani</i>	Yes	Yes	Factors 1, 2, 5	Factor 1
<i>T. a. cahooni</i> vs. <i>T. a. rufescens</i>	Yes	Yes	Factors 3, 4, 5	Factors 1, 2
<i>T. a. brunneicollis</i> vs. <i>T. a. nitidus</i>	No	Yes		Factor 1
<i>T. a. brunneicollis</i> vs. <i>T. a. musculus</i>	Yes	Yes	Factors 1, 2	Factor 1
<i>T. a. brunneicollis</i> vs. <i>T. a. beani</i>	Yes	Yes	Factors 1, 2, 3	Factor 1
<i>T. a. brunneicollis</i> vs. <i>T. a. rufescens</i>	Yes	Yes	Factor 4	Factor 1
<i>T. a. nitidus</i> vs. <i>T. a. musculus</i>	Yes	No	Factors 2, 4	
<i>T. a. nitidus</i> vs. <i>T. a. beani</i>	Yes	Yes	Factors 2, 3	Factors 1, 2
<i>T. a. nitidus</i> vs. <i>T. a. rufescens</i>	Yes	No	Factor 4	
<i>T. a. musculus</i> vs. <i>T. a. beani</i>	Yes	Yes	Factors 2, 3	Factor 1
<i>T. a. musculus</i> vs. <i>T. a. rufescens</i>	Yes	No	Factor 2	
<i>T. a. beani</i> vs. <i>T. a. rufescens</i>	Yes	Yes	Factors 2, 3	Factors 1, 2

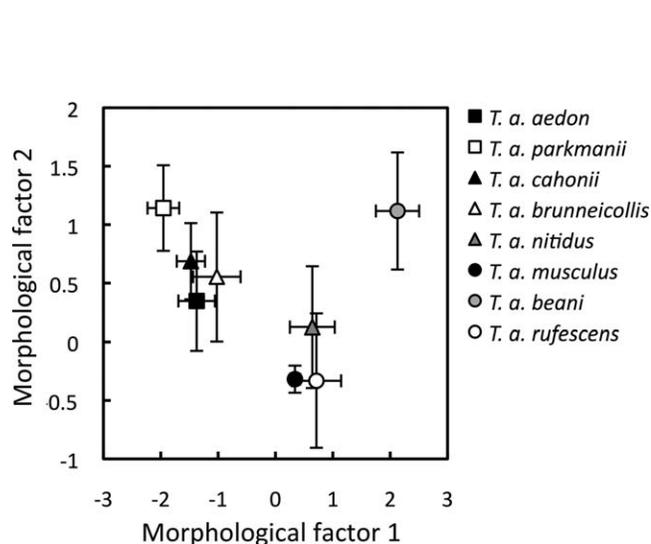


FIGURE 4. Morphological variation between subspecies of *Troglodytes aedon* described by principal component factors summarizing variation in morphological traits between the first two component factors. Points correspond to adjusted means after controlling for latitude. Bars indicate 95% confidence intervals.

to be related to divergence in morphological traits; future comparative studies can provide further insight into whether these factors are involved in morphological trait evolution in House Wrens.

Our results suggest that the subspecies of House Wrens with overlapping or abutting distributions have different songs (i.e. *T. a. aedon* vs. *T. a. parkmanii*, *T. a. parkmanii* vs. *T. a. cahooni*, and *T. a. cahooni* vs. *T. a. brunneicollis*) and morphology (i.e. *T. a. aedon* vs. *T. a. parkmanii* and *T. a. parkmanii* vs. *T. a. cahooni*). Whether variation between sympatric populations represents extremes of a continuum or there is a secondary contact zone between them is still an open question. For instance, vocal divergence in populations with sympatric distribution has been reported in several studies and has often mirrored differences from genetic or playback analyses (e.g., Dingle et al. 2008, 2010, Toews and Irwin 2008, Vázquez-Miranda et al. 2009, Sosa-López et al. 2013). Determining the presence of a secondary contact zone is challenging, particularly in a group like the House Wrens, which exhibit very subtle phenotypic variation that might aid in differentiating multiple forms (e.g., Toews and Irwin 2008). Future

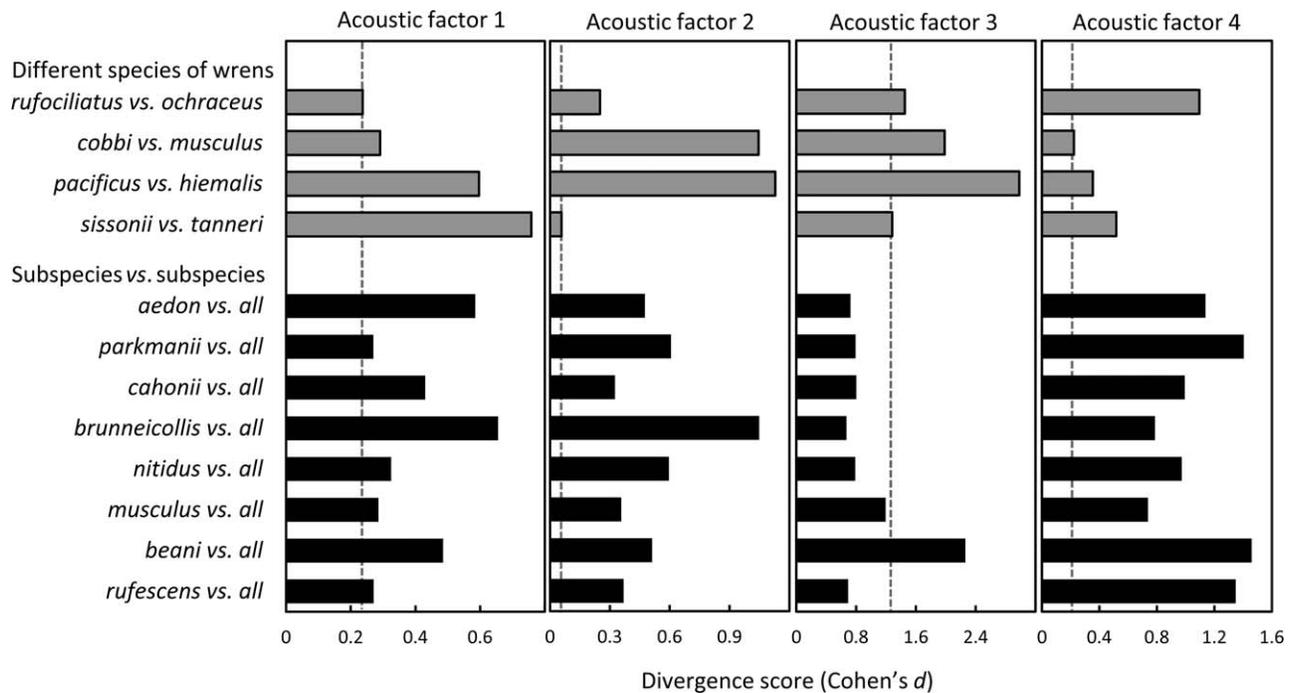


FIGURE 5. Graphs depicting divergence scores between pairs of closely related *Troglodytes* species (top) and between each subspecies of *T. aedon* and all other subspecies (bottom) for the first four principal component factors describing variation in acoustic features of males. Dashed lines indicate the lowest divergence score between pairs of closely related *Troglodytes* species. Divergence scores are expressed as the difference in the two groups' means divided by the pooled standard deviations (i.e. Cohen's *d*).

genetic analyses and playback studies will provide deeper insight into the differences between these taxa in both allopatry and sympatry and the consequences of the acoustic differences with regard to species recognition.

Taxonomically, should some of these bioacoustically divergent groups of House Wrens be considered different species? Our data show that the allopatric subspecies *T. a. nitidus*, *T. a. musculus*, *T. a. beani*, and *T. a. rufescens* can be differentiated from each other acoustically, and from the rest of the subspecies; thus, they may well be treated as different species under the phylogenetic concept (Nixon and Wheeler 1990). On the other hand, the biological species concept requires reproductive isolation between populations for the diagnosis of species (Mayr 1963), and further genetic analysis and playback experiments could help clarify whether complete isolation exists between these taxa. In addition to being acoustically different from one another, we found that the allopatric *T. a. nitidus*, *T. a. musculus*, *T. a. beani*, and *T. a. rufescens* are as different from their counterparts as pairs of recognized *Troglodytes* species, adding support to the hypothesis that they represent reproductively isolated lineages. Whether sympatric subspecies represent independent evolutionary lineages is still an open question, and further genetic analysis is needed before making a clear taxonomic assessment.

Although we lack genetic data to make a rigorous assessment of taxonomic status, it is relevant that many of these subspecies live in allopatry and that they have distinctive acoustic traits. We do not intend to encourage species definitions based on phenotypic dissimilarity (Moritz and Cicero 2004), but instead we conclude that our data strongly suggest that the current taxonomy underestimates the real diversity within the House Wren complex. We believe that future genetic studies will distinguish some of the currently recognized subspecies as full species. We encourage further taxonomic examination of both island populations and sympatric populations in the House Wren complex.

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