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Analysis of plumage, morphology, and voice reveals species-level differences between two subspecies of Prevost's Ground-sparrow *Melozone biarcuata* (Prévost and Des Murs) (Aves: Emberizidae)

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Abstract

Melozone biarcuata (Prevost's Ground-sparrow) has traditionally been divided into two allopatric groups based on differences in vocalizations and plumage characteristics: *M. b. cabanisi* in Costa Rica and *M. b. biarcuata/M. b. hartwegi* in northern Central America. However, the relationship between these subspecies has not been studied using a modern taxonomic approach. In this study, our objective was to provide the first detailed taxonomic comparison between these three subspecies using an integrative multi-trait analysis. We analyzed morphometric features, qualitative plumage patterns, and quantitative plumage measurements using spectral reflectance from all three subspecies, and we analyzed vocalizations for subspecies *M. b. biarcuata* and *M. b. cabanisi*. Our results show that *M. b. cabanisi* can be readily distinguished from the two other subspecies on the basis of morphometrics (*M. b. cabanisi* are smaller), plumage patterns (*M. b. cabanisi* have different facial markings and plumage patches), color differences (*M. b. cabanisi* have plumage patches that differ in color and brightness), and vocalizations (*M. b. cabanisi* have songs and calls that are acoustically distinct from those of *M. b. biarcuata*). By contrast, the two northern subspecies *M. b. biarcuata* and *M. b. hartwegi* were very similar for most traits, supporting previous suggestions that the two northern subspecies should be considered a single subspecies. Our data reveal that the differentiation in phenotypic characteristics between *M. b. cabanisi* versus *M. b. biarcuata* and *M. b. hartwegi* is similar to that reported for other complexes of subspecies where species status has been recognized. We argue that *M. b. cabanisi* should be treated as a species separate from *M. biarcuata* and propose that it be called *Melozone cabanisi*, White-faced Ground-sparrow. Our findings will contribute to the conservation efforts of the White-faced Ground-sparrow, which is endemic to Costa Rica's Central Valley and Turrialba Valley, by bringing focus to conservation policies that preserve ground-sparrow habitat (thickets, shade coffee plantations, and young secondary forest).

Key words: color differences, Emberizidae, ground-sparrows, *Melozone biarcuata*, *Melozone cabanisi*, morphology, plumage patterns, vocalizations

Introduction

The taxonomy of the family Emberizidae, which includes sparrows and buntings, has been the focus of several recent studies at different hierarchical levels. These studies have significantly altered our understanding of the family, such that species that were previously considered members of the Emberizidae have been moved into other families, and species from other families have been moved into Emberizidae (Klicka *et al.* 2000; 2007; García-Moreno *et al.* 2001; Barker *et al.* 2013, Klicka *et al.* 2014). Recent research has suggested that New World sparrows should be classified as a new family called Passerellidae (Barker *et al.* 2013). The evaluation and reorganization of species relationships within the family has involved (1) disentangling species relationships within such problematic genera as *Aimophila* and *Pipilo* (DaCosta *et al.* 2009), and (2) studying subspecies relationships in depth, such as in the genus *Arremon* (Cadena *et al.* 2007; Cadena & Cuervo 2010). Although these important studies provide us with a better understanding of the relationships between species of the Emberizidae, it is still necessary to carry out work on other species and genera to develop a more comprehensive understanding of species relationships within this family.

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The *Melozone* group (Chesser *et al.* 2010), sometimes known as the *Melozone-Pyrgisoma* group (DaCosta *et al.* 2009; Rising 2011), requires careful taxonomic examination. Previous studies have failed to resolve the species relationships within *Melozone* (e.g. DaCosta *et al.* 2009; Klicka *et al.* 2014). Furthermore, there are unresolved relationships among subspecies within this taxonomic group. An obvious example is the controversial subspecies complex comprised of *Melozone biarcuata biarcuata* (Prévost & Des Murs), *M. b. hartwegi* (Brodkorb), and *M. b. cabanisi* (Sclater & Salvin). *Melozone b. cabanisi* has been argued, at times, to be a separate species from the other two subspecies based on anecdotal observations of vocal and plumage differences (Sclater & Salvin 1868; Stiles & Skutch 1989; Howell & Webb 1995; AOU 1998; Sánchez *et al*. 2009). Another problematic issue is that the subspecies boundary between *M. b. biarcuata* and *M. b. hartwegi* is not clear (Fig. 1). In its original description, *M. b. hartwegi* is referred to as a lowland species of Chiapas with no overlap with *M. b. biarcuata* (Bodkorb 1938). Based on this allopatric distribution, and some plumage color differences, *M. b. hartwegi* was considered a separate subspecies (Bodkorb 1938). We now know, however, that *M. b. hartwegi* occurs continuously from 100 m to 2500 m throughout its distribution (Howell & Webb 1995), ruling out the argument that *M. b. hartwegi* is geographically disjunct from *M. b. biarcuata* (Fig. 1; Bodkorb 1938). For this reason, previous investigators have argued that M. *b. hartwegi* is not a valid subspecies, and have grouped it together with *M. b. biarcuata* (Hellmayr 1938; Rising 2011).

The taxonomic status of *M. b. cabanisi* has been problematic since this taxon's description. As early as 1868, Sclater and Salvin believed that *M. b. cabanisi* was a species separate from *M. b. biarcuata*, declaring: "it is unfortunate that all the naturalists who have met with specimens of [*M. b. cabanisi*] should have identified it wrongly." Nonetheless, *M. b. cabanisi* has generally been treated as a subspecies of *M. biarcuata*, rather than a separate species (Rising 2011). Despite the morphological differences exhibited by *M. biarcuata* (including obvious plumage differences on the head and breast), which have been acknowledged since its original description (Sclater & Salvin 1868; Stiles & Skutch 1989; Howell & Webb 1995; Rising 2011), to the best of our knowledge, the relationships between the three subspecies have never been studied using a quantitative taxonomic approach. As a consequence, this group's taxonomic status remains unclear (AOU 1998).

The objective of this investigation is to provide the first detailed and rigorous taxonomic study of the three *M. biarcuata* subspecies using an integrative multi-trait approach assessing phenotypic characteristics (plumage traits and spectrophotometry, morphology, and vocalizations). We evaluate whether *M. b. cabanisi* exhibits a unique combination of characters and may be better understood as a separate species from the two northerly subspecies *M. b. biarcuata* and *M. b. hartwegi*. We also evaluate whether *M. b. biarcuata* and *M. b. hartwegi* should be considered a single subspecies, using the methods of Patten (2010).

Methods

We compared morphometric measurements, plumage patterns, and plumage reflectance characteristics of adult specimens of *M. b. biarcuata*, *M. b. hartwegi*, and *M. b. cabanisi*, from the following museums: Museo de Zoología Universidad de Costa Rica, Museo Nacional de Costa Rica, Field Museum of Natural History in Chicago, University of Michigan Museum of Zoology, and Musée National d'Histoire Naturelle in Paris (Appendix A). We increased our sample size by including morphometric data collected from two adult male *M. b. cabanisi* captured in Costa Rica, because morphological data from live individuals and museum specimens showed no significant differences (Sandoval & Mennill 2013). Because the subspecies boundary between *M. b. biarcuata* and *M. b. hartwegi* is not clear (Fig. 1; see maps in Howell & Webb 1995; Rising 2011), we used the border between Mexico and Guatemala as the boundary between *M. b. biarcuata* (in Guatemala, Honduras, and El Salvador) and *M. b. hartwegi* (in Mexico).

Morphometry. We measured the culmen length (exposed culmen), culmen width and depth (at nares), tarsus length (from the intertarsal joint to the middle of the sole of the foot), tail length, and wing chord length (unflattened) from 22 *M. b. biarcuata*, 20 *M. b. hartwegi*, and 21 *M. b. cabanisi* museum specimens and two males captured in the field. The measurements taken from the live specimens fell within the range of the measurements taken from the museum skins. All measurements were taken to the nearest 0.1 mm following the methods of Sandoval & Mennill (2013). We conducted multivariate analyses of variance (MANOVA) to analyze which morphological measurements differed between the three subspecies. We conducted separate analyses for each sex,

because males are slightly larger than females (Sandoval & Mennill 2013). We conducted post-hoc tests (Dunn's tests) to compare the differences between morphological measurements between subspecies, for all morphological measurements that were different according to the MANOVA. Finally, for morphometric measurements that had different averages we calculated the pairwise diagnosability index proposed by Patten (2010), where diagnosability values ≥ 0 reveal that one population is diagnosable from the other, and values < 0 reveal no diagnosability.

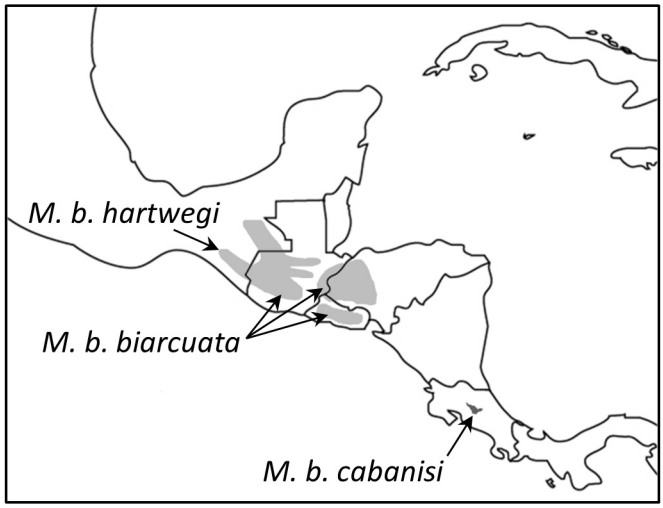

FIGURE 1. Distribution of the *Melozone biarcuata* subspecies from Mexico to Costa Rica, based on data from Stiles & Skutch (1989), Howell & Webb (1995), and Rising (2011). The distribution of *M. b. hartwegi* and *M. b. biarcuta* is continuous, whereas the southeastern distribution of *M. b. biarcuata* is discontinuous. The southern subspecies, *M. b. cabanisi*, is separated by approximately 550 km from the northern subspecies by the Nicaraguan depression.

Plumage traits and spectrophotometry. We performed a qualitative assessment of plumage patterns by visually evaluating a subsample of museum specimens; we selected specimens with plumage that showed no visible degradation or damage and where all plumage features were visible after preparation. We assessed adult birds of both sexes (11 *M. b. biarcuata*: 7 males and 4 females; 9 *M. b. hartwegi*: 4 males and 5 females; and 11 *M. b. cabanisi*: 6 males and 5 females). We focused our attention on body regions that have been reported to differ between *M. b. cabanisi* and other two subspecies (Sclater & Salvin 1868; Stiles & Skutch 1989; Howell & Webb 1995; Rising 2011), notably the head and the breast. No differences between plumage patterns have been reported between *M. b. biarcuata* and *M. b. hartwegi* (Bodkorb 1938).

To objectively quantify differences in plumage coloration, we measured plumage color using reflectance spectrophotometry focusing on ten body regions: throat, breast, belly, undertail coverts, forehead, crown, mantle, pre-ocular spot, cheek (because the cheek of *M. b. biarcuata* fades from black to rust, we targeted both areas of the cheek to obtain the measurements), and the lower flank (the side of the body, just below the tip of the folded wing). We measured the plumage characteristics for each of these ten body regions for 11 *M. b. biarcuata*, 9 *M. b. hartwegi*, and 11 *M. b. cabanisi* museum specimens. For each body region, we collected five measurements, moving the probe at least 3 mm between measurements, and keeping the probe at a fixed distance perpendicular to the feathers' surface using a rubber stopper (Andersson & Prager 2006). We collected these reflectance data using an Ocean Optics S2000 spectrophotometer combined with a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA), operated using OOIBase software on a laptop computer. We measured the reflectance as the percentage of light reflected in reference to a Spectralon pure white standard (WS-2, Ocean Optics).

All spectral analyses were conducted using the R package pavo (Maia *et al.* 2013). We used a tetrahedral color-space visual model to compare plumage coloration between the three groups; these visual models allowed us to compare colors while considering how the birds themselves would perceive them, unlike standard colorimetric approaches that consider only the properties of the reflective surface. We compared the characteristics of plumage patches between the three subspecies using the tetrahedral color-space model (Burkhardt 1989; Goldsmith 1990; Stoddard & Prum 2008) instead of the color opponency model developed by Vorobyev & Osorio (1998) because the color opponency model requires more species-specific information, little of which is available for *Melozone*

species. Determining the position of a color in tetrahedral color-space required us to make assumptions about: (1) peak sensitivities of the four cone photoreceptors of the animal's retina; (2) characteristics of the ambient light; and (3) characteristics of the background coloration in the ground-sparrows' habitat. (1) We used cone peak sensitivities of the average avian visual system for birds that possess an ultraviolet cone type because most passerines, and the species most closely related to *Melozone*, have an ultraviolet cone type with a peak sensitivity near 370nm (Hart 2001). (2) We used a "forest shade" ambient illumination because these *Melozone* ground-sparrows are found in relatively dense thickets. (3) We used an ideal (wavelength-independent) background because it allows plumage patches to be compared without the influence of a background, which in the case *M. biarcuata*, might change among and within locations. We calculated the achromatic component based on the stimulation of the two longest wavelength cones (Vorobyev & Osorio 1998).

We compared the colors of the same body region between individuals by subspecies using the Euclidean distance separating their three-dimensional coordinates in color-space. To avoid independence problems, we compared the plumage characteristics of each individual against all others, using a bootstrapping mean of the distance between them according to their index of similarity. Then we used one-way analysis of variance (ANOVA) to determine the mean differences in the chromatic component of body region per subspecies. If we found differences between subspecies, we conducted a post-hoc test (Dunn's test) to evaluate which subspecies were chromatically different. We compared the brightness value (achromatic component) per body region between subspecies using another ANOVA. For significant differences, we conducted a post-hoc test (Dunn's test) to evaluate which subspecies differed in brightness. Finally, for plumage spectrophotometry measurements that had different averages we calculated the pairwise diagnosability index, where diagnosability values ≥ 0 reveal that one population is diagnosable from the other, and values < 0 reveal no diagnosability.

Vocalizations. For our acoustic analyses we used recordings from 11 *M. b. biarcuata* and 32 *M. b. cabanisi*. We were unable to obtain recordings of *M. b. hartwegi* from the field or from sound libraries. We collected recordings in the field using a solid state digital recorder (Marantz PMD661) and a shotgun microphone (Sennheiser ME66/K6). We recorded *M. b. biarcuata* in Reserva Los Tarrales, Suchitepéquez, Guatemala (10°31'N, 91°08'W), and we recorded *M. b. cabanisi* in four Costa Rica locations: Getsemani, Heredia (10°01´N, 84°06'W); Calle Tiquisia, Heredia (10°02'N, 84°04'W); Aserrí, San José (9°51'N, 84°06'W); and Universidad de Costa Rica campus, San José (10°02'N, 84°04'W). All recordings that were collected by L. Sandoval are deposited in Laboratorio de Bioacústica Universidad de Costa Rica (see Appendix B). We supplemented our recordings with recordings from the private collections of colleagues, from the Macaulay Library of Natural Sounds Cornell Laboratory of Ornithology, and from the Laboratorio de Bioacústica Universidad de Costa Rica (catalogue number for all recordings are provided in Appendix B).

We measured the fine-structural properties (see below) of both the calls and the male solo songs for both *M. b. biarcuata* and *M. b. cabinisi*, which showed high levels of consistency in another *Melozone* species (Sandoval *et al.* in review). Although these birds produce duets (see Sandoval *et al.* 2013), we did not obtain high quality recordings of the duets for *M. b. biarcuata* during our field research, and therefore we could not compare this vocalization type quantitatively. For each vocalization we measured the duration (s), the minimum frequency (Hz), the maximum frequency (Hz), and the frequency of maximum amplitude (Hz). For male solo songs we measured the number of elements and the number of unique types of element per song. We collected acoustic measurements using Raven Pro 1.4 sound analysis software (Cornell Lab of Ornithology, Ithaca, NY, USA). We used the following settings in Raven to achieve frequency resolution of 188 Hz and temporal resolution of 5.8 ms: Hann window with 50% overlap and 256 kHz sampling frequency with 16 bit accuracy.

Following studies of *Buteo nitidus* (Latham; Millsap *et al.* 2011) and *Arremon torquatus* (Lafresnaye & D'Orbigny; Cadena & Cuervo 2010), we conducted a discriminant function analysis (DFA) to evaluate vocal variation between subspecies. We compared the two types of vocalizations between the two subspecies by calculating an average value for each measurement per individual, and using these values as our dependent variables in the DFA. We used a backward stepwise DFA to select the acoustic measurements that best distinguished *M. b. cabanisi* from *M. b. biarcuata*. We sequentially excluded from the analysis the variable with the lowest F value, one at the time, and re-ran the analysis after each deletion until we obtained the model with the lowest number of variables and highest correct assignment. We report the proportion of individuals correctly assigned to their correct taxonomic group based on a jackknife approach for all the analyzed cases. As a post-hoc test, we used a two sampled t-test to compare between both subspecies the differences between the acoustic

variables selected by DFA in the best model. We used SYSTAT (version 11.00.01; SYSTAT Software, Chicago, IL, USA) for all statistical analyses. Data are reported as means ± SE, and all tests are two-tailed.

Results

Morphometry. We found significant, although not diagnosable, morphometric differences between *Melozone biarcuata cabanisi*, *M. b. biarcuata*, and *M. b. hartwegi* in both sexes. For females, multivariate analysis of variance revealed that the best morphometric measurement to distinguish the groups was tail length (MANOVA: $F_{18,31} = 51.27$, $P < 0.001$). Post-hoc tests showed that female tail length was significantly longer in *M. b. biarcuata* and *M. b. hartwegi* than in *M. b. cabanisi* (Table 1). Our analysis of diagnosability, however, showed that this characteristic was not diagnosably different between subspecies (Table 2). The other five morphometric measurements were not diagnosably different between females of three subspecies (Table 1). For males, the morphometric measurements that best distinguished groups were tarsus length, tail length, culmen length, and beak height ($F_{18,102} = 106.82$, $P < 0.001$); but again these characteristics were not diagnosably different between subspecies. Post-hoc tests showed that tarsus, tail, and culmen were longer in male *M. b. biarcuata* and *M. b. hartwegi* than in *M. b. cabanisi* (Table 1). Bill depth was greater in male *M. b. hartwegi* than in *M. b. biarcuata* and *M. b. cabanisi* (Table 1). The other two morphometric measurements were not diagnosably different between males of all subspecies (Table 1).

TABLE 1. Mean (± SE) morphometric measurements by sex in three subspecies of *Melozone biarcuata*. Bold text indicates significant differences between subspecies; brackets in letters show the results of pair-wise post-hoc tests (Dunn's tests) where subspecies with different letters are statistically different.

	M. b. biarcuata	*M. b. hartwegi*	*M. b. cabanisi*
Females			
Tarsus (mm)	24.6 ± 0.5	24.1 ± 0.2	23.9 ± 0.3
Tail length (mm)	**60.2 ± 1.0 (a)**	**62.3 ± 0.8 (a)**	**56.7 ± 1.2 (b)**
Wing cord length (mm)	65.8 ± 1.8	64.3 ± 0.9	67.2 ± 0.9
Culmen length (mm)	12.6 ± 0.3	13.2 ± 0.2	12.3 ± 0.3
Beak width (mm)	8.1 ± 0.4	8.7 ± 0.2	7.9 ± 0.2
Beak depth (mm)	8.2 ± 0.4	7.7 ± 0.2	8.3 ± 0.2
Males			
Tarsus (mm)	**24.9 ± 0.2 (a)**	**25.1 ± 0.4 (a)**	**23.9 ± 0.3 (b)**
Tail length (mm)	**65.9 ± 0.9 (a)**	**67.3 ± 0.7 (a)**	**60.0 ± 0.8 (b)**
Wing cord length (mm)	69.5 ± 0.6	69.4 ± 0.5	68.4 ± 0.8
Culmen length (mm)	**13.0 ± 0.2(a)**	**13.5 ± 0.1 (b)**	**12.6 ± 0.1 (c)**
Beak width (mm)	7.9 ± 0.2	8.4 ± 0.2	8.3 ± 0.2
Beak depth (mm)	**8.3 ± 0.1 (a)**	**8.9 ± 0.1 (b)**	**8.3 ± 0.2 (a)**

Plumage patterns. *Melozone b. biarcuata* and *M. b. hartwegi* were indistinguishable in their plumage patterns, but showed considerable differences in plumage patterns compared to *M. b. cabanisi*. The most marked differences in plumage patterns were on the face and breast (Fig. 2). Around the eye, *M. b. cabanisi* exhibited a thin white eye ring, a small white postocular spot, and a large white preocular spot, whereas *M. b. biarcuata* and *M. b. hartwegi* exhibited a large white facial mask. *M. b. cabanisi* displayed a black moustachial stripe, a white malar stripe, and a black lateral throat stripe; both black stripes were lacking in *M. b. biarcuata* and *M. b. hartwegi*, which instead had a contrasting bicolored auricular patch (black fading to rust) above an incomplete white nape collar. The breast of *M. b. cabanisi* displayed a large circular black patch below the throat whereas *M. b. biarcuata* and *M. b. hartwegi* had no contrasting markings on a white breast. There was no evidence for sexual dimorphism in plumage features between males and females of each subspecies.

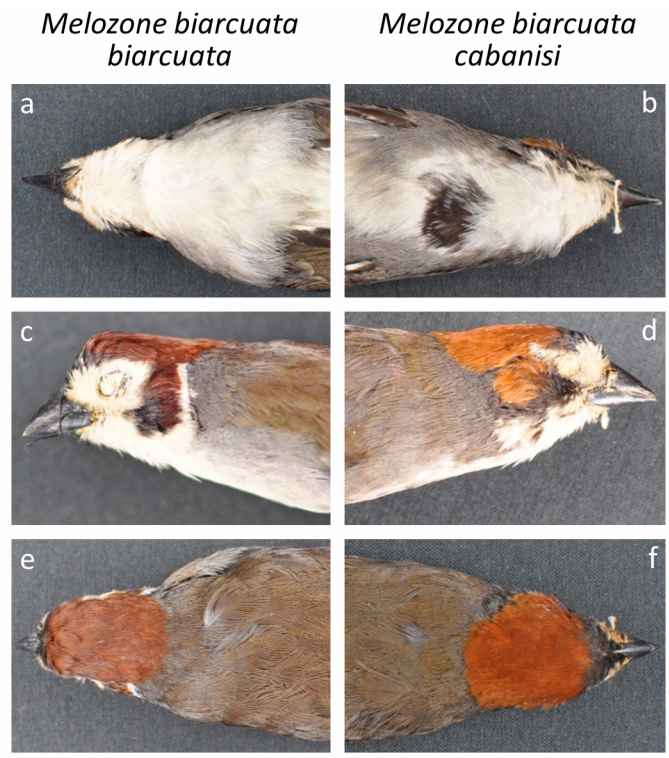

FIGURE 2. Plumage color and pattern differences between *M. b. biarcuta* (left) and *M. b. cabanisi* (right). Photographs were taken under the same light conditions at the Musée National d'Histoire Naturelle in Paris, France. The top row compares the ventral surfaces, the middle row shows lateral surfaces, and the bottom row shows dorsal surfaces. Note the black spot on the white breast of *M. b. cabanisi*, and the numerous head pattern differences between the subspecies.

Plumage color. Our visual models revealed notable differences in reflectance for some body regions between groups (Fig. 3). Our analyses revealed that the most pronounced differences in color were in the cheek and the breast. *Melozone b. biarcuata*/*M. b. hartwegi* showed bicolored cheeks (black fading to rust), whereas *M. b. cabanisi* showed rust-colored cheeks. In the breast, *M. b. biarcuata*/*M. b. hartwegi* showed a grey to white breast, but *M. b. cabanisi* showed a black breast spot on a white background color. For the chromatic component of reflectance, our visual models show that cheek color ($F_{2,27}$ = 8.60, P = 0.001) and breast color ($F_{2,27}$ = 5.54, P = 0.01) differed significantly between *M. b. biarcuata*/*M. b. hartwegi* and *M. b. cabanisi* (post-hoc pair-wise comparisons; Cheek: *biarcuata vs cabanisi*: P = 0.001; *hartwegi vs cabanisi*: P = 0.001; and *biarcuata vs hartwegi*: P = 0.88. Breast: *biarcuata vs cabanisi*: P = 0.007; *hartwegi vs cabanisi*: P = 0.01; and *biarcuata vs hartwegi*: P = 0.95). The breast was diagnosably different between all subspecies and the cheek was diagnosably different between *biarcuata* and *cabanisi* and between *biarcuata* and *hartwegi* (Table 2). For the achromatic component, the brightness of both the breast ($F_{2,28}$ = 36.99, P < 0.001) and undertail coverts ($F_{2,28}$ = 4.43, P = 0.02) differed significantly between *M. b. biarcuata*/*M. b. hartwegi* and *M. b. cabanisi* (Breast: *biarcuata vs cabanisi*: P < 0.001; *hartwegi vs cabanisi*: P < 0.001; and *biarcuata vs hartwegi*: P = 0.42. Undertail coverts: *biarcuata vs cabanisi*: P = 0.048; *hartwegi vs cabanisi*: P = 0.007; and *biarcuata vs hartwegi*: P = 0.30). The breast was diagnosably different between *biarcuata* and *cabanisi* and between *hartwegi* and *cabanisi* (Table 2). The brightness of the belly was significantly different between *M. b. biarcuata*/*M. b. cabanisi* and *M. b. hartwegi* ($F_{2,28}$ = 8.18, P = 0.001; *biarcuata vs cabanisi*: P = 0.33, *hartwegi vs cabanisi*: P = 0.004; and *biarcuata vs hartwegi*: P = 0.001; see Table 2 for diagnosability results). Finally, the brightness of the cheeks was significantly different between *M. b. hartwegi*/*M. b. cabanisi* and *M. b. biarcuata* ($F_{2,28}$ = 4.82, P = 0.02; *biarcuata vs cabanisi*: P = 0.006; *hartwegi vs cabanisi*: P = 0.55; and *biarcuata vs hartwegi*: P = 0.04; see Table 2 for diagnosability results). For all other body patches our visual models revealed no significant differences for the chromatic or achromatic component of reflectance (P > 0.05 for all tests).

TABLE 2. Results of diagnosability tests for morphometric and plumage color features that differed in our post-hoc comparisons for morphometric and plumage color analysis between the three *Melozone biarcuata* subspecies: *M.b. biarcuata, M. b. cabanisi, and M.b. hartwegi*. Bold text indicates significant diagnosability differences between subspecies. Diagnosability values ≥ 0 reveal that one population is diagnosable from the other (shown in bold), and values < 0 reveal populations that are not diagnosable, following Patten (2010).

Morphometric features	***biarcuata* versus *cabanisi***	***cabanisi* versus *biarcuata***	***biarcuata* versus *hartwegi***	***hartwegi* versus *biarcuata***	***hartwegi* versus *cabanisi***	***cabanisi* versus *hartwegi***
Female tail length	-9.2	-3.9	-7.2	-3.4	-7.5	-6.0
Male tarsus length	-2.5	-2.2	-3.6	-3.1	-2.4	-2.6
Male tail length	-3.7	-4.2	-13.3	-10.2	-3.2	-6.8
Male culmen length	-1.0	-1.4	-1.6	-1.5	-0.5	-1.0
Male beak depth	-1.6	-1.5	-1.2	-1.0	-1.1	-1.1
Plumage features						
Breast brightness	**0.1**	-0.1	-0.2	-0.4	**0.2**	**0.1**
Under-tail covert brightness	-0.3	-0.3	-0.2	-0.2	-0.3	-0.3
Belly brightness	-0.2	-0.1	-0.1	-0.1	-0.1	-0.1
Cheek brightness	-0.1	**0.0**	-0.1	-0.1	-0.1	-0.1
Breast color	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Cheek color	**0.0**	**0.0**	**0.0**	**0.0**	-0.1	-0.1

Voice. *Melozone b. cabanisi* exhibited significant acoustic differences in comparison to *M. b. biarcuata* (Fig. 4). For calls, we found that the fine structural measurement that best distinguished *M. b. biarcuata* calls from *M. b. cabanisi* calls was the maximum frequency (DFA: Wilks' $\lambda = 0.50$, $F_{1,14} = 14.10$, $P = 0.002$). This measurement correctly classified 82% of *M. b. biarcuata* to the correct group (9 of 11) and 100% of the *M. b. cabanisi* in the correct group (5 of 5). In post-hoc analyses of calls, maximum frequency ($t_{14} = 3.8$, $P = 0.002$), minimum frequency ($t_{14} = 3.0$, $P = 0.01$), and frequency of maximum amplitude ($t_{14} = 3.0$, $P = 0.01$) exhibited higher values in *M. b. cabanisi* than in *M. b. biarcuata* (Table 3). Call duration was not diagnosably different between subspecies ($t_{14} = 1.10$, $P = 0.29$, Table 3).

TABLE 3. Mean (± SE) values of male solo song and call fine acoustic measurements by sex and *Melozone biarcuata* subspecies. Bold text variables indicate significant differences between subspecies.

Solo songs	***M. b. biarcuata***	***M. b. cabanisi***
Number of elements	6.06 ± 0.38	7.91 ± 0.66
Number of unique element types	3.21 ± 0.22	3.60 ± 0.13
Duration (s)	**1.76 ± 0.22**	**1.46 ± 0.08**
Minimum frequency (Hz)	2277 ± 81	2814 ± 225
Maximum frequency (Hz)	**8582 ± 360**	**10460 ± 234**
Frequency of maximum amplitude (Hz)	**4726 ± 376**	**5456 ± 188**
Calls		
Duration (s)	1.33 ± 0.28	0.81 ± 0.32
Minimum frequency (Hz)	**3248 ± 444**	**5535 ± 570**
Maximum frequency (Hz)	**9080 ± 433**	**11719 ± 394**
Frequency of maximum amplitude (Hz)	**5212 ± 324**	**6943 ± 456**

For male solo songs, we found that the fine structural measurements that best separated *M. b. biarcuata* from *M. b. cabanisi* were song duration, maximum frequency, and frequency of maximum amplitude (DFA: Wilks' $\lambda = 0.28$, $F_{6,15} = 6.39$, $P < 0.001$). Together, these three measurements correctly classified 100% of *M. b. biarcuata* to

the correct group (9 of 9) and 92% of *M. b. cabanisi* to the correct group (12 of 13). Post-hoc tests revealed that *M. b. cabanisi* had higher maximum frequencies (t_{20} = 4.6, P < 0.001) and more song elements (t_{20} = 2.2, P = 0.04), as well as non-significant tendencies for higher frequencies of maximum amplitude (t_{20} = -1.9, P = 0.07) and higher minimum frequencies (t_{20} = 1.9, P = 0.07; Table 2). Solo song duration (t_{20} = 1.5, df = 20, P = 0.16) and number of element types (t_{20} = 1.6, P = 0.12) were not diagnosably different between the subspecies (Table 3).

We did not obtain a sufficient number of high quality recordings of the duets of ground-sparrows in the field, in part because their duets are very quiet sounds (see Sandoval *et al.* 2013). We heard *M. b. cabanisi* perform duets on a few occasions; to our ear, they differed from the duets of southern birds, and based on one recording of intermediate quality, they appear to differ structurally (Fig. 4g, h).

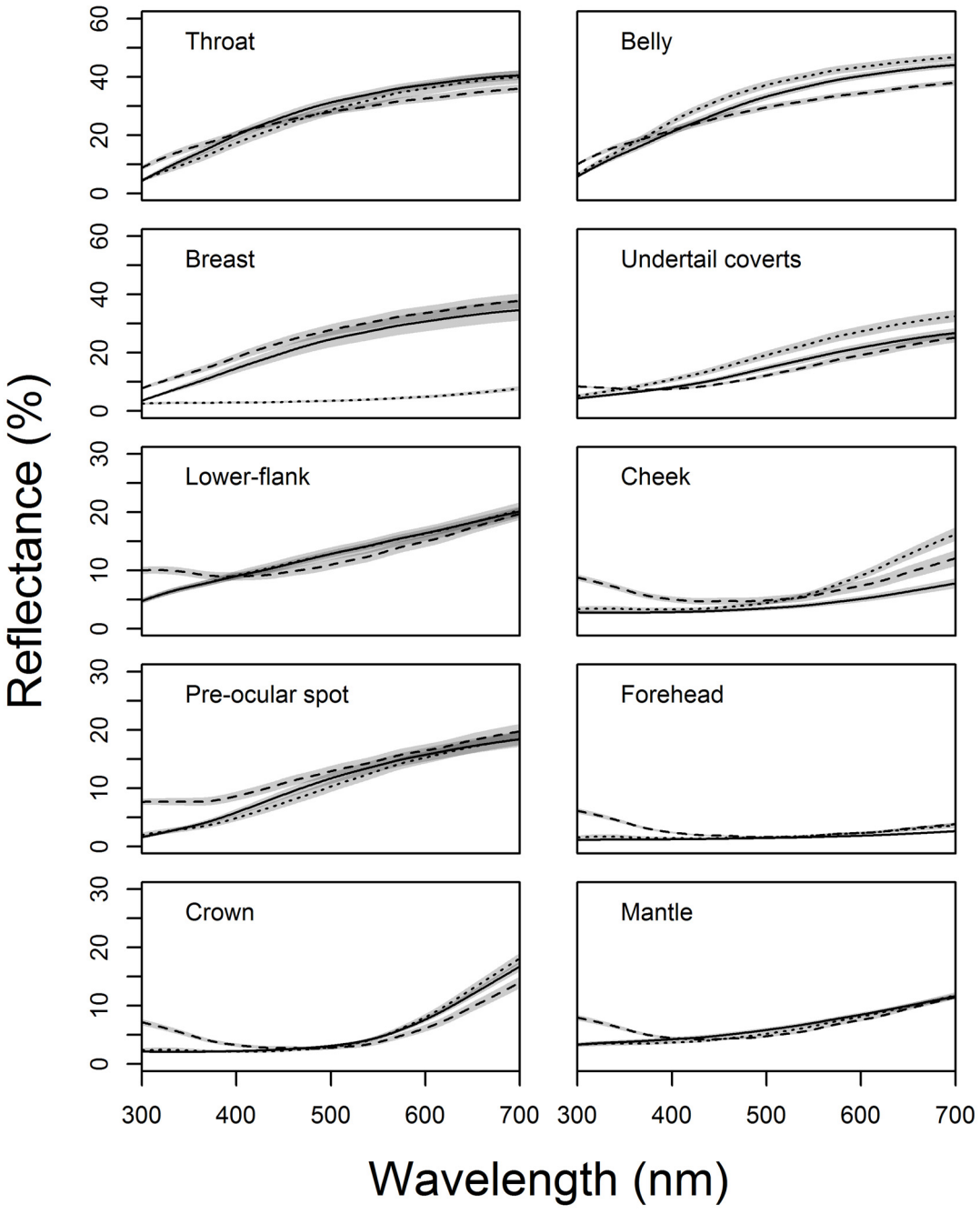

FIGURE 3. Average reflectance spectra for ten body regions measured in 11 *M. b. biarcuata,* 9 *M. b. hartwegi*, and 11 *M. b. cabanisi*. The gray area around each line represents standard error of the mean calculated at every 1nm. Solid lines show *M. b. biarcuata*; dashed lines show *M. b. hartwegi*; and dotted lines show *M. b. cabanisi*. Note that *M. b. cabanisi* and *M. b. biarcuata/M. b. hartwegi* show particularly divergent patterns for in their breast and cheek reflectance patterns.

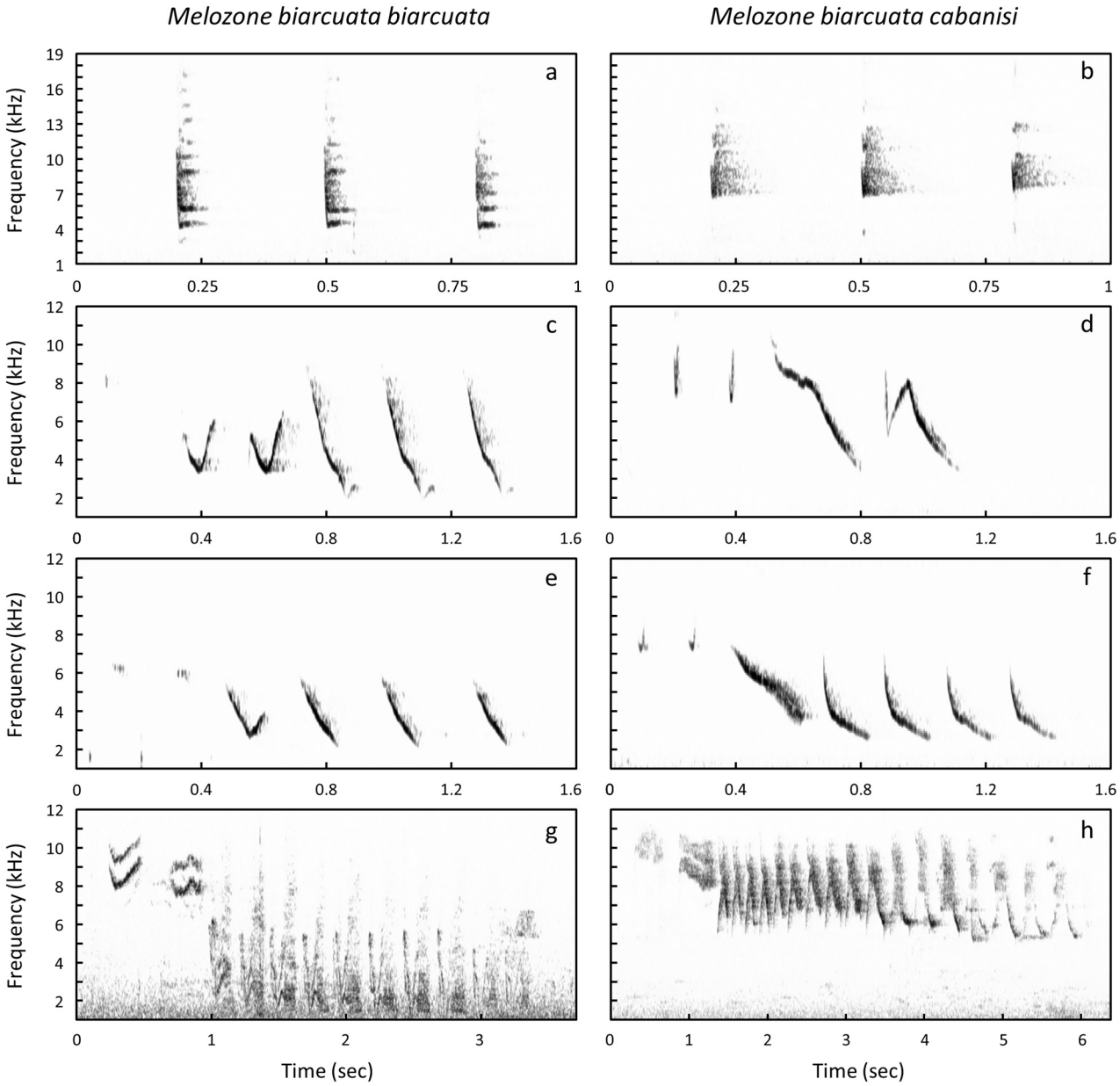

FIGURE 4. Sound spectrograms of calls (a, b), male solo songs (c–f), and duets (g, h) of *M. b. biarcuata* (left) and *M. b. cabanisi* (right). See text for a detailed explanation of the differences between subspecies.

Discussion

Our data show that *Melozone biarcuata cabanisi* in Costa Rica is diagnosable from the *M. b. biarcuata*/*M. b. hartwegi* group in Mexico, Guatemala, El Salvador, and Honduras based on phenotypic characteristics. *Melozone b. cabanisi* can be readily distinguished by morphology, plumage patterns, plumage color differences, and vocalizations, and is geographically isolated from the *M. b. biarcuata*/*M. b. hartwegi* group by more than 500 km. There are no records to date in the area between the two parts of their range. Based on our results, which include four classes of characters, we conclude that *M. b. cabanisi* and the *M. b. biarcuata*/*M. b. hartwegi* group exhibit differences on par with those of many closely related species. We propose that *M. b. cabanisi* should be elevated to species status as *M. cabanisi*, White-faced Ground-sparrow. We also propose that *M. b. biarcuata* and *M. b. hartwegi* should be grouped in the same subspecies—called *M. b. biarcuata*—based on the high degree of

morphological similarities and lack of any defined boundary in the distribution of these subspecies. Below we explore in more detail each of the differences which point towards a high level of differentiation between *M. b. cabanisi* and *M. b. biarcuata*/*M. b. hartwegi*.

When closely related taxa inhabit similar habitats, they often show high morphological similarity (Mayr 1976; Ricklefs 2012). We found significant differences in morphometric measurements even though the two groups inhabit similar habitats (Stiles & Skutch 1989; Howell & Webb 1995; L. Sandoval pers. obs.). Our results for body size agree with initial reports (Sclater & Salvin 1868), which indicated that *M. b. cabanisi* were smaller than *M. b. biarcuata*/*M. b. hartwegi*. Interestingly, the differences in body size are consistent with Bergmann's rule, which states that individuals at higher latitudes have larger body sizes (Meiri 2011). *Melozone b. biarcuata*/*M. b. hartwegi* were more similar in morphological measurements than either was with *M. b.cabanisi*. The lack of difference in morphometric measurements between *M. b. biarcuata* and *M. b. hartwegi* echo the findings of Brodkorb (1938), who also proposed that these subspecies were not distinguishable based on morphological measurements.

Plumage patterns were markedly different between *M. b. cabanisi* and the *M. b. biarcuata*/*M. b. hartwegi* group, allowing unambiguous diagnosis in the field on the basis of face pattern and breast mark. Furthermore, our visual models revealed differences in two chromatic components (breast and cheek color) and two achromatic components (breast and under-tail cover brightness) of reflectance. The breast in *M. b. cabanisi* showed a black spot lacking in the two northern subspecies. The cheek in *M. b. cabanisi* was bicolored (black fading to rust), while in *M. b. biarcuata*/*M. b. hartwegi* it was rufous throughout. Avian plumage patterns are important signals of species recognition and territory defense (Matyjasiak 2005). Inside the dense habitats that these ground-sparrows inhabit, the breast and facial characteristics are conspicuous features. The observed color and pattern differences in these body regions could therefore be an important component of species recognition, and may serve as important reproductive isolation barriers, were the northern and southern subspecies to come into contact. However, a more detailed experimental study testing these hypotheses is necessary to evaluate the exact function of the plumage traits and color differences in these taxa.

Our analyses of vocal characteristics revealed that differences in frequency and the number of elements in male songs allow discrimination between *M. b. biarcuata* and *M. b. cabanisi* with a very high level of accuracy. In addition, differences in call frequency allowed the proper assignment of subspecies with mean accuracy greater than 90%. Solo songs play an important role in female attraction and territory defense in *Melozone leucotis* (Cabanis), a closely related species (Sandoval & Mennill 2012; Sandoval *et al.* 2013; 2014), and our field observations suggest that the same may be true in *M. b. biarcuata* and *M. b. cabanisi.* Significant differences in the fine structural features of solo songs, such as those we report here, could potentially act as a reproductive barrier for the subspecies, if the subspecies were to come into contact. As with male solo songs, calls of *M. b. biarcuata* and *M. b. cabanisi* were highly divergent. These have previously been demonstrated to work mainly as contact and alarm signals in this genus (Sandoval *et al.* 2013; Sandoval & Mennill 2014), suggesting that selective factors beyond sexual selection may be influencing the evolution of the acoustic characteristics of vocalizations in the genus *Melozone*.

M. b. cabanisi are separated from *M. b. biarcuata*/*M. b. hartwegi* by a gap of *ca.* 550 km. This separation is caused by the disjoint distribution of the montane habitats that these two ground-sparrows inhabit (Stiles & Skutch 1989; Howell & Webb 1995; Rising 2011); regions north of Nicaragua and northern Costa Rica, respectively, are separated by the Nicaragua depression (Ferrez Weinberg 1992; Marshall & Liebherr 2000). Two significant geographical barriers between the subspecies are the humid highlands of southern Honduras and northern Nicaragua, and the dry lowlands of the Nicaragua depression (Stiles & Skutch 1989; Howell & Webb 1995). How this separation occurred is unknown; however, climatic oscillation during the Pleistocene may have influenced the current distribution (Haffer 1974; 1987; Webb & Rancy 1996; Barrantes 2009). A phylogeographic analysis will be needed to confirm how long they have been in allopatry.

In conclusion, we found that *M. b. cabanisi* was fully distinguishable from *M. b. biarcuata*/*M. b. hartwegi* based on our comparisons of discrete and continuous phenotypic characteristics used in different contexts: locomotion (tarsus size), feeding (beak morphology), reproduction and territoriality (solo song and plumage patterns), and alarm communication (calls). These differences are similar to differences in phenotypic characteristics reported for the *Arremon torquatus* sparrow complex (Cadena & Cuervo 2010), which is now recognized as eight different species (Chesser *et al.* 2012; Remsen *et al.* 2013). For example, *A. torquatus assimilis* and *A. t. atricapillus*, and *A. t. poliophrys* and *A. t. torquatus* showed distinct plumage pattern differences without

intermediate individuals (Cadena & Cuervo 2010), just as we observed for *M. b. cabanisi* versus the *M. b. biarcuata*/*M. b. hartwegi* group. *Arremon t. assimilis* and *A. t. atricapillus*, and *A. t. poliophrys* and *A. t. torquatus* showed 100% correct classification between the species groups based on discriminant analysis using acoustic features of songs, whereas our analysis showed 96% correct classification between subspecies *M. b. biarcuata* and *M. b. cabanisi*.

Conservation implications for White-faced Ground-sparrow. The White-faced Ground-sparrow is endemic to the Central Valley of Costa Rica (from Atenas and San Ramón in Alajuela province to Paraiso in Cartago province), the Turrialba Valley on the Caribbean side of the country, and the western part of Monteverde mountain range, Guanacaste province, from 500 to 1700 m (Stiles & Skutch 1989; Garrigues & Dean 2007; L. Sandoval pers. obs.). This ground-sparrow inhabits mainly thickets, shade coffee plantations, and young secondary forest (Stiles & Skutch 1989; Garrigues & Dean 2007; Sánchez *et al.* 2009), habitats that are not protected by any conservation laws in Costa Rica. The intense levels of urbanization in Costa Rica's Central Valley endanger these thicket habitats and coffee plantations, reducing the total coverage of this habitat and fragmenting what habitat remains (Joyce 2006; Biamonte *et al.* 2011). If urbanization of thicket and shade coffee habitat continues at its current pace, the White-faced Ground-sparrow faces an uncertain future, potentially making this species one of the more endangered birds in Costa Rica. This endemic taxon brings to light the importance of conserving early successional habitats.

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APPENDIX A. List of skins used in this study, measured at Museo de Zoología Universidad de Costa Rica (UCR), Museo Nacional de Costa Rica (MNCR), the Field Museum of Natural History (FMNH), the University of Michigan Museum of Zoology (MZUM), and the Muséum National d'Histoire Naturelle (MNHN).

Melozone biarcuata biarcuata:
Female: FMNH 109482, FMNH 22986, FMNH 109483, FMNH 109480, MNHN 1880-3400.
Male: MZUM 98401, MZUM 108106, MZUM 89016, MZUM 108105, FMNH 212687, FMNH 212685, FMNH 109481, FMNH 23374, FMNH 22988, FMNH 22990, FMNH 22985, FMNH 22987, FMNH 22983, FMNH 22984, FMNH 22989, FMNH 23373, FMNH 212682.
Melozone biarcuata hartwegi:
Female: MZUM 94608, MZUM 103527, MZUM103529, MZUM107783, MZUM 107784, MNHN 1975-798, MNHN 1975-799, MNHN 1975-800.
Male: MZUM 94610, MZUM 94609, MZUM 94607, MZUM 103526, MZUM 103528, MZUM 103530, MZUM 103531, MZUM 107780, MZUM 107781, MZUM 107785, MZUM 103959, MNHN 1975-797.
Melozone biarcuata cabanisi:
Female: UCR 3176, UCR 2577, MNCR 186, FMNH 6834, FMNH 72939, FMNH 72938.
Male: UCR 2436, UCR 2435, UCR 1218, MNCR6335, MNCR23050, MNCR5175, MNCR 23051, MNCR 4561, FMNH 374214, FMNH 6835, FMNH 72940, FMNH 72937, MNHN 1999-2299, MNHN 1999-2297.

APPENDIX B. List of recordings used in this study, obtained from Laboratorio de Bioacústica Universidad de Costa Rica (UCR), the Macaulay Library of Natural Sounds Cornell Laboratory of Ornithology (ML), the private collection of Jesse Fagan (JF), and the private collection of Knut Eisermann (KE). Asterisks indicate recordings made by L. Sandoval that are being archived in Laboratorio de Bioacústica Universidad de Costa Rica and are awaiting catalogue numbers.

Melozone biarcuata biarcuata:
15259ML El Salvador, Santa Ana, Cerro Verde; 106025ML El Salvador, Sonsonate, Finca Altamira; KE57 Guatemala, Tucurú,

Alta Verapaz, Guaxac; KE74 Guatemala, Solitarius; KE90 Guatemala, Solitarius; JF01 Guatemala, Los Fraijanes; JF02 Guatemala, San Juan La Laguna; JF03 Guatemala, Guatemala City; JF04 Guatemala, Guatemala City; JF05 Guatemala, Guatemala City; JF06 Guatemala, Panajatchel; *MBB1 Guatemala, Reserva Los Tarrales; *MBB2 Guatemala, Reserva Los Tarrales; *MBB3 Guatemala, Reserva Los Tarrales; *MBB4 Guatemala, Reserva Los Tarrales.

Melozone biarcuata cabanisi:

UCR01066 Costa Rica, Heredia, Calle Hernández; UCR01067 Costa Rica, Heredia, Calle Hernández; UCR01068 Costa Rica, Heredia, Calle Hernández; UCR01069 Costa Rica, Heredia, Calle Hernández; UCR01070 Costa Rica, Heredia, Getsemani; UCR01071 Costa Rica, Heredia, Getsemani; UCR01072 Costa Rica, Heredia, Getsemani; UCR01073 Costa Rica, Heredia, Getsemani; UCR01074 Costa Rica, Heredia, Getsemani; UCR01075 Costa Rica, Heredia, Getsemani; UCR01076 Costa Rica, Heredia, Getsemani; UCR01077 Costa Rica, Cartago, Ujarras; UCR01078 Costa Rica, Cartago, Ujarras; UCR01079 Costa Rica, Cartago, Ujarras;UCR01080 Costa Rica, Turrialba, CATIE; UCR01081 Costa Rica, Heredia, Getsemani; UCR01082 Costa Rica, Heredia, Getsemani; UCR01083 Costa Rica, Heredia, Getsemani; UCR01084 Costa Rica, Heredia, Calle Hernández; UCR01085 Costa Rica, Curridabat, Las Monjas; UCR01086 Costa Rica, Curridabat, Las Monjas; UCR01087 Costa Rica, Curridabat, Las Monjas; UCR01088 Costa Rica, Curridabat, Las Monjas; UCR01089 Costa Rica, Curridabat, Las Monjas; UCR01090 Costa Rica, Curridabat, Las Monjas; UCR01091 Costa Rica, Heredia, Getsemani; UCR01092 Costa Rica, Heredia, Getsemani; UCR01093 Costa Rica, Heredia, Getsemani; UCR01094 Costa Rica, Heredia, Getsemani; UCR01095 Costa Rica, Heredia, Getsemani; UCR01096 Costa Rica, Heredia, Getsemani; UCR01097 Costa Rica, Heredia, Getsemani; UCR01098 Costa Rica, Cartago, Ujarras; UCR01099 Costa Rica, Cartago, Ujarras;UCR01100 Costa Rica, Cartago, Ujarras;UCR01101 Costa Rica, Cartago, Ujarras; UCR01102 Costa Rica, Heredia, Getsemani; UCR01103 Costa Rica, Heredia, Getsemani; UCR01104 Costa Rica, Heredia, Getsemani; UCR01105 Costa Rica, Heredia, Calle Hernández; UCR01106 Costa Rica, Heredia, Calle Hernández; UCR01107 Costa Rica, Heredia, Calle Cienega; UCR01108 Costa Rica, Heredia, Calle Cienega; UCR01109 Costa Rica, San José, Universidad de Costa Rica campus; UCR01110 Costa Rica, San José, Universidad de Costa Rica campus; UCR01111 Costa Rica, Heredia, Getsemani; *LS1275 Costa Rica, Heredia, Getsemani; *LS1276 Costa Rica, Heredia, Getsemani; *LS1285 Costa Rica, Heredia, Getsemani; *LS1286 Costa Rica, Heredia, Getsemani; *LS1303 Costa Rica, San José, Aserrí; *LS1305 Costa Rica, Heredia, Getsemani; *LS1306 Costa Rica, Heredia, Getsemani; *LS11439 Costa Rica, Heredia, Calle Tiquisia; *LS11440 Costa Rica, Heredia, Calle Tiquisia; *LS11441 Costa Rica, Heredia, Calle Tiquisia; *LS1446 Costa Rica, Heredia, Getsemani; *LS1447 Costa Rica, Heredia, Getsemani; *LS1448 Costa Rica, Heredia, Getsemani; *LS1449 Costa Rica, Heredia, Getsemani; *T11UCR_04_05_2012 Costa Rica, San José, Universidad de Costa Rica campus; *T1GTC_30_03_2012 Costa Rica, Heredia, Getsemani; *T2GTC_09_05_2011 Costa Rica, Heredia, Getsemani; *T4GTC_26_04_2011 Costa Rica, Heredia, Getsemani; *T8GTC_28_04_2011 Costa Rica, Heredia, Getsemani.