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Phenotypic variation and vocal divergence reveals a species complex in White-eared Ground-sparrows (Cabanis) (Aves: Passerellidae)

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Abstract

The taxonomy of the genus *Melozone* has recently been analyzed from genus to subspecies level, leading to a significant revision of our understanding of this group of birds. Previous studies quantified differences in phenotypic traits, behavior, and genotypes, to provide a better understanding of the underappreciated diversity within *Melozone* and the relationship between species within this genus. Yet the relationship between the subspecies of White-eared Ground-sparrows, *Melozone leucotis*, has not received thorough taxonomic scrutiny. In this study, we evaluate the taxonomic status of the three recognized subspecies of *M. leucotis* using multiple morphometric characteristics, plumage color features, and vocalizations. We measured plumage patterns and reflectance from museum specimens, morphometric features from museum specimens and live birds, and vocal characteristics from sound recordings. We observed substantial variation between subspecies in plumage, morphometry, and voice, especially between northern and southern birds. The phenotypic and vocal differences exhibited by *M. l. occipitalis* (from Chiapas, Mexico; Guatemala; and El Salvador) suggest that its taxonomic relationship with the *M. l. leucotis* and *M. l. nigrior* complex (from Costa Rica and Nicaragua, respectively) needs to be reevaluated, because these two groups are highly diagnosable from one another. Additionally, *M. l. occipitalis* is geographically isolated from the other two subspecies, reducing the probability of contact by natural causes in the near future. Based on the clear differences in voice, plumage, and morphometric features reported here, we propose that *M. l. occipitalis* be recognized as a distinct species, *M. occipitalis* (Salvin's Ground-sparrow), diagnosed on the basis of its longer tail, longer bill, duller plumage, and songs with a lower frequency of maximum amplitude.

Key words: Central America, spectrophotometry, subspecies, taxonomic status, vocalizations

Introduction

The high levels of plant and animal biodiversity and endemism that characterize Neotropical ecosystems arise, in part, from the high diversity of tropical habitats (Cadena *et al.* 2007; Hoorn *et al.* 2010). These habitats were created by dynamic geophysical processes (Haffer 1969; Barrantes 2009; Ribas *et al.* 2012), which also contributed to diversification of taxa by separating habitats and isolating populations (Hoorn *et al.* 2010; Smith *et al.* 2014). Within the Neotropics, the Central American region is no exception and shows a high diversity of habitats that are home to many species with disjunct distributions. This is especially true of mid- and high-elevation habitats (García-Moreno *et al.* 2006; Pulgarín *et al.* 2013; Sandoval *et al.* 2014). Species from regions that harbor populations with disjunct distributions are good candidates for evaluating the degree of divergence (e.g., morphological, behavioral, and genetic divergence) that is influenced by this isolation.

The White-eared Ground-sparrow, *Melozone leucotis* Cabanis, 1861, is an endemic Central American bird species, occurring from Chiapas, Mexico in the north, to the Central Valley of Costa Rica in the south. The range of this species is discontinuous, with five isolated populations (Fig. 1). These taxa are found in premontane habitats between 500 and 2000 m above sea level (Stiles & Skutch 1989; Howell & Webb 1995) where they inhabit

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secondary forest edge, shade coffee plantations, and natural thickets (Sandoval & Mennill 2012). Three subspecies are recognized: *M. l. occipitalis* (Salvin, 1878) from south Chiapas, Mexico south through the Pacific slope of Guatemala and El Salvador; *M. l. nigrior* (Miller & Griscom, 1925) in the north-central mountains of Nicaragua; and *M. l. leucotis* from Monteverde south through the Central Valley and Turrialba Valley in Costa Rica (Rising 2011). No geographic overlap occurs between the three subspecies: *Melozone l. occipitalis* is separated by at least 370 km from *M. l. nigrior*, and *M. l. nigrior* is separated by at least 250 km from *M. l. leucotis* (Fig. 1). Historically, these three subspecies have been distinguished by differences in plumage coloration and the size of plumage patches (Rising 2011). All populations are non-migratory, and there is no published record of dispersal between the three subspecies' ranges.

The taxonomy of *Melozone* has been under recent scrutiny with analyses that focus on genetic divergence, morphological features, plumage color variation, and acoustic differences (DaCosta *et al.* 2009; Barker *et al.* 2013, Klicka *et al.* 2014; Sandoval *et al.* 2014). These studies have provided us with a better understanding of the within-species and between-species relationships, and revealed unrecognized taxa or taxonomic affinities (DaCosta *et al.* 2009; Barker *et al.* 2013; Klicka *et al.* 2014; Sandoval *et al.* 2014). The taxonomic status of the three *M. leucotis* subspecies, however, has never been studied using a quantitative taxonomic approach. Therefore, our objective in this study is to compare the morphometric characteristics, plumage traits, plumage color, and vocalizations of the three subspecies of *Melozone leucotis* to evaluate the degree of differentiation between subspecies in the different geographic areas.

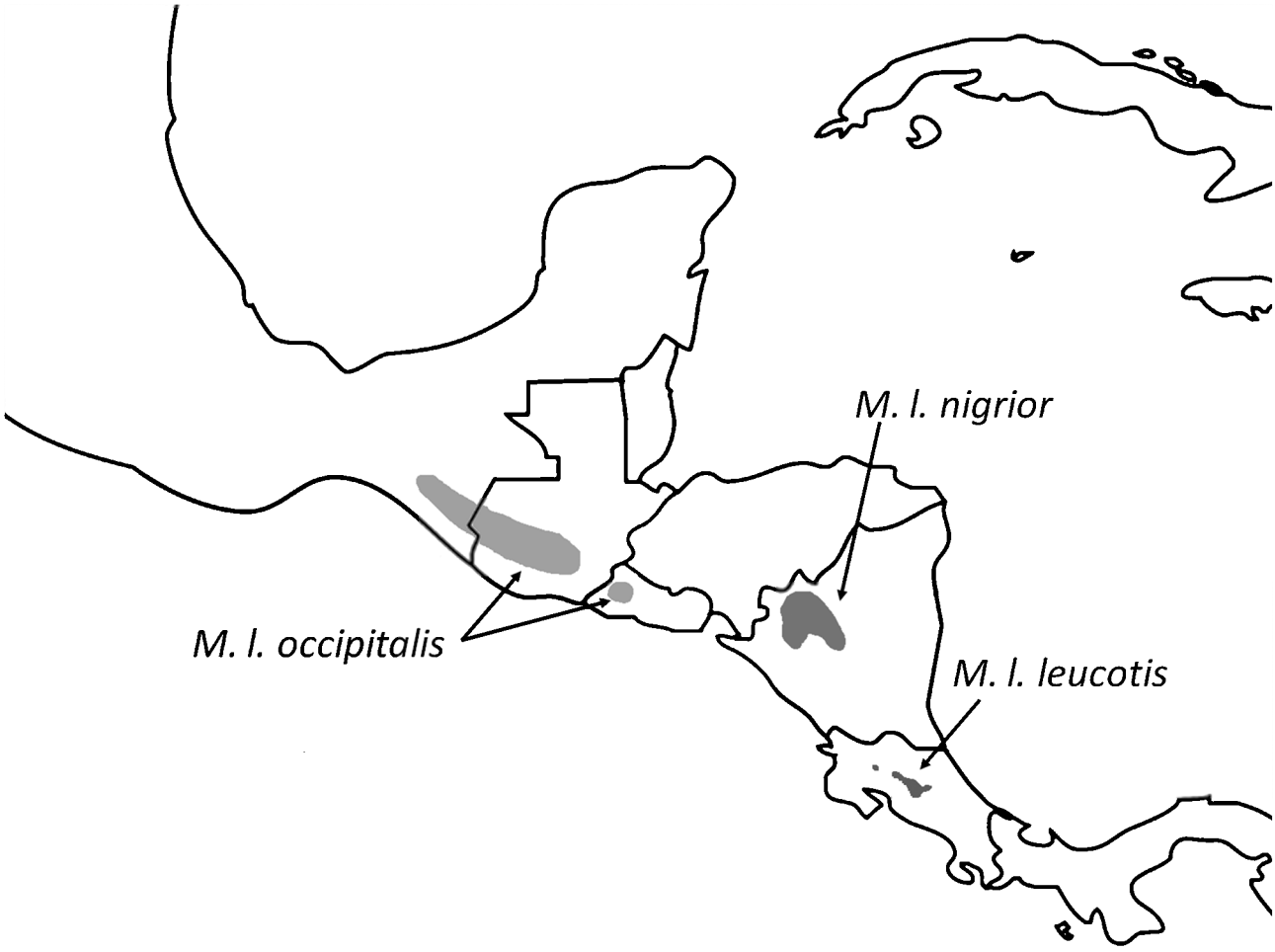

FIGURE 1. Map of the distribution of the *Melozone leucotis* subspecies from southern Mexico to Costa Rica, based on data from Stiles & Skutch (1989), Howell & Webb (1995), and Rising (2011). *Melozone l. occipitalis* is separated from *M. l. nigrior* by at least 370 km, and *M. l. nigrior* is separated from *M. l. leucotis* by at least 250 km.

Methods

We measured plumage patterns, plumage reflectance, and morphometric characteristics from adult specimens of the three subspecies of *Melozone leucotis* from the following museums: Museo de Zoología Universidad de Costa Rica, Museo Nacional de Costa Rica, the Field Museum of Natural History, the University of Michigan Museum of Zoology, and the American Museum of Natural History (Appendix A). We also measured morphometric characteristics from live individuals captured in the field, both for *M. l. leucotis* (n= 53) and *M. l. nigrior* (n= 2). We measured spectro-temporal characteristics of vocalizations from recordings deposited in the Macaulay Library of Natural Sounds, Cornell Laboratory of Ornithology, and from the Laboratorio de Bioacústica Universidad de Costa Rica (Appendix B). We also collected recordings directly in the field, using a solid state digital recorder (Marantz PMD661; sampling rate: 44.1 kHz; accuracy: 24 bit; file format: WAV) and a shotgun microphone (Sennheiser ME66/K6), for *M. l. leucotis* (n= 49), *M. l. nigrior* (n= 7), and *M. l. occipitalis* (n= 5).

Morphometric characteristics. We measured six body parts for each museum specimen and live individual (females: *M. l. leucotis:* N = 28, *M. l. nigrior:* N = 8, and *M. l. occipitalis:* N = 3; males: *M. l. leucotis:* N = 45, *M. l. nigrior:* N = 7, and *M. l. occipitalis:* N = 5) using the same protocol previously described in other studies of *Melozone* species (Sandoval & Mennill 2013; Sandoval *et al.* 2014). Specifically, we measured tarsus length (from the intertarsal joint to the middle of the sole of the foot), tail length, unflattened wing chord length, exposed culmen length, and culmen width and depth at nares. Measurements of live individuals fell within the range of museum specimens for these taxa (Sandoval & Mennill 2013). We compared the morphological measurements between subspecies separately for males and females, because previous research on *M. l. leucotis* revealed that the sexes differ in size (Sandoval & Mennill 2013). We used a backward stepwise discriminant function analysis (DFA) to select the lowest number of morphological measurements that best distinguished between subspecies, as has been used in other studies to evaluate subspecies relationships (Millsap *et al.* 2011; Cadena & Cuervo 2010; Sandoval *et al*. 2014). At each iteration, we excluded from the analysis the variable with lowest F-value, and then re-ran the analysis until we obtained a model with the lowest number of variables that classified the majority of the individuals to the correct subspecies. Additionally, we conducted a binomial test to evaluate if percentage of individuals classified in the correct subspecies using DFA was significantly higher than classification expected by chance (i.e. one divided by the number of subspecies included in each DFA). We conducted analysis of variance (ANOVA) as a post-hoc test, and a pairwise diagnosability index test (Patten 2010) to compare the difference of the morphological variables selected in the best model of the DFA. In the diagnosability index test, negative values reveal no diagnosability (i.e. the subspecies cannot be distinguished) and positive values reveal diagnosability (i.e. at least one subspecies can be distinguished).

Plumage traits and plumage spectrophotometry. We described differences in color patterns observed between the subspecies. We measured plumage patterns in eight *M. l. occipitalis*, 13 *M. l. nigrior*, and 13 *M. l. leucotis* from museum specimens. We ran this analysis with the sexes pooled because these birds are sexually monochromatic and no sex-based differences in their plumage features or color have been reported previously (Sandoval & Mennill 2013) nor recognized by us in the course of this study. We measured physical dimensions of five plumage patches to the nearest mm: width of the pre- and post-ocular white spots (horizontally in the center of the spot), height of the black breast spot (vertically with the top and bottom edges defined as where continuous black feathers were interrupted by feathers of other colors) and width of the black breast spot (horizontally in the center of the spot), length of the black throat patch, and length of the black crown (from the beak base to the neck where cap feather color changes to mantle feather color). Previous studies have suggested that these plumage features show differences between the three subspecies (Salvin 1878; Ridgway 1901; Miller & Griscom 1925; Fig. 2). We used a backward stepwise DFA to select the lowest number of plumage pattern measurements that best distinguished between subspecies, following the same approach outlined above. We also conducted a binomial test to evaluate whether the classification determined by DFA was higher than expected by chance, again using the same approach outlined above. We conducted an analysis of variance (ANOVA) as a post-hoc test and a pairwise diagnosability index test (Patten 2010) to compare the difference of the plumage pattern variables selected in the best model of the DFA.

We used reflectance spectrophotometry to quantify plumage colors. We measured ten body regions: throat, breast, belly, under-tail coverts, forehead, crown, mantle, pre-ocular white spot, cheek, and lower flanks. We measured the plumage characteristics for each of seven *M. l. occipitalis*, 13 *M. l. nigrior*, and 16 *M. l. leucotis* museum specimens. We collected five measurements from each region, randomly moving the probe within the

region between subsequent measurements to obtain a representative average measurement of each patch. The probe was kept at a fixed distance perpendicular to the feather surface using a rubber stopper, which also excluded external light (Andersson & Prager 2006). We used a USB2000 spectrophotometer combined with a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA) to collect spectral measurements with OOIBase32 software. We measured reflectance as the percentage of light reflected relative to a Spectralon pure white standard (WS-2, Ocean Optics). We used a tetrahedral colorspace visual model (Goldsmith 1990; Endler & Mielke 2005, Stoddard & Prum 2008) to compare plumage patches between the three subspecies; these visual models allowed us to estimate how the birds themselves would perceive the differences. Each color is described as its position in three-dimensional space (x, y, z coordinates) and can be compared to other colors by calculating the Euclidean distance between them. As in previous studies of *Melozone* (Sandoval *et al.* 2014), the model assumed the members of this taxa possess photoreceptor peak sensitivities similar to that of the average avian visual system for birds that possess an ultraviolet cone type (Endler & Mielke 2005); the species most closely related to *Melozone* for which data are available have an ultraviolet cone type with a peak sensitivity near 370 nm (Hart 2001). We used a "forest shade" ambient illumination because these *Melozone* ground-sparrows are found in relatively dense thickets. We calculated the achromatic component based on the stimulation of the two longest wavelength cones (Vorobyev & Osorio 1998), and all spectral and visual model analyses were conducted using the package pavo (Maia *et al.* 2013) in R.

We conducted two one-way non-parametric multiple analysis of variance (PERMANOVA) to compare the differences in the mean chromatic and achromatic components of the same body region between individuals by subspecies (Anderson 2001). These analyses allowed us to avoid independence problems in the comparisons of each body region because each individual is compared against all others based on the mean of the distance between them according to a similarity distance. To analyze the chromatic component of plumage reflectance, we used Euclidean distance between three-dimensional coordinates in colorspace as our similarity measurement in our one-way PERMANOVAs. In both chromatic and achromatic PERMANOVAs we conducted 9999 permutations to estimate the values of significance. We calculated the pairwise diagnosability index (Patten 2010) for the body regions where spectrophotometry measurements showed differences in the one-way PERMANOVA analysis for chromatic and/or achromatic components.

Vocalizations. For all three subspecies we measured calls (defined as short duration vocalizations produced by both male and females, and sorted into two types, as explained below), songs (defined as vocalizations that include multiple types of elements and are produced only by males in this species), and duets (defined as the coordinated vocalizations produced simultaneously by male and females; Sandoval *et al.* 2015). We measured calls from 49 *M. l. leucotis* (189 ± 27 calls per individual), seven *M. l. nigrior* (20 ± 11 calls per individual), and five *M. l. occipitalis* (44 ± 14 calls per individual). We measured male solo songs from 46 *M. l. leucotis* (80 ± 10 songs per individual), seven *M. l. nigrior* (29 ± 12 songs per individual), and three *M. l. occipitalis* (26 ± 9 songs per individual). We measured duets from 47 pairs of *M. l. leucotis* (12 ± 2 duets per pair), nine *M. l. nigrior* (2 ± 0 duets per pair), and six *M. l. occipitalis* (6 ± 1 duets per pair). For two call types (*chip* and *tseet*; see Sandoval *et al*. 2016), male solo songs, and duets we extracted four fine-structural acoustic measurements: the duration (s), the minimum frequency (kHz), the maximum frequency (kHz), and the frequency of maximum amplitude (kHz). Additionally, for male solo songs we annotated the total number of elements per song. We collected the fine-structural acoustic measurements using Raven Pro 1.4 sound analysis software (Cornell Lab of Ornithology, Ithaca, NY, USA). We used a combination of waveform, spectrogram, and power spectrum windows with the Hann window set at 50% overlap and 256 kHz sampling frequency with 16 bit accuracy, providing an effective resolution of 188 Hz and 5.8 ms.

For each type of vocalization, we calculated an average measurement per individual for use in our statistical analysis. As in previous studies (Cadena & Cuervo 2010; Millsap *et al*. 2011; Sandoval *et al.* 2014), we compared vocalizations between subspecies using a DFA. We used a backward stepwise DFA to select the lowest number of acoustic measurements that best distinguished between subspecies, excluding from the analysis the variables with lowest F-value (following the same approach outlined above). We also conducted a binomial test to evaluate whether the classification determined by DFA was higher than expected by chance.

We conducted statistical analyses using SYSTAT (version 11.00.01; SYSTAT Software, Chicago, IL, USA) and PAST (version 3.9; Øyvind Hammer, Natural History Museum, University of Oslo, Norway). We report data as means ± SE, and all tests are two-tailed.

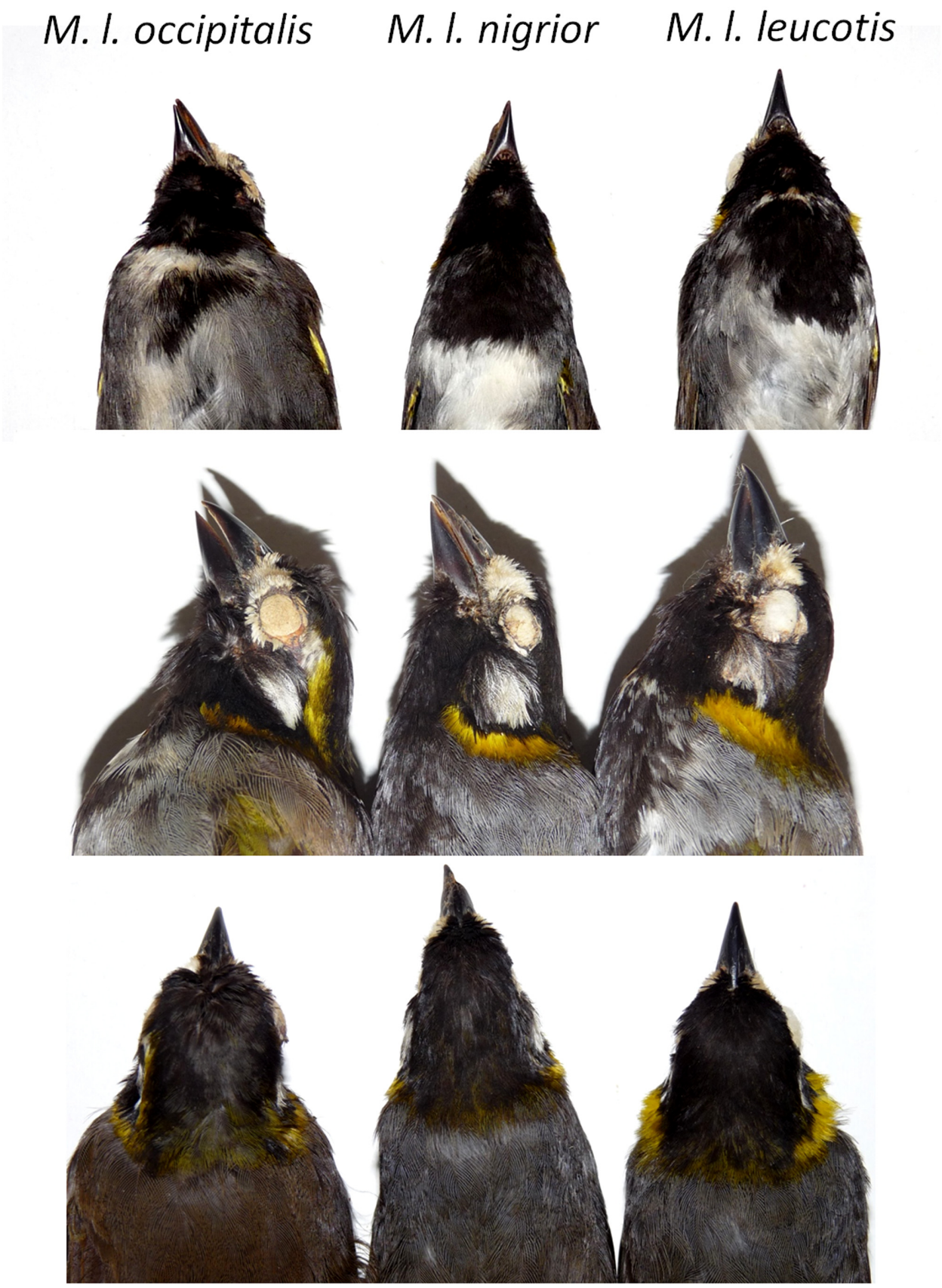

FIGURE 2. Photographs of museum specimens reveal plumage color and pattern differences between the three subspecies of *Melozone leucotis*. Photographs were taken under the same light conditions at the Field Museum of Natural History, Chicago. The top row compares the ventral surfaces, the middle row shows lateral surfaces, and the bottom row shows dorsal surfaces.

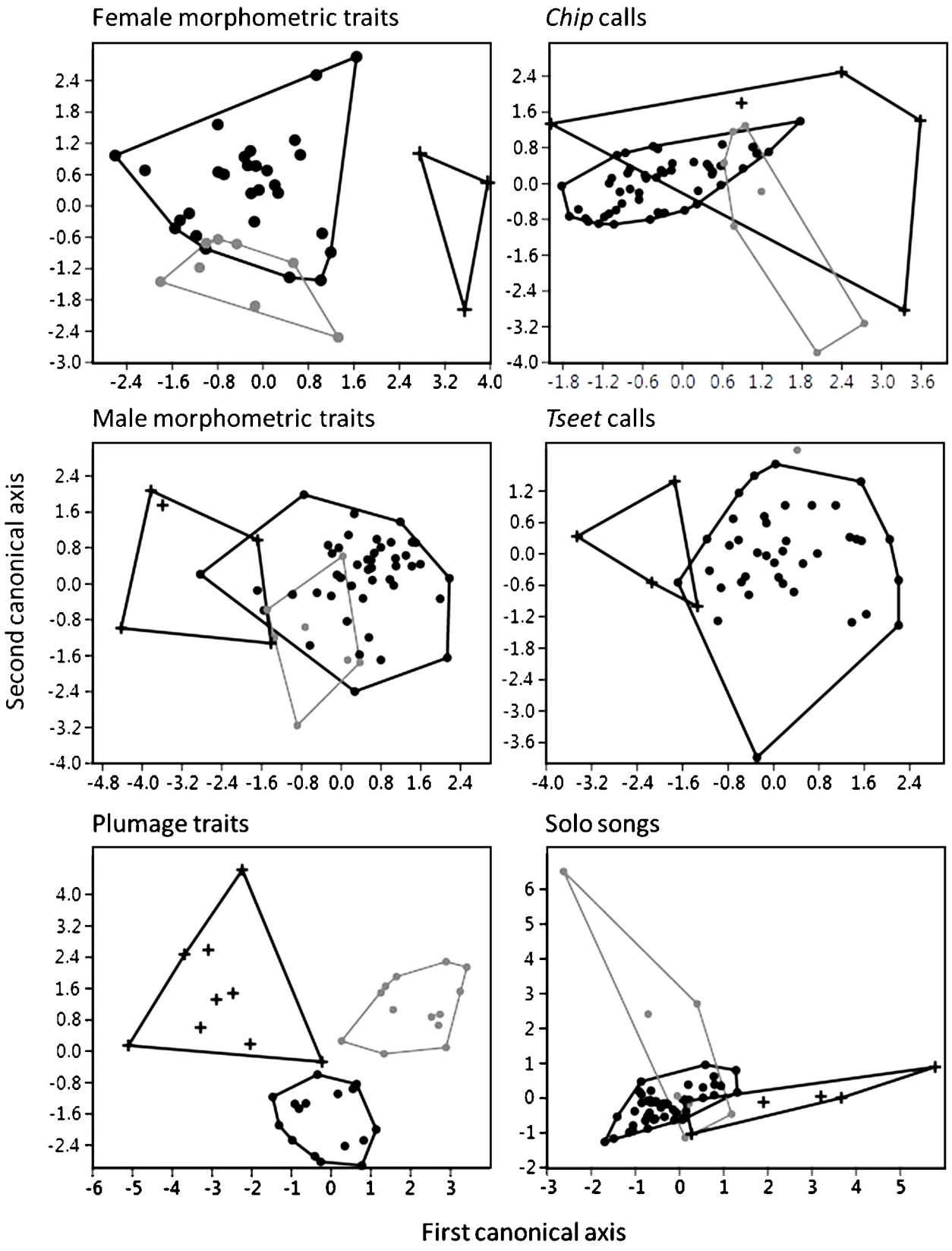

FIGURE 3. Plot of the first two discriminant functions for morphometric measurements, plumage patterns, and vocalizations showing separation between the three subspecies of *Melozone leucotis* (black circles: *M. l. leucotis*, gray circles: *M. l. nigrior*, addition sign: *M. l. occipitalis*) based on the best model for each comparison (see text for details on percentage of classification and variables included in each model).

Results

Morphometric characteristics. We found significant morphological variation across the three *M. leucotis* subspecies in our analyses of both males and females; *M. l. nigrior* grouped together with *M. l. leucotis* whereas *M. l. occipitalis* grouped separately for both sexes.

For females, the model that best distinguished between the three *M. leucotis* subspecies on the basis of morphometric characteristics included tarsus length, tail length, wing chord length, and culmen depth (Wilk's λ = 0.36, $F_{8,66}$ = 5.43, $P < 0.001$, Fig. 3). This model correctly classified 74% of females to their own subspecies (*M. l. leucotis:* 19 of 28 assigned correctly; *M. l. nigrior:* 7 of 8 assigned correctly; and *M. l. occipitalis* 3 of 3 assigned correctly), and exceeded classification expected by chance (Binomial test: $P < 0.001$). Tail length (ANOVA: $F_{2,35}$ = 13.65, $P < 0.001$) and wing chord length ($F_{2,36}$ = 4.71, P = 0.01) were significantly longer in *M. l. occipitalis* than in *M. l. leucotis* and *M. l. nigrior* (Table 1). Tarsus length ($F_{2,36}$ = 4.57, P = 0.02) was significantly longer in *M. l. leucotis* than in *M. l. occidentalis* and *M. l. nigrior*, and culmen depth ($F_{2,31}$ = 5.03, P = 0.01) was significantly longer in *M. l. occipitalis* than in *M. l. leucotis* and *M. l. nigrior* (Table 1).

For males, the model that best distinguished between the three *M. leucotis* subspecies on the basis of morphometric characteristics included: tarsus length, tail length, and wing chord length (Wilk's λ = 0.63, $F_{6,104}$ = 4.54, $P < 0.001$, Fig. 3). This model correctly classified 79% of males to their own subspecies (*M. l. leucotis*: 35 of 45; *M. l. nigrior*: 6 of 7 assigned correctly; and *M. l. occipitalis* 4 of 5 assigned correctly), and exceeded classification expected by chance (Binomial test: $P < 0.001$). Tarsus length ($F_{2,54}$ = 13.78, $P < 0.001$) and wing chord length ($F_{2,54}$ = 5.15, P = 0.01) were significant longer in *M. l. leucotis* than in *M. l. occipitalis* and *M. l. nigrior* (Table 1). However, tail length ($F_{2,53}$ = 7.38, P = 0.002) was significantly longer in *M. l. leucotis* and *M. l. occipitalis* than in *M. l. nigrior* (Table 1).

TABLE 1. Morphological and plumage pattern measurements (mean ± SE) from the three recognized subspecies of *Melozone leucotis*, separated by sex. Boldface signifies statistically different variables between subspecies.

Female	***M. l. leucotis* (n = 28)**	***M. l. nigrior* (n = 8)**	***M. l. occipitalis* (n = 3)**
Tarsus (mm)	**28.1 ± 0.3**	**26.8 ± 0.5**	**26.1 ± 0.8**
Tail length (mm)	**68.2 ± 0.7**	**64.3 ± 1.2**	**76.0 ± 1.9**
Wing chord length (mm)	**76.2 ± 0.5**	**73.5 ± 0.8**	**77.0 ± 1.3**
Culmen length (mm)	**14.1 ± 0.1**	**14.5 ± 0.5**	**14.5 ± 0.4**
Beak width (mm)	9.6 ± 0.1	9.5 ± 0.1	9.5 ± 0.3
Beak depth (mm)	8.5 ± 0.1	8.7 ± 0.5	9.1 ± 0.2
Male	**(n = 45)**	**(n = 7)**	**(n = 5)**
Tarsus (mm)	**29.4 ± 0.2**	**28.4 ± 1.2**	**27.3 ± 0.5**
Tail length (mm)	**72.2 ± 0.5**	**71.0 ± 3.0**	**76.6 ± 1.6**
Wing chord length (mm)	**81.8 ± 0.4**	**81.9 ± 0.2**	**79.9 ± 1.0**
Culmen length (mm)	14.5 ± 0.1	14.4 ± 0.4	14.7 ± 0.3
Beak width (mm)	9.9 ± 0.1	9.5 ± 0.3	9.6 ± 0.3
Beak depth (mm)	10.5 ± 1.7	8.9 ± 0.2	9.6 ± 5.1
Plumage Pattern	**(n =16)**	**(n = 13)**	**(n = 9)**
Pre-ocular white spot (mm)	**5.8 ± 0.1**	**8.1 ± 0.2**	**6.4 ± 0.2**
Post-ocular white spot (mm)	8.6 ± 0.3	9.54 ± 0.3	8.7 ± 0.4
Height of breast spot (mm)	**21.4 ± 1.1**	**19.8 ± 1.2**	**11.8 ± 1.5**
Length of breast spot (mm)	**21.1 ± 0.8**	**26.6 ± 0.9**	**13.3 ± 1.1**
Throat patch length (mm)	21.3 ± 0.8	18.8 ± 0.7	20.8 ± 0.8
Crown length (mm)	**27.8 ± 0.6**	**31.1 ± 0.7**	**29.7 ± 0.8**

Morphometric measurements within each sex were not diagnosably different between subspecies according to the pairwise diagnosability index test (Table 2).

TABLE 2. Diagnosability test results for morphometric features that were selected in the best discriminant function analysis model per sex in the three subspecies of *Melozone leucotis.* 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). Sample size for females (*M. l. leucotis*: n = 28, *M. l. nigrior*: n = 8, and *M. l. occipitalis*: n = 3) and males (*M. l. leucotis*: n = 45, *M. l. nigrior*: n = 7, and *M. l. occipitalis*: n = 5).

Morphometric features	*leucotis* versus *nigrior*	*nigrior* versus *leucotis*	*leucotis* versus *occipitalis*	*occipitalis* versus *leucotis*	*nigrior* versus *occipitalis*	*occipitalis* versus *nigrior*
Female tarsus length	-2.9	-5.5	-16.2	-7.4	-17.3	-5.9
Female tail length	-4.0	-14.3	-35.4	-4.1	-38.2	**3.3**
Female wing chord length	-4.7	-10.0	-18.3	-7.0	-20.7	-4.1
Female beak depth	-2.1	-1.0	-3.3	-0.5	-3.2	-1.5
Male tarsus length	-2.0	-4.8	-2.9	-5.5	-4.4	-4.2
Male tail length	-10.9	-13.9	-31.4	-8.0	-35.8	-9.3
Male wing chord length	-7.1	-9.6	-14.5	-9.9	-17.7	-10.6

Plumage traits. We found significant variation in plumage traits across the three *M. leucotis* subspecies. *Melozone l. occipitalis* has a grey crown stripe, a broader yellow line on the side of the neck, and a small black breast spot, but *M. l. nigrior* and *M. l. leucotis* have a black crown stripe, a thinner yellow line on the side of the neck, and a bigger black breast spot. The model that best separated the subspecies included all six plumage trait measurements (Wilk's $\lambda = 0.06$, $F_{12,60} = 15.67$, $P < 0.001$, Fig. 3). This model correctly classified 92% of individuals to the correct subspecies (*M. l. leucotis*: 13 of 13 assigned correctly; *M. l. nigrior* 12 of 13 assigned correctly; and *M. l. occipitalis* 7 of 9 assigned correctly), and exceeded classification expected by chance (Binomial test: $P < 0.001$). Height of the black breast spot ($F_{2,35} = 14.42$, $P < 0.001$) and width of the black breast spot ($F_{2,35} = 41.60$, $P < 0.001$) were significantly greater in *M. l. leucotis* and *M. l. nigrior* than in *M. l. occipitalis* (Table 1). However, width of pre-ocular white spot ($F_{2,35} = 40.94$, $P < 0.001$) and length of the black crown ($F_{2,35} = 6.40$, $P = 0.004$) were significantly greater in *M. l. nigrior* than in *M. l. leucotis* and *M. l. occipitalis.* The post-ocular white spot ($F_{2,35} = 3.00$, $P = 0.06$) and length of the throat patch ($F_{2,35} = 2.67$, $P = 0.08$) were similar between subspecies (Table 1). None of the plumage trait measurements were diagnosably different between subspecies (Table 3).

Plumage spectrophotometry. We found notable differences in the chromatic component of plumage reflectance for six of the 10 body regions analyzed using a visual model (Fig. 4). The color of the throat (PERMANOVA: $F = 5.48$, $P = 0.001$), breast ($F = 8.99$, $P < 0.001$), and forehead ($F = 4.39$, $P = 0.003$) of *M. l. occipitalis* were more saturated in the ultraviolet region of the spectrum in comparison to *M. l. leucotis* and *M. l. nigrior* (Fig. 4). The color of the pre-ocular white spot ($F = 5.68$, $P = 0.003$) and cheek ($F = 4.59$, $P = 0.01$) of *M. l. nigrior* were more saturated in the 500 nm to 700 nm region of the spectrum in comparison to *M. l. leucotis* and *M. l. occipitalis* (Fig. 4). Finally, the crown color ($F = 10.64$, $P = 0.04$) varied between the three subspecies, showing the highest saturation values in *M. l. occipitalis*, intermediate values in *M. l. leucotis,* and the lowest values in *M. l. nigrior.* According to the pairwise diagnosability index test, only the breast and crown patch color difference between *M. l. occipitalis* and *M. l. nigrior* was diagnosably different between subspecies (Table 3). The other four body regions showed similar values of color chromatic variation between subspecies (mantle: $F = 1.42$, $P = 0.25$; belly: $F = 2.30$, $P = 0.07$; lower flank: $F = 2.76$, $P = 0.07$; and under-tail coverts: $F = 0.65$, $P = 0.56$).

We found notable differences in achromatic reflectance for five of the 10 body regions analyzed: breast ($F = 7.95$, $P < 0.001$), crown ($F = 10.36$, $P = 0.005$), and forehead ($F = 7.26$, $P = 0.01$) showed higher brightness values in *M. l. occipitalis* than in *M. l. leucotis* and *M. l. nigrior.* Cheeks ($F = 10.74$, $P = 0.0005$) and pre-ocular white spot ($F = 16.68$, $P < 0.001$) showed higher values of achromatic reflectance in *M. l. nigrior* than in *M. l. leucotis*. From these five measurements, none were diagnosably different between subspecies (Table 3). The other five body regions showed similar values of achromatic reflectance between subspecies (mantle: $F = 0.29$, $P = 0.75$; belly: $F = 3.30$, $P = 0.06$; under-tail coverts: $F = 2.36$, $P = 0.11$; lower flank: $F = 0.90$, $P = 0.42$; and throat: $F = 0.23$, $P = 0.80$).

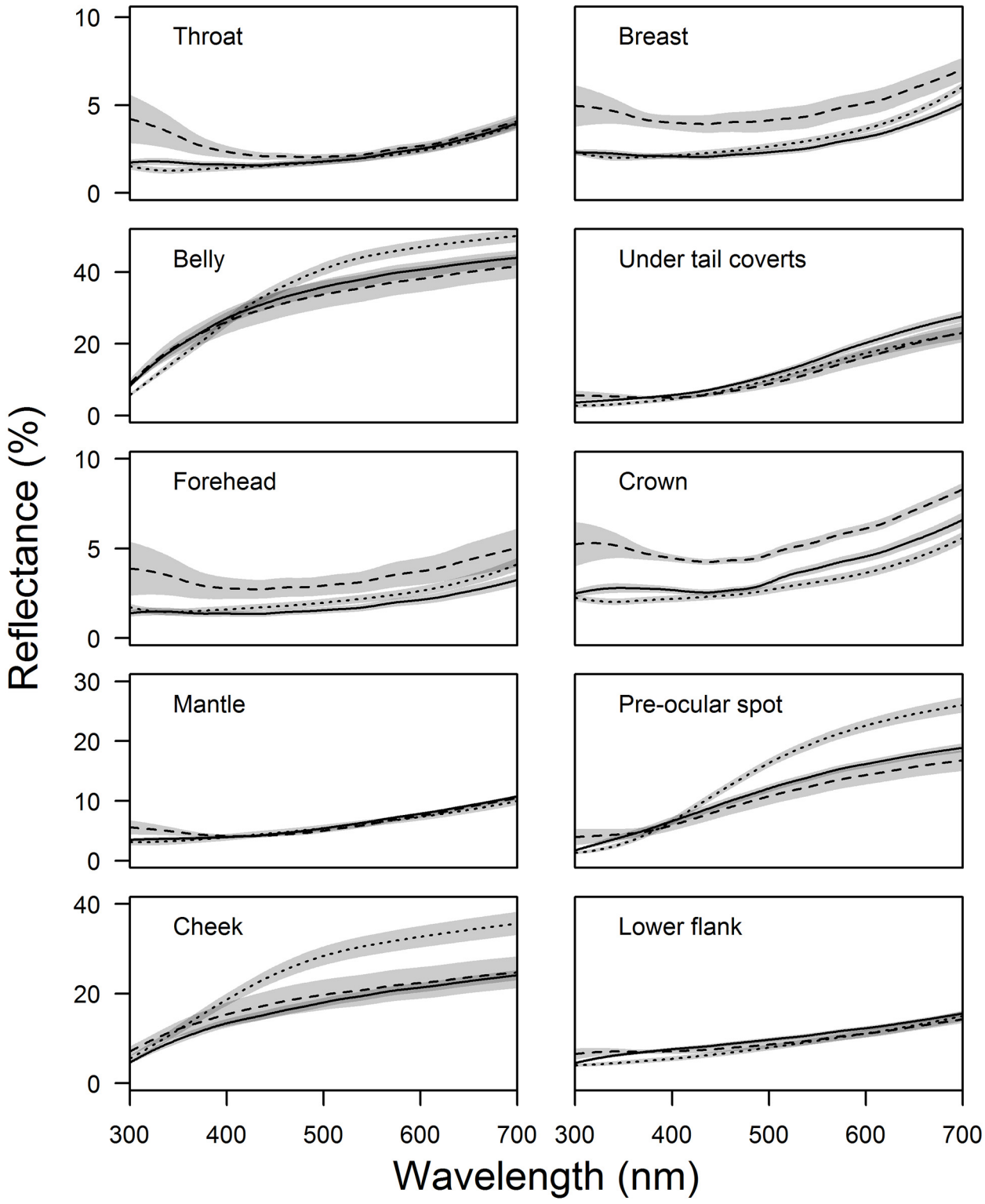

FIGURE 4. Mean reflectance spectra for ten body regions measured from three *Melozone leucotis* subspecies, *M. l. leucotis* (solid lines, $N = 13$), *M. l. nigrior* (dotted lines, $N = 13$), and *M. l. occipitalis* (dashed lines, $N = 8$). The gray area around each line represents standard error of the mean calculated at every 1nm.

Vocalizations. We found notable differences in calls (both *chip* and *tseet* calls), songs, and duets between the three subspecies (Fig. 5). The best DFA model that separated *chip* calls between subspecies included: minimum frequency and frequency of maximum amplitude (Wilk's $\lambda = 0.58$, $F_{4,114} = 9.04$, $P < 0.001$, Fig. 3). This model

correctly classified 85% of *chip* calls to the correct subspecies (*M. l. leucotis*: 48 of 49 assigned correctly; *M. l. nigrior* 2 of 7 assigned correctly; and *M. l. occipitalis:* 2 of 5 assigned correctly), and exceeded classification expected by chance (Binomial test: $P < 0.001$). Minimum frequency ($F_{2,58} = 14.14$, $P < 0.001$) and frequency of maximum amplitude ($F_{2,58} = 10.99$, $P < 0.001$) were significantly lower in *M. l. leucotis* than in both *M. l. nigrior* and *M. l. occipitalis* (Table 4). In contrast, duration and maximum frequency were similar between subspecies (Table 4). The best DFA model that separated *tseet* calls between subspecies included the four acoustic measurements (Wilk's $\lambda = 0.60$, $F_{8,76} = 2.72$, $P = 0.01$, Fig. 3). This model correctly classified 93% of *tseet* calls to their own subspecies (*M. l. leucotis*: 39 of 39 assigned correctly*, M. l. nigrior*: 0 of 1 assigned correctly; *M. l. occipitalis*: 2 of 5 assigned correctly), and exceeded classification expected by chance (Binomial test: $P < 0.001$). However, none of the four acoustic measurements showed differences between subspecies in a post-hoc analysis of variance (minimum frequency: $F_{2,41} = 0.05$, $P = 0.95$; maximum frequency: $F_{2,41} = 2.41$, $P = 0.10$; frequency of maximum amplitude: $F_{2,41} = 0.58$, $P = 0.56$; duration: $F_{2,41} = 3.11$, $P = 0.06$).

TABLE 3. Diagnosability test results for plumage traits and plumage features that were selected in the best discriminant function analysis model comparing two of the three subspecies of *Melozone leucotis.* 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). Sample size for plumage pattern (*M. l. leucotis*: n = 16, *M. l. nigrior*: n = 13, and *M. l. occipitalis*: n = 9) and plumage features (*M. l. leucotis*: n = 16, *M. l. nigrior*: n = 13, and *M. l. occipitalis*: n = 7).

Plumage patterns	*leucotis* versus *nigrior*	*nigrior* versus *leucotis*	*leucotis* versus *occipitalis*	*occipitalis* versus *leucotis*	*nigrior* versus *occipitalis*	*occipitalis* versus *nigrior*
Pre-ocular white spots	-4.3	**0.5**	-3.8	-1.4	-1.6	-4.1
Post-ocular white spots	-3.3	-1.9	-5.5	-3.5	-4.4	-3.7
Height of breast black spot	-12.1	-18	-3.6	-25.8	-4.1	-20.4
Length of breast black spot	-14.2	-4.3	-8.1	-19.3	-2.1	-23.2
Throat patch length	-4.4	-9.7	-10.9	-8.8	-13.2	-5.7
Crown length	-10.4	-5.3	-11.8	-7.2	-7.9	-8.4
Plumage features						
Breast color	-0.01	-0.02	-0.04	-0.01	-0.04	**0.00**
Crown color	-0.01	-0.03	-0.05	-0.02	-0.05	**0.00**
Forehead color	-0.02	-0.03	-0.04	-0.03	-0.04	-0.02
Pre-ocular spot color	-0.03	-0.03	-0.02	-0.03	-0.02	-0.03
Throat color	-0.03	-0.04	-0.08	-0.04	-0.08	-0.03
Cheek color	-0.05	-0.02	-0.03	-0.2	-0.02	-0.05
Breast brightness	-0.03	-0.02	-0.09	-0.02	-0.08	-0.02
Crown brightness	-0.03	-0.05	-0.05	-0.02	-0.06	-0.01
Forehead brightness	-0.04	-0.03	-0.09	-0.02	-0.08	-0.03
Pre-ocular spot brightness	-0.18	-0.03	-0.16	-0.12	-0.10	-0.22
Cheek brightness	-0.41	-0.06	-0.37	-0.18	-0.27	-0.43

The model that best separated male solo songs between subspecies included frequency of maximum amplitude, duration, and number of elements (Wilk's $\lambda = 0.45$, $F_{6,108} = 8.96$, $P < 0.001$, Fig. 3). This model correctly classified 93% of male solo songs to the correct subspecies (*M. l. leucotis*: 46 of 46 assigned correctly; *M. l. nigrior* 3 of 7 assigned correctly; and *M. l. occipitalis* 3 of 3 assigned correctly), and exceeded classification expected by chance (Binomial test: $P < 0.001$). Frequency of maximum amplitude ($F_{2,56} = 4.74$, $P = 0.01$) showed the highest values in *M. l. leucotis,* followed by *M. l. nigrior*, and the lowest values were found in *M. l. occipitalis*. Song duration ($F_{2,56} = 14.64$, $P < 0.001$) was longer in *M. l. occipitalis* than in *M. l. leucotis* and *M. l. nigrior.* The number of elements in each song ($F_{2,56} = 14.64$, $P < 0.001$) was higher in *M. l. nigrior* than in *M. l. leucotis* and *M. l. occipitalis* (Table 4). In contrast, minimum and maximum frequencies were similar between subspecies (Table. 4).

TABLE 4. Values (mean ± SE) of the acoustic characteristics in four vocalization types in the three recognized subspecies of *Melozone leucotis* separated by sex. Bold data mean statistically different variables between subspecies. Sample size (number of individuals analyzed) for calls (*M. l. leucotis*: n = 49, *M. l. nigrior*: n = 7, and *M. l. occipitalis*: n = 5), solo songs (*M. l. leucotis*: n = 46, *M. l. nigrior:* n = 7, and *M. l. occipitalis*: n = 3), and duets (*M. l. leucotis*: n = 47, *M. l. nigrior*: n = 9, and *M. l. occipitalis*: n = 6).

	Minimum freq. (kHz)	Maximum freq. (kHz)	Freq. maximum amplitude (kHz)	Duration (s)	Number of elements
***Chip* calls**					
M. l. leucotis	**7341.7 ± 43.9**	12151.4 ± 93.8	**8294.3 ± 38.2**	0.078 ± 0.001	
M. l. nigrior	**7615.2 ± 101.7**	12349.2 ± 219.3	**9019.3 ± 213.6**	0.075 ± 0.003	
M. l. occipitalis	**8034.7 ± 273.5**	12237.1 ± 405	**8924.4 ± 447.2**	0.077 ± 0.005	
***Tseet* calls**					
M. l. leucotis	8102.7 ± 95.3	10595.1 ± 94.7	9263.6 ± 78.4	0.29 ± 0.01	
M. l. nigrior	8194.7	9892.9	9345.4	0.41	
M. l. occipitalis	8017 ± 142	11133.9 ± 178.4	8998.3 ± 160.3	0.35 ± 0.04	
Solo songs					
M. l. leucotis	3472.1 ± 587.6	11142 ± 704.3	**6064.6 ± 580.3**	**1.9 ± 0.2**	**7.7 ± 0.9**
M. l. nigrior	3725.7 ± 599.3	11032.4 ± 1009.4	**5904.3 ± 627.4**	**2.0 ± 0.1**	**11.0 ± 5.6**
M. l. occipitalis	3364.5 ± 978.4	10392.1 ± 1629.5	**5408.7 ± 479.3**	**2.2 ± 0.8**	**7.6 ± 1.2**
Duets					
M. l. leucotis	5093.2 ± 107.4	11547.4 ± 81.2	**7444.2 ± 170.7**	5.8 ± 0.2	
M. l. nigrior	5963.7 ± 225.7	10421.5 ± 246.9	**7497.5 ± 274.6**	5.8 ± 0.4	
M. l. occipitalis	5500.7 ± 214.8	11351.7 ± 226.5	**7168.7 ± 467.2**	5.8 ± 0.5	

The model that best separated duets between subspecies included only maximum frequency (Wilk's $\lambda = 0.72$, $F_{2,59} = 11.47$, $P < 0.001$). This model correctly classified 81% duets to the correct subspecies (*M. l. leucotis*: 46 of 49*, M. l. nigrior:* 3 of 7 assigned correctly; and *M. l. occipitalis:* 0 of 6 assigned correctly), and exceeded classification expected by chance (Binomial test: $P < 0.001$). Maximum frequency ($F_{2,59} = 11.47$, $P < 0.001$) showed higher values in *M. l. nigrior* than in *M. l. leucotis* and *M. l. occipitalis* (Table. 4). Minimum frequency, frequency of maximum amplitude, and duration were similar between subspecies (Table 4).

Discussion

We found substantial variation between the northern subspecies and the two southern subspecies of *Melozone leucotis* in terms of morphometric features, plumage patterns, plumage color, and vocalizations. In particular, *Melozone leucotis occipitalis* from Chiapas, Mexico, Guatemala and El Salvador is distinguishable from the two more southern subspecies, *M. l. leucotis* from Costa Rica and *M. l. nigrior* from Nicaragua (Stiles & Skutch 1989; Howell & Webb 1995; Rising 2011), on the basis of morphometric characteristics (the southern subspecies has longer tarsi, but shorter tail), plumage patches (the northern subspecies has a shorter breast spot), plumage color (the southern subspecies exhibit less brightness in the black throat, breast, and forehead, but higher brightness in the crown), and voice (the northern subspecies has calls with higher minimum frequency and frequency of maximum amplitude; songs with lower frequency of maximum amplitude but longer song duration; and duets with lower maximum frequency). These phenotypic and vocal differences indicate significant divergence between the northern and southern taxa, and suggest the need to reconsider the taxonomic relationship between the northerly subspecies, *M. l. occipitalis*, and the complex of the southerly subspecies, *M. l. leucotis* and *M. l. nigrior*.

Melozone l. occipitalis (Salvin, 1878) was originally described as a different species from *M. l. leucotis* based on morphological differences (longer tails than *M. l. occipitalis*) and plumage patterns (e.g., pileum color, distinct yellow neck stripe, and smaller black breast spot in *M. l. occipitalis*; Ridgway 1901). As in the description of *M. l. occipitalis*, our results support the differences in morphology and plumage patterns between subspecies, even when

considering the sex of the individuals. Additionally, we found differences in plumage reflectance and vocalizations, characteristics not previously compared between these groups. Together, these differences allow reliable identification of *Melozone l. occipitalis* and *M. l. leucotis* in the field and in museum specimens. On the other hand, *Melozone l. nigrior* and *M. l. leucotis* differed in the amount of black they displayed on their breast spot, the principal characteristic used to describe *M. l. nigrior* as a new subspecies (Miller & Griscom 1925). The two subspecies appear to be inseparable in terms of plumage patterns, although they show some deviation in song characteristics and morphology (e.g., tarsus length; Table 1). This, combined with the fact that they are separated by a distance of approximately 300 km, and the fact that Rising (2011) and others have considered them distinct subspecies, seems sufficient grounds to continue to treat them as two separate subspecies.

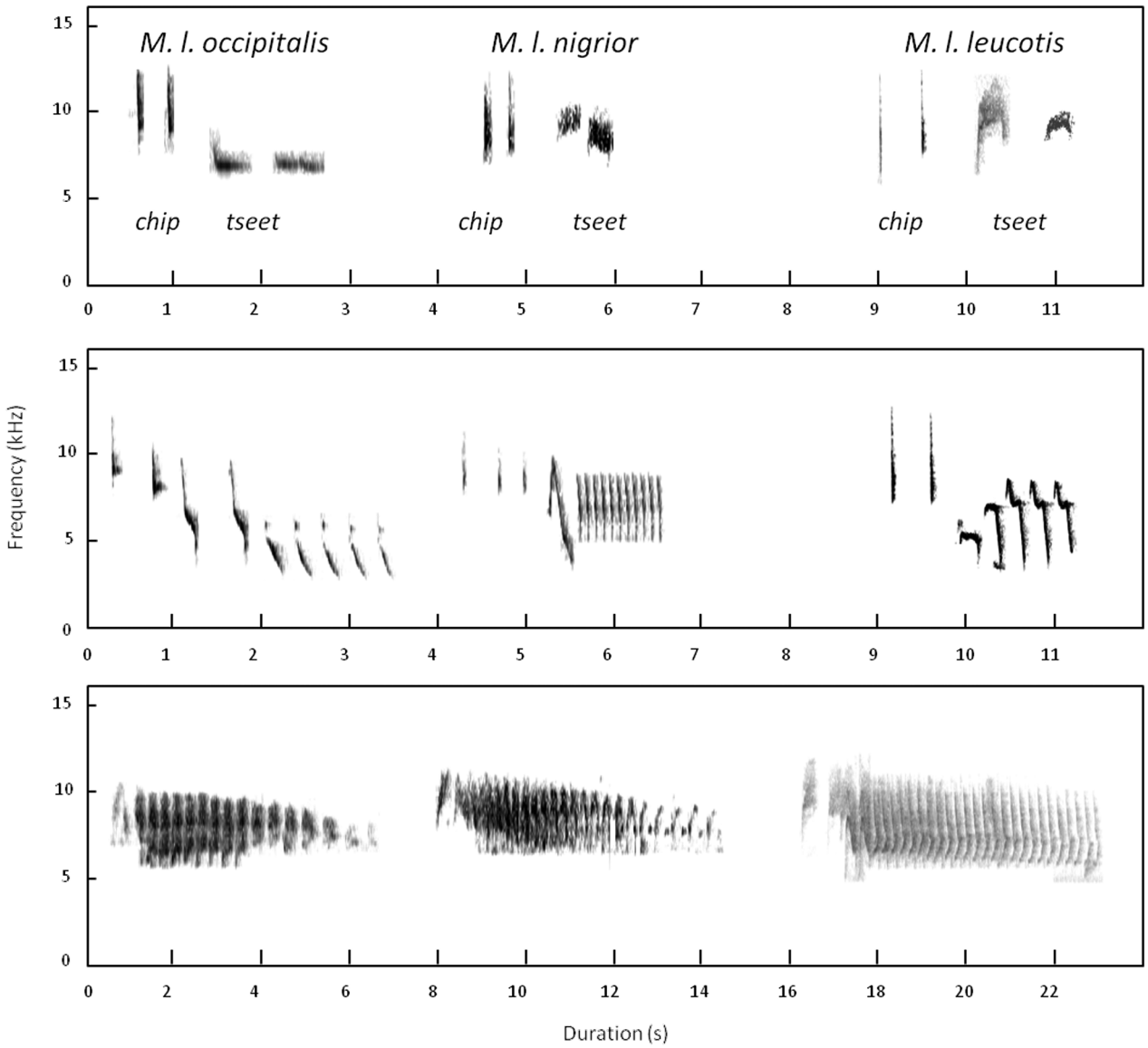

FIGURE 5. Sound spectrograms of two call types, male solo songs, and duets of the three subspecies of *Melozone leucotis.*

Members of the genus *Melozone* often show similar plumage between species, which historically has led to underestimation of the number of species in the genus. For example similar plumage patterns and coloration have been reported for Canyon Towhee *M. fusca* (Swainson) in comparison with California Towhee *M. c. crissalis* (Vigors), *M. c. albigula* (Baird) and White-throated Towhee *M. albicollis* (Sclater) (Rising 2011). These similarities could be the result of recent divergence (DaCosta *et al.* 2009; Klicka *et al.* 2014) limiting the time to evolve different plumage patterns, or similarities may persist because the species inhabit similar environments (Rising 2011; Sandoval *et al*. 2014), which favor certain plumage patterns, possibly through selection for crypsis.

Future comparative studies will be required to evaluate this hypothesis. Plumage characteristics that appear to be important for species recognition in *Melozone* sparrows (Sandoval *et al*. 2013, 2014) nevertheless show high levels of similarity across species.

Body size of individuals of the same or closely related species is generally affected by latitudinal or altitudinal distribution, where individuals at higher altitudes or latitudes have larger body sizes (Meiri 2011; Gutiérrez-Pinto *et al.* 2014). However, the differences in body size that we have documented between *M. l. occipitalis* and *M. l. leucotis/nigrior* appear to be unrelated to distribution, given that both taxa occur at the same altitudinal distribution (500–2000 m; Stiles & Skutch 1989; Howell & Webb 1995; Rising 2011), and that northern subspecies (*M. l. occipitalis*) is smaller in tarsus size (indicating smaller body size), in contrast to the expected pattern for species with northerly distributions. The *M. l. leucotis/nigrior* group also possesses larger beaks in comparison to *M. l. occipitalis*, which could be the result of different subspecies consuming different types of seeds. In Darwin's finches, for example, when different individuals of the same species consume different types of seeds, bill size diverges (Grant & Grant 1996; Abzhanov *et al.* 2006).

Vocalizations play an important role in social interactions within *Melozone* (Marshall 1964; Benedict 2010; Sandoval *&* Mennill 2014). This is especially true for *M. leucotis*, which defends territories in pairs year-round and inhabits very densely vegetated habitats (Sandoval *et al.* 2015, 2016), where visual communication is highly constrained in comparison to other types of habitats. Our results show that the divergence in vocalizations between taxa has been extensive, which could act as an isolating barrier between the taxa in the event of secondary contact by facilitating species recognition for mating or territory defense (Ptacek 2000; Pfennig & Pfennig 2009). Future research that involves playback to explore the reactions of the northern versus southern forms to each other's vocalizations would help to clarify the importance of vocal differences to the animals themselves.

In conclusion, we found divergence in morphological characteristics (body size, plumage color, and plumage pattern) and vocalizations (calls, songs, and duets) at levels that suggest a consistent separation of *M. l. occipitalis* from *M. l. leucotis/nigrior.* Additionally, the differences we document are at levels similar to those reported for other closely-related species that were once were recognized as subspecies of a species, but are now recognized as different species, for example, *M. fusca* and *M. crissalis*, and *M. cabanisi* (Sclater & Salvin) and *M. biarcuata* (Prévost & Des Murs) (Rising 2010; Sandoval *et al.* 2014). Based on the clear phenotypic differences reported here, and allopatric distributions that reduce the probability of future contact by natural causes, we propose that *M. l. occipitalis* be recognized as a different species called *M. occipitalis* (Salvin's Ground-sparrow) separate from *M. leucotis* (White-eared Ground-sparrow) which would encompass the two more southerly subspecies *M. l. leucotis* and *M. l. nigrior.* Our proposal is also supported by other sources, which recognize both groups as different species based on plumage differences (del Hoyo & Collar 2017) and genetic distances (Sandoval *et al.* 2017).

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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 American Museum of Natural History (AMNH).

Melozone leucotis leucotis
Female: UCR 932, UCR 383, MNCR 27250, FMNH 72935, FMNH 72933.
Male: UCR 3190, MNCR 27318, MNCR 27320, MNCR 26972, MZUM 89017, MZUM 210627, FMNH 72936, FMNH 72932.

Melozone leucotis nigrior
Male: FMNH 22185, AMNH 103040, AMNH 103498, AMNH 103821, AMNH 103822, AMNH 423592.
Female: AMNH 103041, AMNH 103231, AMNH 103233, AMNH 103234, AMNH 103499, AMNH 103823, AMNH 144637

Melozone leucotis occipitalis
Female: MZUM 94606, MZUM 110057, FMNH 434112
Male: MZUM 110056, MZUM 94605, MZUM B494a, FMNH 189850, FMNH 189849.

APPENDIX B. LIST OF RECORDINGS USED IN THIS STUDY, OBTAINED FROM THE MACAULAY LIBRARY OF NATURAL SOUNDS IN THE CORNELL LABORATORY OF ORNITHOLOGY (ML). 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 leucotis leucotis
*H1–*H11 Costa Rica, Heredia, Getsemaní; *H12–*H15 Costa Rica, Heredia, Calle Hernández; *JBL1–JBL6, JBL8, JBL9 Costa Rica, Cartago, Jardín Botánico Lankester; *MTV1–*MTV5, *MTV7-*MTV11 Costa Rica, Puntarenas, Monteverde; *UCR1–*UCR7, *UCR11, *UCR13–*UCR15, *UCR17, *UCR18 Costa Rica, San José, Universidad de Costa Rica Campus.

Melozone leucotis nigrior
*SN2, *SN32, *SN100–*SN104 Nicaragua, Matagalpa, Hotel Selva Negra.

Melozone leucotis occipitalis
ML 95083 Mexico, Chiapas, El Triunfo Biosphere Reserve; ML 105969–ML 105974 El Salvador, Santa Ana, Las Lajas wildlife refuge; *LT200–*LT202 Guatemala, Suchitipéquez, Reserva Los Tarr