FILLING THE GAP: MOLTING BEHAVIOR OF COLIMA WARBLERS AND RESEARCH OPPORTUNITIES FOR UNDERSTUDIED NORTH AMERICAN SONGBIRDS

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ABSTRACT—We implemented stable isotope analysis to evaluate the molt behavior of the Colima warbler (*Leiothlypis crissalis*), an understudied migratory songbird occurring in Mexico and recently discovered breeding in the southern part of Texas, USA. We built a geostatistical model showing variation in deuterium precipitation values (δ^2H_p) across a latitudinal gradient within the Colima warbler breeding range in northeastern Mexico. Then, based on stable isotope ratios of deuterium in feathers (δ^2H_f), we assigned wintering Colima warblers captured in Central Mexico to possible molting areas near the southwestern portion of the species' breeding range. To the best of our knowledge, this is the first field study documenting Colima warbler captures and recaptures within the Parque Ecológico de la Ciudad de México, near the mountain ranges surrounding the Basin of Mexico. Overall, our study demonstrates the potential of winter ecology fieldwork in conjunction with molecular study techniques, such as stable isotope analysis, for revealing the migratory and molting behavior of warblers with restricted distribution ranges.

RESUMEN—Implementamos un análisis de isótopos estables para evaluar el comportamiento de muda del chipe crisal (*Leiothlypis crissalis*), un ave migratoria poco estudiada que ocurre en México y que se descubrió recientemente reproduciéndose en la parte sur de Texas, EE. UU. Construimos un modelo geoestadístico que muestra la variación en los valores de precipitación de deuterio (δ^2H_p) a lo largo de un gradiente latitudinal dentro del área de reproducción del chipe crisal en el noreste de México. A continuación, con base en las proporciones de isótopos estables de deuterio en plumas (δ^2H_f), asignamos chipes crisal invernantes capturados en el centro de México a posibles sitios de muda cerca de la porción suroeste del área de reproducción de la especie. Hasta donde sabemos, este es el primer estudio de campo que documenta las capturas y recapturas del chipe crisal en el Parque Ecológico de la Ciudad de México, cerca de las sierras que rodean la cuenca de México. En general, nuestro estudio demuestra el potencial del trabajo de campo sobre la ecología invernal en combinación con técnicas de estudio molecular, como el análisis de isótopos estables, para revelar el comportamiento migratorio y de muda de los chipes con áreas de distribución restringidas.

Much effort has been invested in understanding the decline of North American migratory songbird species in the past decades (Rosenberg et al., 2019). However, data deficiencies often impede an initial assessment for conservation plans (Ballard et al., 2003; Faaborg et al., 2010). Thus, filling key gaps in basic knowledge advances our ability to prioritize and effectively use resources for management and conservation. Studies of migratory connectivity between breeding, molting, and wintering grounds provide useful information for population-level management (Rushing et al., 2016; Contina et al., 2019). Although studies that adopt miniaturized tracking technologies (e.g., geolocators) or other types of external tags also provide important information about migratory connectivity (e.g., Kramer et al., 2018), weight limitations, low return rates of birds, and high costs of tags (especially for devices that transmit data and thus do not require recapture) combine to limit the gathering of information that is essential for conservation (Bridge et al., 2013). Molecular techniques such as stable isotope analyses can help circumvent these problems by inferring bird movements between two points based on a single capture because a single feather sample provides information of its growing origin (Hobson et al., 2004).

Stable isotope analyses of naturally abundant elements such as carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and hydrogen ($\delta^{2}H$) have been widely used to investigate the ecology and movement patterns of migratory species across taxa (Bearhop et al., 2004; Hobson, 2005; Hobson and Wassenaar, 2018). By exploiting a precipitation latitudinal gradient in deuterium ($\delta^{2}H$) that exists globally, and notably across Central and North America, it is possible to assign inert biological tissues such as claws or feathers to the region where they were grown and thus reconstruct individual molting and movement patterns (Bowen et al., 2005; Zenzal et al., 2018). Using stable isotope ratio analyses in conjunction with the timing of capture and morphometric data can provide further information about how migrants manage their journeys. For example, Smith et al. (2003) found that age and size of birds differed by breeding latitude as estimated through stable isotope analysis. Additionally, the amount of energy reserves carried before and during migration might be proportional to the distance a migrant must travel to its breeding location and be related to sex-based differences in the timing of migration (Wilson et al., 2008).

The Colima warbler (*Leiothlypis crissalis*) is a small (about 10 g), short-distance migratory songbird occurring in Mexico and the southern United States for which managers lack natural history data necessary for informing management needs. It is currently listed as a species of least concern (BirdLife International, 2020), although it was listed as Near Threatened from 2004 to 2012 and is included in the "subject to special protection" category by the Mexican government (Secretaría de Medio Ambiente y Recursos Naturales, 2010). Additionally, it is listed as a species of continental concern by Partners in Flight (Rosenberg et al., 2016). Notably, almost the entirety of the known Colima warbler breeding and wintering range is in Mexico. The breeding range follows the Sierra Madre Oriental, and the majority lies within the northeastern Mexican states of Coahuila, Nuevo León, Zacatecas, and San Luis Potosi, although a small portion extends into southwestern Texas (Howell and Webb, 1995). The recognized wintering range includes the Western Mexican Pacific slopes of the Sierra Madre Occidental, from Southern Sinaloa south to Michoacán, Guerrero, and Oaxaca, but also to the east along the Trans-Mexican Volcanic Belt, although stopping well outside Mexico City (Fig. 1). Adult Colima warblers molt twice per year, although the spring molt most likely takes place on the wintering grounds and is thought to be limited to the head (Pyle, 1997). Wing and tail feathers are molted in late summer (Pyle, 1997). The Colima warbler's primary habitat consists of oak (*Quercus*) forests between 1,500 and 3,600 m elevation, with drier conditions on the breeding range than the wintering range. With both breeding and wintering terrains extremely difficult to traverse, studies examining population dynamics and migration timing are challenging to conduct. Furthermore, research is complicated by the fact that the Colima warbler breeding range spans across the United States–Mexico border into Texas, where laws and conservation management practices differ significantly from those in the interior of Mexico (Abbitt et al., 2000).

In the present study, we searched for a distinguishable $\delta^{2}H$ precipitation pattern within the currently recognized Colima warbler breeding range, an area covering about 102,000 km^2. Such a pattern would be useful in linking Colima warblers captured at their wintering ground in central and southwestern Mexico to their breeding and molting grounds in northeastern Mexico. These geographic assignments are warranted because the Colima warbler is considered among the most understudied species of songbirds in Mexico and information on its behavioral and movement ecology is needed for conservation planning (Beason and Wauer, 1998; International Union for Conservation of Nature [IUCN], 2019). There is no current reliable population estimate, though studies note that Colima warblers may be locally common (Lanning et al., 1990). To the best of our knowledge, our study is the first stable isotope investigation of the Colima warbler, which we present in conjunction with banding and biometric data documenting the first records of captures and recaptures within Parque Ecológico de la Ciudad de México. We provide an insight into the migratory and molting behavior of this understudied Neotropical species and discuss the implications of merging winter-ecology field studies with molecular research techniques.

Materials and Methods—*Study Area*—The main study site is part of a natural protected area called Parque Ecológico de la Ciudad de México—Mexico City Ecological Park—henceforth, PECM. The park, also known as Ajusco Medio, is situated in one of the mountain ranges that surround the Basin of Mexico and is located approximately 21 km SSW from Mexico City's historic downtown (Fig. 1). Altitudes in the park range from 2,400 to 2,800 m, the mean annual temperature is 14°C, and the mean annual precipitation ranges from 717 to 918 mm, with 80% of the precipitation occurring between June and October (González-Hidalgo et al., 2002; Mendoza-Hernández et al., 2014). The park is 727 ha in size and is covered by three vegetation types: oak forests, pine (*Pinus*)–oak forests, and xerophytic shrub vegetation, which are present in fragments of different sizes because of the human pressure in the area resulting from urban sprawl (Arizmendi et al., 2007; Mendoza-Hernández et al., 2013). We captured birds at the Roca volcánica y cañada study site (henceforth, ROCA) within this park (Fig. 1), which was operated as part of the Monitoreo de Sobrevivencia Invernal (MoSI) program between November 2010 and December 2013 (DeSante et al., 2005). This site (19°15′04.03″N, 99°11′52.52″W; elevation 2,742 m) encompasses approximately 25 ha covered by patches of oak forest interspersed with open secondary xerophilous shrubland.

In addition to samples collected at ROCA, morphometric information for one additional Colima warbler individual came from Arcos del Sitio, henceforth abbreviated as ARCO (19°44′52.51″N, 99°20′32.89″W; elevation 2,507 m), an MoSI

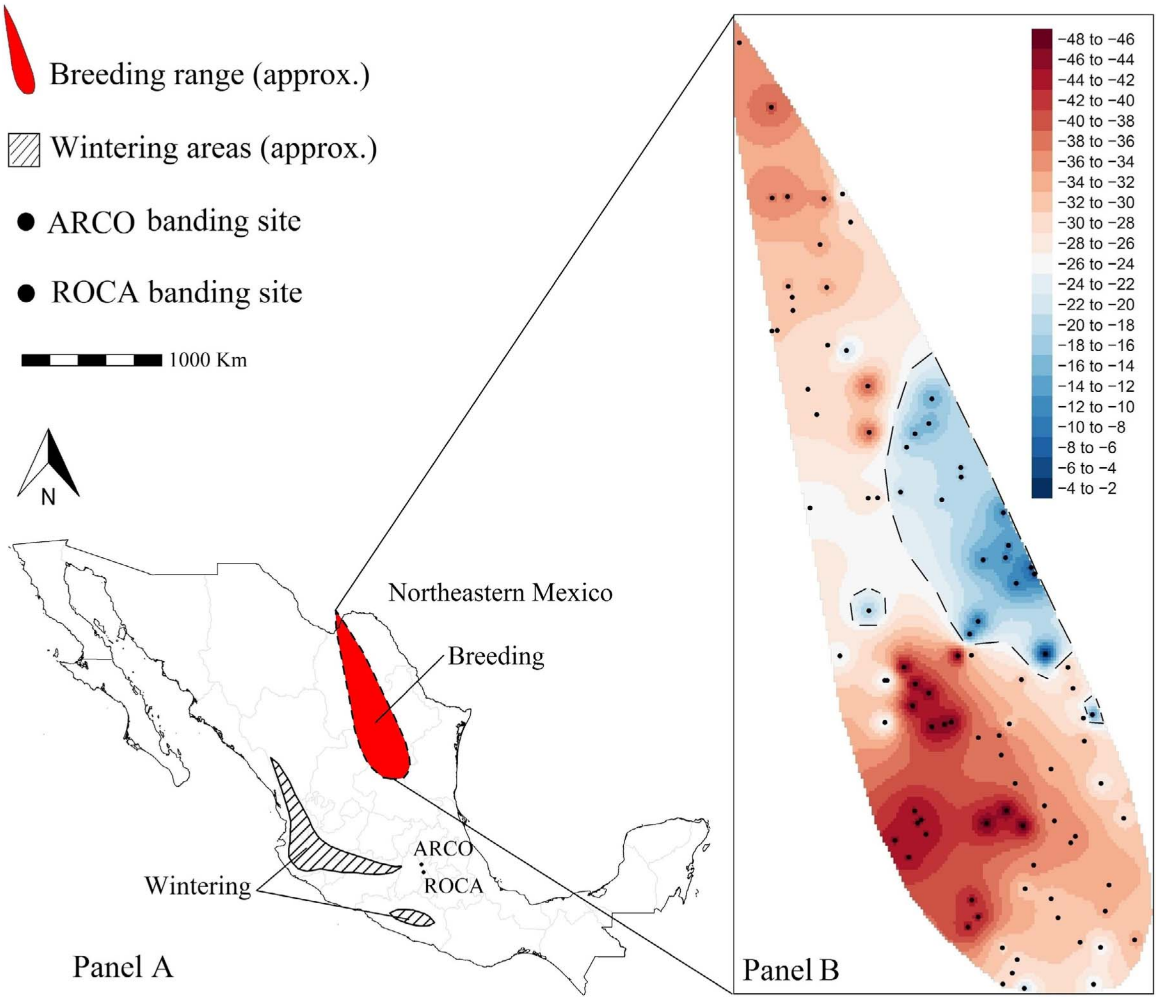

FIG. 1—Banding sites (panel A) and geospatial distribution of δ^2H_p values within the Colima warbler breeding range before rescaling. Note the overall north–south gradient and the clear differentiation between the isotopically enriched eastern region (indicated in blue and enclosed with a dashed border) and the isotopically depleted southwestern region indicated in red (panel B). The breeding range extends from approximately 23.5 to 30°N and from 100 to 103°W, while the wintering range is composed of two sections that together extend from approximately 17.5 to 24°N and 99 to 106°W. Gray lines within the map of Mexico represent state borders (panel A). Roca volcánica y cañada (ROCA) and Arcos del Sitio (ARCO) are Monitoreo de Sobrevivencia Invernal (MoSI) banding stations located in the Parque Ecológico de la Ciudad de México (PECM) and The Sierra de Tepotzotlan, respectively. (Color version is available online.)

station located within the Parque Estatal Sierra de Tepotzotlan, another natural protected area located 58 km NNW of PECM (see Fig. 1). This park is 9,783 ha, and its climate is temperate humid with rain in summer (precipitation of 703 mm) and frosts in winter, and it has an average annual temperature of 16°C. The area contains three vegetation types: oak forest, cactus scrub, and grassland (Gobierno del Estado de México, 2004). Geographically, both PECM and the Parque Estatal Sierra de Tepotzotlan lie in the Eje Neovolcánico Transversal, a volcanic mountain chain that crosses the country east–west, near the parallel 19°N.

Sample Collection—We sampled Colima warbler tail feathers during the nonbreeding season of 2013 at the ROCA banding station. Beginning in 2010, we caught birds using mist-nets placed along preexisting trails, but we started feather collection only during the last year of the station operation in 2013. We plucked two feathers from the captured birds: the outer rectrix of one side of the tail and the central of the other side. We marked all individuals with unique identification (ID) nine-digit United States Fish and Wildlife Service bands or five-digit and three-digit custom-made bands and aged them (second year [SY] vs. after second year [ASY]) using plumage criteria (Pyle, 1997). We also collected morphological data for each individual, including weight, unflattened wing chord, and culmen (DeSante et al., 2009). We also obtained morphological data for a single individual captured and recaptured at ARCO in December 2005 and January 2008, respectively. In total, we obtained morphological data on 18 captured Colima warblers (Table 1). For our stable isotope investigation, we analyzed nine feather samples from eight of these birds, resulting in one processed feather per individual except for COLW-06–COLW-09, which we sampled again upon recapture on 6 November 2013 (Supplemental Table S1).

Stable Isotope Analysis—We removed oil contaminants from

TABLE 1—δ^2H_f values and first-capture morphometric data for 18 Colima warblers captured between December 2005 and December 2013 (COLW-09* is a recapture of COLW-06 but was given a unique ID because a new feather sample was taken upon recapture). Roca volcánica y cañada (ROCA) and Arcos del Sitio (ARCO) are Monitoreo de Sobrevivencia Invernal (MoSI) banding stations located in the Parque Ecológico de la Ciudad de México (PECM) and The Sierra de Tepotzotlan, respectively. — = no data available; isotopic data are missing from COLW-10 through COLW-18 because no feather samples were taken from these birds. Morphological measurements and visual fat assessments were conducted following standard procedures of the MoSI banding stations (DeSante et al., 2009). Capt. = capture(s).

Bird ID (laboratory)	Band ID (field)	δ^2H feather (‰)	No. of capt.	Date of first capt.	Capt. location	Wing cord (mm)	Mass (g)	Tail (mm)	Tarsus (mm)	Culmen (mm)	Fat assessment
COLW-01	831	−59.61	3	18 Nov. 2010	ROCA	66	10.7	59	20.7	8.4	Half
COLW-02	CP072	−61.49	1	12 Apr. 2013	ROCA	61	7.6	48	17.6	8.7	None
COLW-03	CP094	−57.66	2	12 Sep. 2013	ROCA	64	10	56	17.7	7.9	None
COLW-04	CP097	−55.84	1	9 Oct. 2013	ROCA	63	10.3	55	16.6	9	Trace
COLW-05	CP098	−53.60	1	10 Oct. 2013	ROCA	62	10.1	53	18.8	8.7	None
COLW-06	CP095	−62.80	3	13 Sep. 2013	ROCA	62	10.5	55	17.7	8.3	Trace
COLW-07	CP099	−96.87	2	6 Nov. 2013	ROCA	64	10.2	55	16.7	9.4	None
COLW-08	CP062	−46.85	2	13 Dec. 2012	ROCA	64	9.5	55	17.6	9.2	None
COLW-09*	CP095	−64.03	3	13 Sep. 2013	ROCA	62	10.5	55	17.7	8.3	Trace
COLW-10	841	—	1	19 Nov. 2010	ROCA	64	10.1	56	19.6	8	Trace
COLW-11	869	—	1	16 Sep. 2011	ROCA	65	10.3	67	18.6	8.4	Light
COLW-12	875	—	2	3 Nov. 2012	ROCA	65	8.8	49	18.8	8.9	None
COLW-13	183177802	—	2	9 Dec. 2005	ARCO	65	8.9	55	—	—	None
COLW-14	237076668	—	1	21 Nov. 2012	ROCA	61	10.1	56	16.5	9.5	Trace
COLW-15	237076687	—	5	5 Dec. 2010	ROCA	62	9.7	54	19.3	9.3	Trace
COLW-16	CP036	—	1	24 Aug. 2012	ROCA	64	10.3	57	19.8	8.1	Trace
COLW-17	CP053	—	1	24 Aug. 2012	ROCA	56	9.1	48	16.3	8.5	Half
COLW-18	CP055	—	1	13 Sep. 2012	ROCA	61	9.9	51	18.3	8.8	None
COLW-19	CP063	—	1	6 Nov. 2013	ROCA	63	10.1	53	19	8.9	None

feathers by bathing them for about 30 s in a chloroform–methanol solution under a fume hood (to limit exposure to hazardous vapors). We then proceeded with three additional bathing steps in detergent solution and deionized water and dried all the feathers at room temperature for 48 h following a standardized protocol (Chew et al., 2019). We cut and weighed the distal tip of each feather to 200 μg (±10 μg), packed them into silver capsules, and analyzed them using a Thermo Finnigan analyzer connected through an interface (Finnigan ConFlo II, Thermo Electron Corporation, Waltham, Massachusetts) with a continuous-flow isotope ratio mass spectrometer (Finnigan Delta Plus XL). We reported all isotopic values in delta notation of parts per million (‰) from the standards with an estimated laboratory error of ±2‰, where $\delta D_{sample} = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$, relative to a standard (Vienna standard mean ocean water [VSMOW]). We used Caribou Hoof Standard ($\delta^2H_{CHS} = -197.0$‰) and Kudu Horn Standard ($\delta^2H_{KHS} = -54.1$‰) as internal calibration standards (United States Geological Survey, Reston Stable Isotope Laboratory, Virginia). We performed stable isotope analysis at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University (Flagstaff). We stored all feathers for a minimum of 2 weeks before analysis to allow equilibration to the local environment.

Development of a Precipitation Isoscape—We inferred a northeastern Mexico isoscape precipitation model for δ^2H in IsoMap (http://isomap.org) over a latitude range of 4.3–39°N and a longitude range of 84.4–125.3°W. We incorporated temperature, elevation, and vapor pressure into the model as independent variables and restricted δ^2H data to April–August for the years 1980–2009 (for a complete list of precipitation model parameters, see IsoMap job key = 73538). To extract maximum isotopic information in Central Mexico, we then computed a finer scale model interpolation in the R package *gstat* (Pebesma, 2004) by computing a grid with a single δ^2H value per km^2 within the species' breeding range. We used an inverse distance weighting algorithm (Gräler et al., 2016) to spatially interpolate unsampled grid cells and develop a δ^2H_p isoscape geographically restricted to the Colima warbler breeding range in Northern Mexico.

Stable Isotope Geographic Assignments—Using the R statistical environment (R Core Team, 2018), we computed a probability density surface for each individual captured at ROCA. We acknowledge that, whenever possible, species-specific isoscape models should be calibrated by using δ^2H values of feathers grown at a known location. However, to the best of our knowledge, there are no δ^2H_f data from Colima warblers currently available. Therefore, to estimate the probability of molting origin of wintering individuals, we rescaled Colima warbler δ^2H_f values using isotopic parameters computed for the Wilson's warbler (*Wilsonia pusilla*) against δ^2H_p using the equation $\delta^2H_f = -14.47 + 1.4 \times \delta^2H_p$ (Paxton et al., 2007). This approach is widely adopted whenever species-specific feather calibration data are not obtainable (Hobson et al., 2012*a*; Paxton and Moore, 2015). We then masked the assignment results outside the uncertainty-adjusted species breeding range (BirdLife International, 2016) and normalized the probability surfaces in the package *raster* (Hijmans, 2019) before plotting them in base R.

Morphological Traits—Delingat et al. (2011) demonstrated that morphometric measurements, particularly of the wing, can

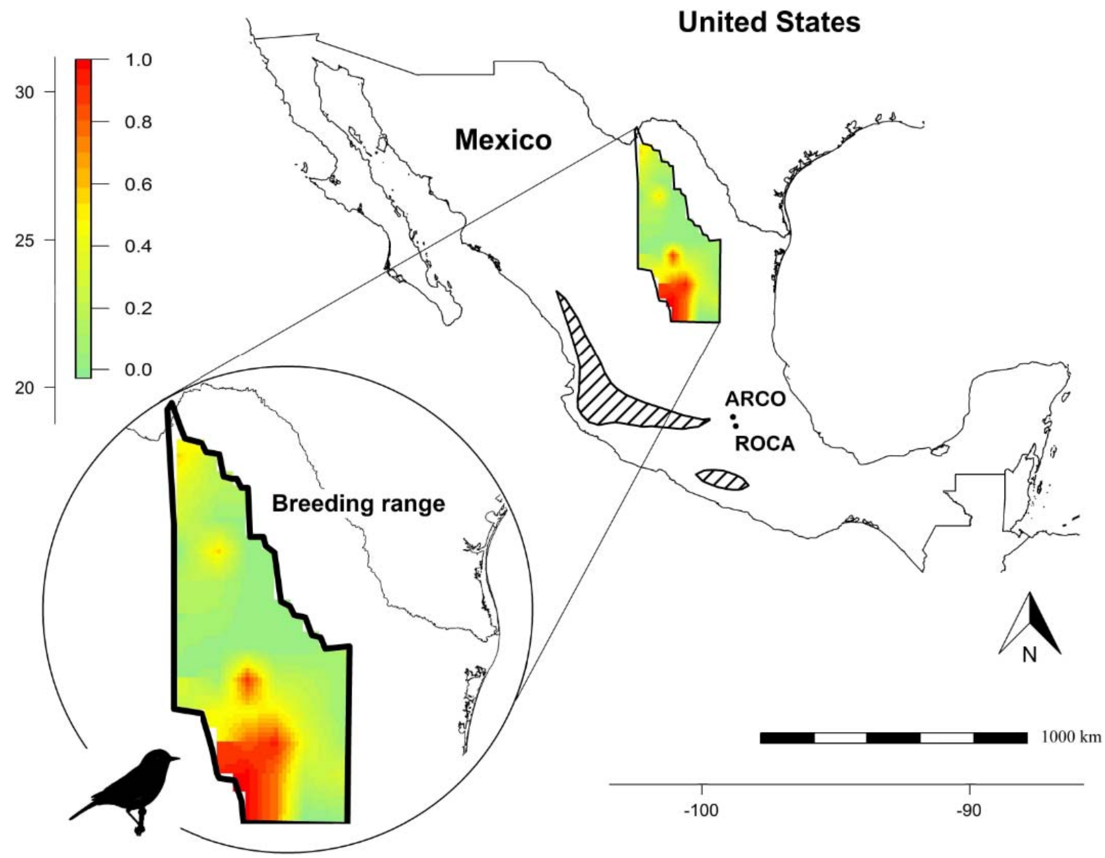

FIG. 2—Composite assignment map traces Colima warblers sampled at Roca volcánica y cañada (ROCA) to the southwestern edge of the breeding range (dark red). Lower assignment probabilities exist to the north and east (yellow and green). The breeding distribution range is modified and slightly extended from BirdLife International (2016; Version 6.0) to better incorporate species occurrence uncertainty. The wintering range is indicated with diagonal black lines in two disjunct areas in southwestern Mexico. ROCA and Arcos del Sitio (ARCO) are Monitoreo de Sobrevivencia Invernal (MoSI) banding stations located in the Parque Ecológico de la Ciudad de México (PECM) and The Sierra de Tepotzotlan, respectively. (Color version is available online.)

supplement stable isotope studies that aim to establish migratory links between breeding and wintering grounds. Previous research also found wing morphometrics to be useful in separating populations geographically (Tellería et al., 2001) and predicting migration distance (Leisler and Winkler, 2003). Therefore, we report the first-capture morphometric measurements for 18 Colima warblers captured between 2005 and 2013 to provide the initial baseline of data for our wintering population (Table 1). Additionally, we computed correlations between Colima warbler wing chord and mass, tarsus, culmen, and tail length and plotted the data using the R packages *ggplot2* (Wickham, 2016) and *ggpubr* (Kassambara, 2019). These plots are presented below, along with estimates of the Pearson correlation coefficient.

RESULTS—*Geographic Assignments Inferred from Hydrogen Stable-Isotope Ratio*—We highlighted areas within the recognized Colima warbler breeding range that are characterized by a latitudinal gradient in δ^2H_p values (Fig. 1). We exploited this gradient to assign migratory Colima warblers wintering at our study site in Mexico (ROCA) back to their molting locations (Fig. 2) using our rescaled precipitation isoscape containing δ^2H_p values ranging from −66.2 to 10.7‰. Our results show that all sampled individuals likely originated from the southwestern part of the breeding distribution range, from approximately 23.5 to 25.5°N and 100.5 to 102°W (Fig. 2 and Supplemental Fig. S1).

Individual Variation in Hydrogen Stable-Isotope Ratio—The feather hydrogen stable-isotope ratios (δ^2H_f) for the Colima warblers captured in Mexico (ROCA banding station) ranged between −96.87 and −46.85‰ (with mean δ^2H_f and $\pm SD$ values of -62.08 ± 14‰; Table 1). COLW-07 had the most depleted δ^2H value (−96.87‰) of all birds studied, resulting in high assignment probabilities to the southwestern edge of the breeding range (23.5–24°N, 101.25–101.75°W) and possibly even from a location south of the established breeding boundaries (Fig. S1). Conversely, COLW-08 exhibited the most enriched δ^2H value (−46.85‰) of all birds studied, pushing its geographic assignment slightly northward, with maximum assignment probabilities between 24 and 25.5°N and moderate to high assignment probabilities extending across most of the southern half of the breeding range (Fig. S1).

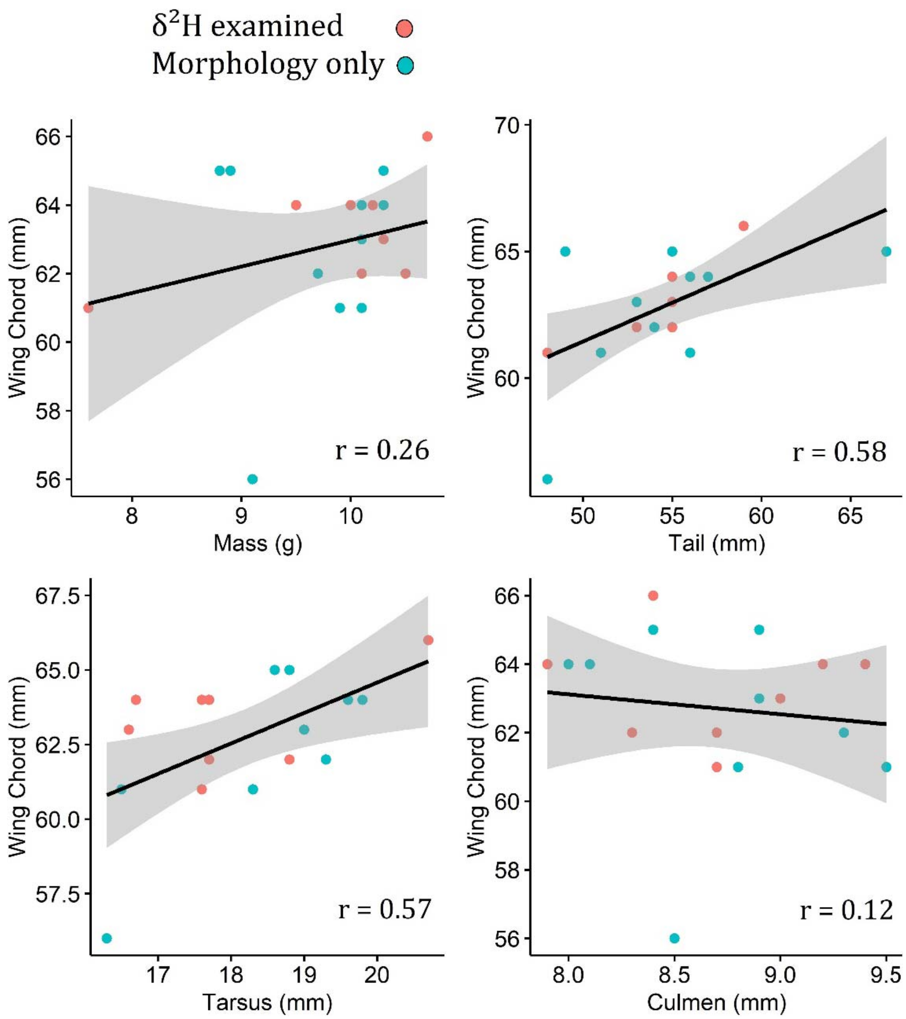

FIG. 3—Wing chord regressed against mass and tail, tarsus, and culmen length for 18 Colima warblers captured between December 2005 and December 2013. Because COLW-09 is a recapture of COLW-06, it is plotted only once. Points are colored according to whether only morphological data were collected (teal) or whether isotopic data were also collected (pink). Regression lines with 95% confidence limits are plotted and the Pearson correlation coefficient (r) is reported in the bottom right corner of each plot. (Color version is available online.)

Capture Dates and Morphological Variation—The occurrence of Colima warblers at our main study site (ROCA) ranged from late August to mid-April across years (Table 1). Our descriptive analysis of individual morphological variation shows that wing chord has a positive linear relationship with tail length ($r = 0.58$; 95% confidence interval [CI] = 0.15, 0.82) and tarsus length ($r = 0.57$; 95% $CI = 0.12$, 0.82). However, its correlations with mass ($r = 0.26$; 95% $CI = -0.23$, 0.65) and culmen ($r = 0.12$; 95% $CI = -0.38$, 0.57) are much weaker. The correlations between these variables are shown in Fig. 3.

DISCUSSION—We assigned all the birds in our data set to the southwestern part of the species' breeding range, supporting a migratory link with our banding site at Roca volcánica y cañada (ROCA), which we present as a suitable wintering ground for Colima warblers to the southwest of Mexico City. We showed that the latitudinal variation in deuterium existing even within the restricted species breeding distribution range is useful in developing probabilistic geographic assignments of migratory individuals. The results of our analysis support the use of stable isotope inferences for studying broad-scale Colima warbler movement and molting patterns.

Notably, our study is the first to report repeated recaptures within the wintering season of Colima warblers in such proximity to Mexico City. The multiple recaptures of Colima warblers at ROCA, which historically and until now had not been well-documented as part of the Colima warbler wintering range (Bangs, 1925; Peterson and Navarro-Sigüenza, 2006), supports a re-evaluation and careful examination of Colima warbler wintering distribution. If the wintering range has indeed shifted or expanded in recent years, implications of habitat suitability or adaptation to climate change should be considered (Lehikoinen et al., 2013). In particular, stable isotope analysis of museum specimens might shed light on historical molting grounds and allow long-term temporal comparisons (Rocque and Winkler, 2005).

Interestingly, our analysis revealed that 78% of the birds in our data set exhibited δ^2H values between −64.03 and −53.60‰, and all the birds were assigned to the southern edge of the breeding range. The more extreme δ^2H values exhibited by two individuals in our sample (COLW-07 and COLW-08 in Table 1) may indicate that these birds adopted a slightly different molting strategy compared with the rest of the birds captured at our banding site; for a discussion of molting strategies, see Bridge (2011). Although any inferences regarding those two sample outlier values should be taken with caution to avoid overinterpretation of the movement patterns, which currently lack cross-validation data from direct observations, our stable isotope approach might reveal new molting areas on a broad scale within the breeding range. Furthermore, stable isotope inferences might point to overlooked molting grounds in the northern portion of the species distribution or highlight a molt migration pattern in areas outside the currently recognized species range.

The accuracy of the stable isotope assignments of the present study cannot be explicitly evaluated with our current data because we lack any information on the molting location of the Colima warblers being examined. When available, data on molting sites can be used to establish a "known-origin" baseline to be compared with the estimated origin. However, the broad range of environmental-hydrogen stable-isotope rescaled values (δ^2H_p spanning from −66.2 to 10.7‰) present within the species' breeding distribution (Fig. 1) supports our confidence that we captured the approximate origin area in the southwestern part of the breeding range.

Our study was limited by its small sample size and we could not exhaustively describe the molt and migration system of the Colima warbler. However, our goal was primarily to test a stable isotope approach that could improve our understanding of the migration and molt pattern of a warbler species with restricted distribution range and facilitate the implementation of more effective management action plans. The collection of morphometric data can supplement our analysis by helping to distinguish different breeding populations migrating through Mexico over the winter. Additionally, our morphometric data indicated high variation in mass-to-wing ratios, which may be informative for population demographics (Gilbert and West, 2015). Thus, the body measurements that we reported here established a population baseline record for future research that might combine molecular and morphological data to identify the origin of migratory individuals (Smith et al., 2003).

Overall, the current paucity of data about Colima warbler migration calls attention to the importance of continuing and expanding the monitoring effort of wintering songbird populations in Mexico. Furthermore, there is still a poor understanding of migratory connectivity for nearly all members of the genus *Leiothlypis* and many questions on the evolution of migration in Neotropical "wood-warblers" remain unresolved (but see Winger et al., 2012). Of the five other species within the *Leiothlypis* genus, the Colima warbler is most closely related to the Nashville warbler (*L. ruficapilla*) and Virginia's warbler (*L. virginiae*; Lovette et al., 2010). Nashville warblers have a broader breeding and wintering range than do Virginia's, yet no breeding and wintering site connectivity studies have been attempted with either species, to the best of our knowledge. Orange-crowned warblers (*L. celata*) have been studied isotopically during migration (Kelly, 2006), but migratory links between breeding and wintering areas remain unknown. Thus far, only a stable isotope assessment of wintering Tennessee warblers (*Leiothlypis peregrina*) in Venezuela has been completed (Hobson et al., 2014). However, sampling multiple wintering sites will lead to the best map of connectivity, and therefore there is ample opportunity especially within the *Leiothlypis* genus to fill these important knowledge gaps.

Conclusions—We recommend that future isotopic assessments of the Colima warbler consider multi-isotope elements, including carbon and nitrogen, to add inferences on diet shifts between breeding and winter seasons (Phillips and Eldridge, 2006; Hobson et al., 2012*b*). Furthermore, stable isotope analyses, particularly in understudied species, should be integrated within a larger investigative framework accounting for recent molecular ecology advances that refine the resolution of geographic assignments and incorporate information from genetic studies on migratory populations (Rundel et al., 2013; Contina et al., 2016). We lack essential natural history information on the Colima warbler as a result of low population densities and difficulties accessing breeding and molting grounds. Stable isotope analysis is a cost-efficient (approximately US$20/sample) and reliable method to assess large-scale population connectivity in declining migratory species. Our repeated captures of Colima warblers at previously unrecognized areas also highlight the value of long-term wintering field studies in Central America.

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