MIGRATORY CONNECTIVITY IN EUROPEAN BIRD POPULATIONS: FEATHER STABLE ISOTOPE VALUES CORRELATE WITH BIOMETRICS OF BREEDING AND WINTERING BLUETHROATS *LUSCINIA SVECICA*

CONECTIVIDAD MIGRATORIA EN POBLACIONES DE AVES EUROPEAS: CORRELACIÓN ENTRE EL VALOR DE ISÓTOPOS ESTABLES EN PLUMAS Y BIOMETRÍA DE PECHIAZULES *LUSCINIA SVECICA* EN ZONAS DE CRÍA E INVERNADA

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Summary.—Understanding the migratory connectivity of migrant species is fundamental to their effective conservation. Analysis of individual traits that can vary geographically, such as biometrics and stable isotopic values of tissues, can help establish migratory connections. The bluethroat *Luscinia svecica* is a species of conservation concern in Europe (Annex I Birds Directive). Our aim was to identify the possible migratory connectivity of bluethroats *Luscinia svecica* breeding in central and western Europe (subspecies *L. s. namnetum*, *L. s. azuricollis* and *L. s. cyanecula* in part) with their wintering areas

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in southern Europe and Africa using biometric and stable isotopic (δ^2H) analyses. Overall, the morphological and stable isotopes analyses provided two clusters of localities, one for the Atlantic French, Portuguese and Moroccan localities, corresponding to the breeding and winter quarters of *L. s. namnetum*, and another for the remaining localities (Spain, The Netherlands, Germany and Senegal), corresponding to the ranges of *L. s. azuricollis* and *L. s. cyanecula*. Migratory connectivity of *L. s. namnetum* is strong but it is much weaker for the other two subspecies. Biometric data were positively correlated to the stable isotope values, suggesting that the results derived from both methodological approaches lead to similar conclusions.

Key words: biogeography, biological conservation, body size, deuterium, migrant passerines, stable isotopes, wing length.

RESUMEN.—Comprender la conectividad migratoria es fundamental desde el punto de vista de la conservación de las especies que migran. El análisis de caracteres individuales que varían geográficamente, como la biometría o el valor de determinados isótopos estables en tejidos, es útil a la hora de establecer la conectividad migratoria. El pechiazul *Luscinia svecica* es una especie que está incluida en el Anexo I de la Directiva de Aves. El objetivo de nuestro estudio es determinar el grado de conectividad migratoria entre las áreas de cría de la especie en el centro y oeste de Europa (subespecies *L. s. namnetum*, *L. s. azuricollis* y *L. s. cyanecula*) y puntos de invernada en Europa meridional y África. Para ello se utilizaron datos biométricos e isótopos estables (δ^2H). En conjunto, se obtuvieron dos grupos que aglutinaron, respectivamente, (1) las localidades del área atlántica de Francia, así como Portugal y Marruecos (para este país no se obtuvieron datos biométricos), correspondientes al área de distribución de cría e invernada de la subespecie *L. s. namnetum*; (2) el resto de localidades (España, Alemania, Holanda y Senegal), correspondientes al área de distribución de *L. s. azuricollis* y *L. s. cyanecula*. La conectividad migratoria en *L. s. namnetum* es alta, no así para las subespecies *L. s. azuricollis* y *L. s. cyanecula*. Para el conjunto de puntos de muestreo, hubo correlación entre el valor biométrico y el obtenido a partir de isótopos estables, lo que sugiere que el resultado de ambos métodos lleva a conclusiones similares.

Palabras clave: biogeografía, biología de la conservación, deuterio, isótopos estables, longitud alar, paseriformes migratorios, tamaño corporal.

INTRODUCTION

Compared to animals that spend their entire lives within a small home range, migrant species are affected by the conditions existing across huge geographical areas (Newton, 2004). For instance, conditions in tropical Africa during the boreal winter can have a severe impact on survival rates of trans-Saharan migrants breeding in Europe (Peach *et al*., 1991; Cowley and Siriwardena, 2005; Zwarts *et al*., 2009). Migratory connectivity is deemed high or strong when individuals from a breeding population overwinter together in a particular region, distinct from that used by others (for examples see e.g., Zwarts *et al*., 2009). By contrast, migratory connectivity is weak when individuals from one breeding population disperse to different wintering areas where they may mix with individuals from other breeding populations. Patterns of migratory connectivity between breeding and wintering sites of these migrants are highly significant in terms of their ecology, evolution and conservation (Webster *et al*., 2002).

To date, the use of technologies which would allow us to obtain the geographical position (GPS) of small migratory birds directly is unavailable. The transmitters required are still too heavy. Other devices that enable the accurate determination of the position of particular individuals throughout the year (e.g., light-level geolocators) (Bächler *et al*., 2010; Schmaljohann *et al*., 2012;

Tøttrup *et al*., 2012) are less expensive but large sample sizes are needed since usually fewer than 20% of the birds are recaptured (Bächler *et al*., 2010; Schmaljohann *et al*., 2012; Tøttrup *et al*., 2012). Although useful (e.g., Bairlein, 2001), ring recovery data is insufficient when the number of recoveries is low and/or when the recovery rate varies geographically (Arizaga and Tamayo, 2013). Instead, individual traits, such as biometrics (Delingat *et al*., 2010; Arizaga *et al*., 2012) or the stable isotopic values of certain tissues (Hobson and Wassenaar, 1997; Chamberlain *et al*., 2000; Pain *et al*., 2004; Yohannes *et al*., 2005; Hobson and Wassenaar, 2008; Hobson *et al*., 2009) may be used. These vary geographically and hence can be utilised to ascertain the origin of an individual if food web isotopic patterns or "isoscapes" are known.

The bluethroat *Luscinia svecica* is an Holarctic songbird of conservation concern in Europe (Tucker and Heath, 2004) (Annex I Birds Directive). It breeds from Iberia in Europe, eastwards to Alaska and Canada in North America, and overwinters in southern Europe, Africa or Asia (Collar, 2005). About 11 subspecies have been reported (Collar, 2005). Three subspecies breed in western and central Europe: *L. s. cyanecula*, typically inhabiting lowland wetlands in central and western Europe (east to southern Russia); *L. s. namnetum*, which breeds along the Atlantic part of France; and *L. s. azuricollis*, found in north-western and central Iberia (Collar, 2005). This last population occupies a very small breeding range (Arizaga *et al*., 2011). The wintering areas of these subspecies are still not well known from a population-specific viewpoint. *L. s. cyanecula* spends the winter in southern Europe, northern Africa and the Sahel (Cramp, 1988), but exactly which populations go to which wintering sites is still unknown, because ring-recovery data seem to be insufficient to test this (Arizaga and Tamayo, 2013). According to biometric analyses, *L. s. namnetum* migrates

via the coast of the Bay of Biscay (Arizaga *et al*., 2006a) to overwinter along the coast of Portugal and in the wetlands on the coast of north-western Africa (Zucca and Jiguet, 2002; Arizaga and Tamayo, 2013). Morphological studies suggest that *L. s. azuricollis* is a long-distance migrant and so may overwinter in tropical Africa (Arizaga *et al*., 2006b), but this has not been confirmed.

With respect to morphology, the wing is longer in *L. s. azuricollis*, intermediate in *L. s. cyanecula*, and shorter for the smaller *L. s. namnetum* bluethroats (Arizaga *et al*., 2006b). A similar pattern may apply to body size (Cramp, 1988) although this has not been tested specifically. The δ^2H values of feathers grown in Europe decrease with increasing latitude and from west to east (Hobson *et al*., 2004). Primary feathers in bluethroats are replaced after breeding in adults but not in first-year birds, which moult these feathers after breeding in their second year of life (Jenni and Winkler, 1994). Hence, bluethroats caught in winter and during the subsequent breeding season before moulting, either as adults or first-year birds, will have δ^2H values associated with the end of the previous breeding season. Thus, their feathers would have the same (or similar) isotopic values to first-year birds, although some age-associated isotopic variations can happen (Meehan *et al*., 2003; Studds *et al*., 2012; Haché *et al*., 2014). Theoretically, these δ^2H values would be expected to be high in *L. s. azuricollis*, intermediate in *L. s. namnetum* and low in *L. s. cyanecula*.

Accordingly, it can be hypothesised that if migratory connectivity is high for the central-western European bluethroat populations, we should expect a high association between particular breeding localities and certain wintering regions. However, if migratory connectivity is low we would find high overlap between the wintering regions of birds from particular breeding localities. In particular, if migratory connectivity is

strong, and if *L. s. azuricollis* spends the winter in tropical Africa (Arizaga *et al*., 2006b), we expect at least three morphological/isotopic clusters, grouping (a) the *L. s. azuricollis* breeding sites with their tropical winter quarters; (b) the *L. s. namnetum* Atlantic French breeding sites with their wintering areas in south-western Iberia (mainly Portugal) and Morocco (Zucca and Jiguet, 2002);

and (c) the *L. s. cyanecula* breeding grounds from central-western Europe with their wintering sites within the circum-Mediterranean region. The first cluster would be formed by localities with long-winged, large body-sized bluethroats, having the lowest feather δ^2H values. The second by sites with short-winged, small body-sized bluethroats and intermediate δ^2H values. The third cluster
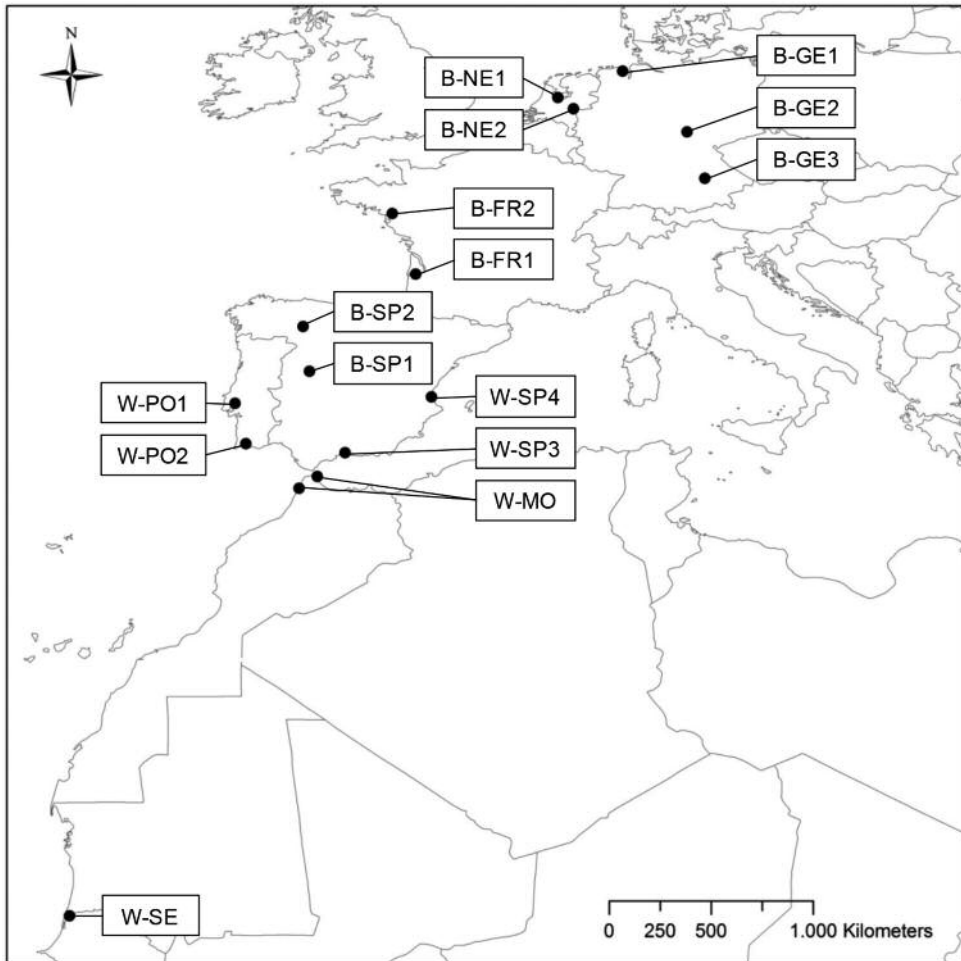

FIG. 1.—Location of the breeding (B) and wintering (W) sites where the bluethroats were caught from Germany to Senegal. Site codes as shown in table 1.
[Localización de puntos de cría (B) e invernada (W) muestreados, desde Alemania hasta Senegal. Código de sitios, como en la tabla 1.]

would have intermediate morphological values but higher δ^2H values. If the overlap between *L. s. azuricollis* and *L. s. cyanecula* is high during the winter, then we would expect to find two clusters grouping their breeding sites separately, and another grouping all their wintering areas, or alternatively, one for the circum-Mediterranean wintering sites including only *L. s. cyanecula* birds and another for the tropical wintering sites including *L. s. azuricollis* and *L. s. cyanecula*. (2) If migratory connectivity is weak, we should expect at least four clusters, one relative to each subspecies-specific breeding ground plus a large cluster for the wintering areas where all populations would occur in sympatry.

MATERIAL AND METHODS

Sampling sites

This study was carried out across much of the distributional range of white-throated bluethroat populations (*L. s. cyanecula*, *L. s. namnetum*, *L. s. azuricollis*) (Cramp, 1988), both at their breeding areas (nine localities; fig. 1) and at their winter quarters (7 localities; fig. 1). A wide geographical area, from Germany to Senegal (fig. 1), was covered. Due to the small sample sizes from Morocco, the data from the two localities in this area were lumped for the analysis and considered as a single locality (W-MO).

Data collection

The sampling was carried out during the breeding seasons of 2010 and 2011, and during the winters of 2010/11 and 2011/12. Winter data were obtained from December to February of the next year (for details see table 1), to ensure that only wintering bluethroats were sampled (Cramp, 1988).

Bluethroats were captured both at their breeding and wintering sites, in humid areas, either inland wetlands or coastal marshes (Cramp, 1988). An exception to this rule were the bluethroats captured as breeders in Spain. Such birds occupied areas of bushy vegetation in northwestern Spain, in forests of holm oaks *Quercus rotundifolia* (locality: B-SP2) (for details see García *et al.*, 2008) or in high-mountain habitats with a dense cover of broom species (*Cytisus* spp.) (locality: B-SP1) (Arizaga *et al.*, 2011).

At each site, bluethroats were captured with either mist nets (normally 16 mm-mesh) or spring traps baited with a mealworm (*Tenebrio* spp.). Once captured, bluethroats were ringed (or the ring was recorded if the bird had been ringed before) and their sex and age were determined. Each individual was used only once for the analyses. Birds were aged as either first-years (birds with some juvenile feathers in the greater coverts and/or tertials) or adults (older birds, with no juvenile flight feathers or coverts) (Jenni and Winkler, 1994). We then measured the lengths of the wing, tail, third primary feather (numbered from outermost to innermost) and tarsus. Plumage measurements were recorded with 0.5 mm accuracy and tarsus length with 0.1 mm precision. We also extracted P10 (i.e., the innermost primary feather) from each wing for the stable isotopes analyses. It was not possible to obtain both biometric data and feather samples for all sampling localities. Therefore, we used data from 14 localities (all except W-MO) for the biometric analyses and from 14 localities (all except B-GE1) for the stable isotopes analyses (for details see table 1).

Stable isotopes

All feathers were cleaned of surface oils using a triple 2:1 chloroform:methanol rinse and prepared for stable-hydrogen isotope

TABLE 1

Sample sizes for the sites where bluethroats were caught to obtain biometric variables and/or feathers for the isotopic (δ^2H) analyses.
[Tamaño muestral para cada uno de los sitios de donde se obtuvieron muestras biométricas y de isótopos estables en plumas (δ^2H).]

Site Code	Coor.	Biometrics[1]		Stable isotopes	
		Sample size	Year	Sample size	Year
B-GE1	53º35'N 08º07'E	9	2010	—	—
B-GE2	50º21'N 10º44'E	14	2011	10	2011
B-GE3	50º07'N 11º18'E	14	2011	10	2011
B-NE1	52º26'N 05º15'E	16	2010, 2011	10	2011
B-NE2	51º56'N 05º57'E	86	2010, 2011	10	2011
B-FR1	44º39'N 01º01'W	20	2010, 2011	10	2011
B-FR2	47º19'N 02º02'W	7	2011	10	2012
B-SP1	40º22'N 05º41'W	267	2010, 2011	10	2011
B-SP2	42º20'N 05º58'W	110	2010, 2011	10	2011
W-SP3	36º46'N 04º07'W	41	2010, 2011	9	2010
W-SP4	39º14'N 00º19'W	62	2010, 2011	10	2010
W-PO1	38º57'N 08º58'W	6	2010	10	2010
W-PO2	37º10'N 08º29'W	20	2010, 2011	10	2010
W-MO	35º12'N 06º09'W				
	35º43'N 05º21'W	—	—	7	2011
W-SE	16º22'N 16º16'W	21	2010	10	2010

1   Biometrics: Number of bluethroats caught with wing length, tail length, tarsus length and P3 measured, and with their age and sex known.

2   Abbreviations for the sampling localities: B, breeding; W, winter; GE, Germany; NE, Netherlands; FR, France; SP, Spain; PO, Portugal; MO, Morocco; SE, Senegal.

3   Breeding localities-associated subspecies: *L. s. namnetum,* B-FR1 and B-FR2; *L. s. cyanecula*, B-GE1 to B-GE3, B-NE1 and B-NE2; L. s. *azuricollis*, B-SP1 and B-SP2.

analysis at Environment Canada, Saskatoon, Canada. The δ^2H analyses were carried out following Wassenaar and Hobson (2003), and using calibrated keratin-isotope reference materials to account for H-exchange. Measurements of δ^2H were performed on H_2 derived from the high-temperature flash pyrolysis (1350°C) of the feathers with a HEKAtech HT-O Analyzer™ linked to a Elementar Isoprime™ continuous-flow isotope-ratio mass spectrometer. All δ^2H values were given in delta notation, in units per mil (‰), and normalised on the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) scale. Repeated analyses of δ^2H inter-comparison material IAEA-CH-7 (-100‰) and laboratory keratin references yielded within-run repeatability (n = 5) of < 2‰ and a long-term (six-month running average) repeatability approximately ± 3.2‰.

Statistical analyses

In seven localities (corresponding to five obser-vers) we were able to standardise all measurements to a single observer (J. Arizaga). Such localities were: B-GE1, B-NE1, B-FR1, B-SP1, W-PO1, W-PO2, W-SE. Using a paired t-test, we only detected between-observer variations for P3 in two observers and wing length in two observers (results omitted). Biometric variables were corrected for this bias by removing the observer effect manually.

We conducted a Principal Component Analysis (PCA) with all the biometric variables. This PCA provided a single Component (PC1) with an eigenvalue >1 (3.029), and explained 75.7% of the variance. The PC1 scores were positively correlated with all the biometric variables and therefore could be used as a body size proxy (PC1 scores: tarsus length, +0.701; wing length, +0.935; tail length, +0.892; P3, +0.932).

We then tested to what extent year might impact on the biometrics of bluethroats at a single site, compared to age and sex. To test this, we considered the data from one breeding locality in Spain (B-SP1), where there was a sufficiently high sample size both in 2010 and 2011 (table 1). We conducted an ANOVA on the PC1 scores with age, sex and year as factors. Year did not have any significant effect on the PC1, whereas age and sex did (table 2), so we considered that including data from more than one year per site, in order to increase sample size, was well justified from a statistical perspective. Therefore, we considered all the data ob-

TABLE 2

ANOVA conducted to test the effect of year (Y), age (A) and sex (S) on bluethroats biometry (PC1). The data used here come from a single breeding locality from Spain (B-SP1).
[ANOVA empleado para comprobar el efecto del año (Y), edad (A) y sexo (S) en la biometría (PC1) de pechiazules. Para llevar a cabo este análisis se utilizaron los datos que se obtuvieron en la localidad (B-SP1).]

Source of variance	SS	df	F	P
Y	0.041	1	0.234	0.629
A	0.842	1	4.786	0.030
S	17.121	1	97.271	< 0.001
Y×A	0.043	1	0.245	0.621
Y×S	0.242	1	1.377	0.242
A×S	0.008	1	0.045	0.832
Y×A×S	0.019	1	0.106	0.744
Error	45.588	259		

tained both in 2010 and 2011, either in winter or during the breeding season (table 1).

We conducted an ANOVA on the PC1 with age, sex and site as factors. From this we found that, overall, first-year birds were smaller than adult birds (*B*-parameters ± SE; first-years: –0.326 ± 0.043). Males were also larger than females (*B*; males: +1.000 ± 0.044). Thereafter, we corrected all the PC1 scores for the effect of age and

TABLE 3

Mean (±SD) values of the biometric variables (PC1 scores) and first primary feather δ^2H values of bluethroats breeding in central-western Europe, captured both at their breeding sites and in wintering localities.
[Media (±SD) biométrica (PC1) y de isótopos estables (δ^2H) de los pechiazules que crían en el oeste y centro de Europa, capturados tanto en áreas de cría como de invernada.]

Locality	Subspecies	Biometrics: PC1 scores			δ^2H (‰)		
		Mean	SD	n	Mean	SD	n
Breeding							
B-FR1	*namnetum*	–2.79	0.51	20	–58.3	10.3	10
B-FR2	*namnetum*	–2.31	0.45	7	–73.0	16.1	10
B-GE1	*cyanecula*	–0.88	0.51	9	—	—	—
B-GE2	*cyanecula*	–0.74	0.43	14	–81.7	13.5	10
B-GE3	*cyanecula*	–1.08	0.63	14	–89.0	11.9	10
B-NE1	*cyanecula*	–0.85	0.49	16	–82.3	17.3	10
B-NE2	*cyanecula*	–1.25	0.48	86	–74.3	18.2	10
B-SP1	*azuricollis*	+0.17	0.44	267	–56.1	14.8	10
B-SP2	*azuricollis*	–0.56	0.49	110	–70.0	10.9	10
Winter							
W-PO1		–2.30	0.38	6	–57.9	11.9	10
W-PO2		–1.53	0.94	20	–64.1	13.3	10
W-MO		—	—	—	–45.0	7.7	7
W-SE		–0.18	0.34	21	–91.7	11.3	10
W-SP3		–0.80	0.57	41	–90.8	14.4	9
W-SP4		–1.09	0.62	62	–79.0	13.0	10

sex (i.e. we removed these effects), calculated the mean for each sampling locality, and ran a Hierarchical Analyses of Clusters based on the Euclidean distance between sampling localities (paired-group algorithm).

To minimise the possible year-associated biases on δ^2H values, the feathers used in this study were from a single generation, grown in 2010. Exceptions to this rule were the localities W-MO, where the birds were caught during the winter of 2011, and B-FR2, where birds were caught during the breeding season in 2012. In these two cases the feathers were grown in 2011.

For feather δ^2H values, we ran an ANOVA to test for variations between localities and, with the mean value from each locality, a Hierarchical Analyses of Clusters based on the Euclidean distance between sampling localities (paired-group algorithm). This analysis was done with the aim of having clusters of localities which could group birds with similar isotopic values, and hence possibly similar geographic origins.

Finally, we conducted a Mantel test in order to detect any correlation between the results obtained from the biometrics and the stable isotopes analyses. A significant correlation would mean that the two methodological approaches used here lead us to the same conclusions.

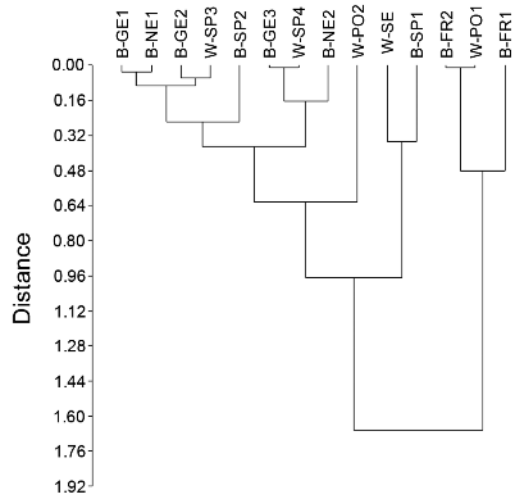The statistical packages used for these analyses were SPSS 18.0 and PAST (Hammer *et al.*, 2001).

FIG. 2.—Diagram obtained from a Hierarchical Analysis of Clusters on the mean PC1 scores of each population, after correction for observer (where possible), age and sex effects.
[Diagrama derivado de un Análisis Jerárquico de Clusters basado en la media de los valores del PC1 para cada zona de muestreo, una vez corregida para efectos de observador (cuando se pudo), edad y sexo.]

RESULTS

Bluethroat body size (PC1 scores) differed between sampling sites ($F_{13} = 104.870$, $P < 0.001$). The smallest birds were found at a breeding locality in France (B-FR1) and the largest at a breeding locality in Spain (B-SP1; table 3).

The Hierarchical Analysis of Clusters on PC1 scores revealed two clusters: (1) one

including the two breeding points in France (B-FR1 and B-FR2) plus one of the winter localities in Portugal (W-PO1), and (2) one containing the other localities (fig. 2). In the latter cluster we noted that one of the breeding localities in Spain (B-SP1) and the winter site in Senegal (W-SE) were separated from the rest, as was one winter site in Portugal (W-PO2) (fig. 2).

The feather δ^2H values differed between sampling localities ($F_{13} = 10.275$, $P < 0.001$), ranging between -91.7 ± 7.0 ($n = 10$) in W-SE (Senegal) and -45.0 ± 5.7 ($n = 7$) in W-MO (Morocco) (table 3). The Hierarchical Analysis of Clusters on mean δ^2H values revealed two clusters, with one including a breeding site in France and another in Spain and the winter localities from Portugal and Morocco (fig. 3).
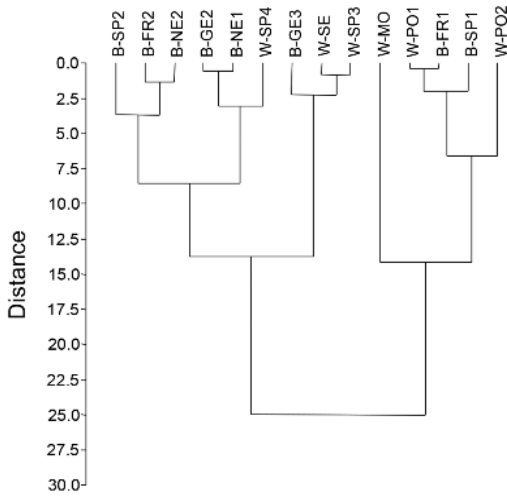
F<small>IG</small>. 3.—Diagram obtained from a Hierarchical Analysis of Clusters on the mean first primary feather δ^2H values of each population.
[Diagrama derivado de un Análisis Jerárquico de Clusters basado en el valor medio de δ^2H para cada zona de muestreo.]

We detected a significant positive moderate correlation between PC1 scores and stable isotopes (Mantel test on distance matrices: r = 0.35, P = 0.008; localities B-GE1 and W-MO1 excluded).

D<small>ISCUSSION</small>

As expected, the largest, longest-winged breeding bluethroats were observed in Spain (*L. s. azuricollis*), the next largest being *L. s. cyanecula* from central-western Europe. The smallest, shortest-winged bluethroats belonged to the *L. s. namnetum* population breeding in France. The lowest δ^2H values were reported in bluethroats breeding in Germany/The Netherlands, and the highest were detected in the southern breeding localities, in both France and Iberia. The values obtained in one of the French and one of the breeding localities in Iberia were slightly

higher than –60‰, so similar to those found in feathers from Savi's warblers *Locustella luscinioides* grown in Portugal (Neto *et al*., 2006). This supports a relatively origin-associated consistency in isotopic signatures of feathers of marsh passerine birds grown in Europe.

With some slight variations, all the Hierarchical Analyses of Clusters revealed two clusters, both morphologically and isotopically, one grouping the French breeding localities and the Portuguese (and for the stable isotopes analyses also the Moroccan) winter quarters, and the other the remaining localities. This supports the existence of relatively high migratory connectivity for the French population (*L. s. namnetum*), but the situation is not so clear for the other two subspecies, which could live in sympatry at their wintering sites, at least in part. We also detected a positive moderate (Mantel test) correlation between the two methodological approaches (biometry, stable isotopes), supporting a relative consistency of the two methodological approaches.

Our results support the idea that *L. s. namnetum* occupies a relatively small wintering range, spread along the coast of Portugal and northern Africa, as suggested in previous studies (Zucca and Jiguet, 2002; Arizaga and Tamayo, 2013). This has direct conservation implications, since these birds depend on mainly Atlantic coastal marshes located across a relatively restricted geographical area. Global climate change and its associated projected sea-level increase (Huntley *et al*., 2007), together with habitat loss (e.g., Green *et al*., 2002), mean that the conservation of this subspecies is particularly challenging. Future research should be aimed at quantifying, in detail, the population size throughout the wetlands situated across this region, in order to ascertain where the chief wintering areas are situated.

The second cluster grouped both the breeding grounds of *L. s. cyanecula* and

L. s. azuricollis, and their wintering areas in Iberia and Africa. The Afrotropical wintering of *L. s. azuricollis* is still unclear given the results derived from the morphological and stable isotopes analyses. Regarding body size, one of the Spanish breeding points (B-SP1) was well differentiated from the rest, clustering in addition with birds wintering in Senegal. This supports the idea that at least some birds (populations) breeding in Spain could spend the winter in tropical Africa, as suggested by previous morphological analyses (Arizaga *et al*., 2006b). However, this result was not confirmed when we analysed δ²H values. Furthermore, the other breeding point in Spain (B-SP2) was not differentiated from the rest of the breeding sampling points in Germany and The Netherlands and the winter localities in southern Spain (W-SP3, W-SP4) and Senegal. Leap-frog migration is supported in part, at least for the birds of the *L. s. namnetum* and *L. s. cyanecula* populations. Further research is needed to discover the precise wintering areas of Iberian bluethroats.

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