WORLDWIDE
DRAGONFLY
ASSOCIATION

Patterns of variation in wing venation of Iberian *Cordulegaster boltonii* (Donovan, 1807) (Odonata: Cordulegastridae)

Luisa Ferreira Nunes[a,b], Tomás Santamaría[c], Patricia Casanueva[d],
Luis Fernando Sánchez-Sastre[e], Manuel Ferreras-Romero[f], Ángel Romero[g],
Francisco Campos[d] & M. Ángeles Hernández[h*]

[a] Instituto Politécnico de Castelo Branco – Escola Superior Agrária. Qta Sra Mercules, 6000-900 Castelo Branco, Portugal

[b] Centre for Applied Ecology Prof. Baeta Neves (CEABN-InBIO), School of Agriculture, University of Lisbon, Tapada da Ajuda, Lisbon, 1349-017, Portugal

[c] Universidad Católica de Ávila, Calle Canteros s/n, 05005 Ávila, Spain

[d] Departamento de Ciencias Experimentales, Universidad Europea Miguel de Cervantes, 47012 Valladolid, Spain

[e] Departamento de Ingeniería Agrícola y Forestal, Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid, 34004 Palencia, Spain

[f] c/ Gerona 9, 41003 Sevilla, Spain

[g] Consejería de Sostenibilidad, Medio Ambiente y Economía Azul – Junta de Andalucía. Parque Nacional y Parque Natural de Sierra Nevada. 18191 Pinos Genil, Granada, Spain

[h] Departamento de Biología Ambiental, Facultad de Ciencias, Universidad de Navarra, 31080 Pamplona, Spain

*Corresponding author: Email: mahermin@unav.es

Research Article

Abstract. Some structural characters, such as wing venation, have been used in odonates to differentiate taxa. In *Cordulegaster boltonii*, a species widely distributed throughout the western Palaearctic, the main characteristics of its wing venation have not been quantified until now. A six-variable analysis of wing venation (number of antenodal and postnodal cross-veins, number of cells in the anal triangle and in the anal loop) in specimens from several European countries was carried out. The results showed that: (a) females had a greater number of transversal veins and cells in the anal loop than males; (b) the values of these four variables were significantly lower in males from the Iberian Peninsula than in those from elsewhere; (c) within the Iberian Peninsula two groups of populations can be distinguished: one covers the north and the other the rest of the peninsula, the latter with two subgroups, one in the centre and one in the south and east. The number of cells in the anal loop is a valid variable for analyzing geographic differences in this species.

Key words. Anisoptera, dragonflies, Iberian Peninsula, odonates, wings

Introduction

Patterns of body coloration (Mayr & Ashlock, 1991) have been used in some genera of Odonata, e.g., *Cordulegaster* Leach, 1815, for differentiating species (Abbott & Hibbitts, 2011; Boudot, 2001; Corso, 2019). Characters based on coloration can be controlled by various genes, some of which may be expressed or not depending on environmental conditions (Sánchez-Guillén et al., 2005), so that it would appear preferable to use structural characteristics instead.

Among the structural characteristics, the wings of odonates present particularly elaborate vein patterns (Hoffman et al., 2018), which have been used for phylogenetic and systematic analyses (e.g., Carle & Kjer, 2002; Ninomiya & Yoshizawa,

2009; Trueman, 1996; Rehn, 2003). In recent decades such analyses have been largely—but not entirely—superseded by genetic analyses (e.g., Bybee et al., 2008; Dijkstra & Kalkman, 2012; Kohli et al., 2021; Suhling et al., 2015; Suvorov et al., 2022). The functioning of the odonates' wings has been extensively studied (e.g., Blanke, 2018; Norberg, 1972; Wootton 1991, 1992). Some wing structures, such as anal loop, anal triangle, and node position, have been used to distinguish taxa (e.g., Abbott, 2006; Carle & Kjer, 2002; Tillyard, 1917). Other structural characteristics, such as male reproductive appendages (hamuli) and anal appendages, are also used because they can be species-specific (Corbet, 2004). In some *Cordulegaster* species, differentiation based on these structures agreed with that based on genetic characteristics (Pilgrim et al., 2002). However, in other species, a clear discordance was found between phylogeny based on morphological characteristics and one suggested by genetic characters (Huang et al., 2020; Sánchez-Herrera et al., 2020).

The genus *Cordulegaster* includes 30 species distributed throughout the northern hemisphere (Schorr & Paulson, 2020). Two main groups of species have been established: *boltonii* and *bidentata*, distinguishable mainly by characteristics of their abdominal appendages (Askew, 2004; Dijkstra & Lewington, 2006).

Cordulegaster boltonii is distributed throughout the Western Palaearctic (Boudot & Holuša, 2015). In the Iberian Peninsula, it occupies all mountain ranges, and up to four subspecies differentiated by morphological characteristics have been described from southwestern Europe: *C. b. boltonii*, *C. b. inmaculifrons*, *C. b. iberica*, and *C. b. algirica* (Boudot, 2001; Boudot & Jacquemin, 1995). However, more recent work has demonstrated that the validity of these subspecies does not have genetic support (Froufe et al., 2014), but the genes analyzed by Froufe et al. are likely not linked to those that produce the different chromatypes. Until this situation is clarified, it seems preferable to speak of *C. boltonii* morphs rather than of subspecies.

The morphology of *C. boltonii* has been extensively studied. Ocharan (1989) demonstrated that there is individual variability in adult coloration. A biometric analysis carried out using exuviae revealed that the Iberian populations are distributed in four distinguishable areas: northern, central, east and middle-south, and southernmost (Casanueva et al., 2020). On the other hand, Hernández et al. (2022) showed that latitude, altitude, and environmental temperature are significantly correlated with the sizes of some exuviant body parts. Based on these last two studies, it might therefore be possible that some adult structural characteristics can also be useful for differentiating Iberian populations. In the Iberian Peninsula there is a great diversity of environments and climatic conditions, as the northern parts belong to the Euro-Siberian region, and a much larger area, occupying the centre and south, is ascribed to the Mediterranean region (Rivas-Martínez, 1987). Both feature mountain ranges with populations of *C. bolto-*

nii that must have adapted to different environmental conditions.

The aim of this paper is to show, using structural characteristics of the wing venation, the level of differentiation existing between *C. boltonii* specimens from six Iberian areas, and possible differences with respect to specimens from populations residing in other European countries.

Material and methods

Our analyses were carried out on adult specimens of *C. boltonii* from 15 entomological collections, both private and in museums, representing ten European countries in which *C. boltonii* occurs (Supplementary Material 1). Of each specimen, the fore- and hindwings were photographed, while track was kept to which collection it belonged, whenever possible.

In the Iberian Peninsula, six distribution zones were selected according to: (a) the geographical distribution of the species, which mainly occupies mountain ranges (Boudot & Holuša, 2015), although it may also occupy non-mountainous areas (Ocharan & Torralba-Burrial, 2004); (b) the groups recovered by means of the biogeographical analysis of *C. boltonii* carried out by Casanueva et al. (2020); and (c) the availability of specimens in the entomological collections reviewed. These six zones have been specified as "Pyrenees", "Cantabrian Mountains", "Central System", "Central Portugal", "Iberian System", and "Sierra Nevada", respectively (Fig. 1). The first two belong to the Euro-Siberian region, and the other four to the Mediterranean region. Specimens from several localities in each zone (from 11 to 64 localities, see Supplementary Material 2) were analyzed.

We follow the wing terminology of Riek & Kukalová-Peck (1984) as updated by Rehn (2003). Six characteristics were analyzed for each specimen: in the hindwing, the number of cells in the anal triangle (AT) (males only) and the anal loop (AL), and in fore- and hindwings, number of antenodal (Ax) and postnodal (Px) cross-veins (Fig. 2). Furthermore, the nodal index (NI) was calculated as NI = Ax/Px (Fraser, 1934). This index was used in *Cordulegaster* by Fraser (1936) to describe species.

In the analysis of geographic differences, only male specimens were used, because females do not have an anal triangle, and the venation of their wings differs from that of males (see below). The use of male specimens only has proved informative in odonates for specific differentiation using the *hamuli*, which are also absent in females (Leong & Hafernick, 1992).

To identify the level of population homogeneity existing within each of the six Iberian areas was compared, calculating the diversity of the number of cells in the anal loop in specimens from each zone with the Shannon-Wiener index ($H' = -\sum p_i \cdot \ln p_i$), where p_i is the proportion of the item 'i' in the total number of specimens.

Using Past 3.15 software (Hammer et al., 2001), a clustered multivariate analysis was performed that

seeks to group the variables in a quest to achieve the maximum homogeneity in each group and show up the greatest difference between the groups. In this analysis, the Unweighted Pair-Group Average (UPGMA) was used as the algorithm, and the Euclidean and constrained distances as the similarity index. Clusters were joined based on the average distance between all members in the two groups. With a bootstrapping of N = 10,000, the percentage of support is given in each of the nodes.

An ANOVA (ANalysis Of VAriance) was performed with the STATA 12.1 software between the studied zones for each of the variables, after verifying that the normality and homogeneity of the variance were met. If this was not met, the non-parametric Kruskal-Wallis analysis was performed. Pairwise comparisons were also made with the STATA 12.1 software for the variables in which significant differences were found between specimens from different areas of the Iberian Peninsula.

Results

A total of 679 adult specimens of *C. boltonii* (588 males, 91 females) were analyzed. Of these, 127 (105 males, 22 females) were from outside the Iberian Peninsula and 552 (483 males, 69 females) from the Iberian Peninsula.

Differences between the sexes

The average numbers of both antenodal and postnodal cross-veins of fore- and hindwings, and of cells in the anal loop of the hindwing were significantly lower in males than in females (Table 1). Therefore, these variables must be analyzed separately for males and females. In contrast, the nodal index (NI) values for both fore- and hindwings were statistically similar in both sexes. Since the females lack an anal triangle, only male specimens were analyzed.

Differences between Iberian vs. non-Iberian male specimens

Average values of all measured variables differed significantly between Iberian and non-Iberian males (Table 2). The numbers of antenodal and postnodal cross-veins in fore- and hindwings were consistently higher in specimens from outside Iberia (Fig. 3). Likewise, the numbers of cells inside the anal triangle and anal loop were higher in specimens from outside Iberia (Fig. 4). However, specimens from within the Iberian Peninsula had higher nodal index (NI) values than those from outside Iberia.

The number of cells of the anal loop (AL) varied between three and ten (Table 1). Only seven specimens from outside Iberia had more than seven cells in the anal loop (Table 3). In the six Iberian zones, anal loops with five cells were the most common configuration, but the frequencies of each class differed significantly between zones (χ^2 = 141.82, p < 0.0001, 30 d.f.). The highest value of cell class diversity (from 3 to > 7), calculated according to the Shannon index, was obtained in the heterogeneous group of specimens from outside Iberia, and the lowest in Central Portugal (Table 3), but the number of samples studied from this last zone was small (n = 16).
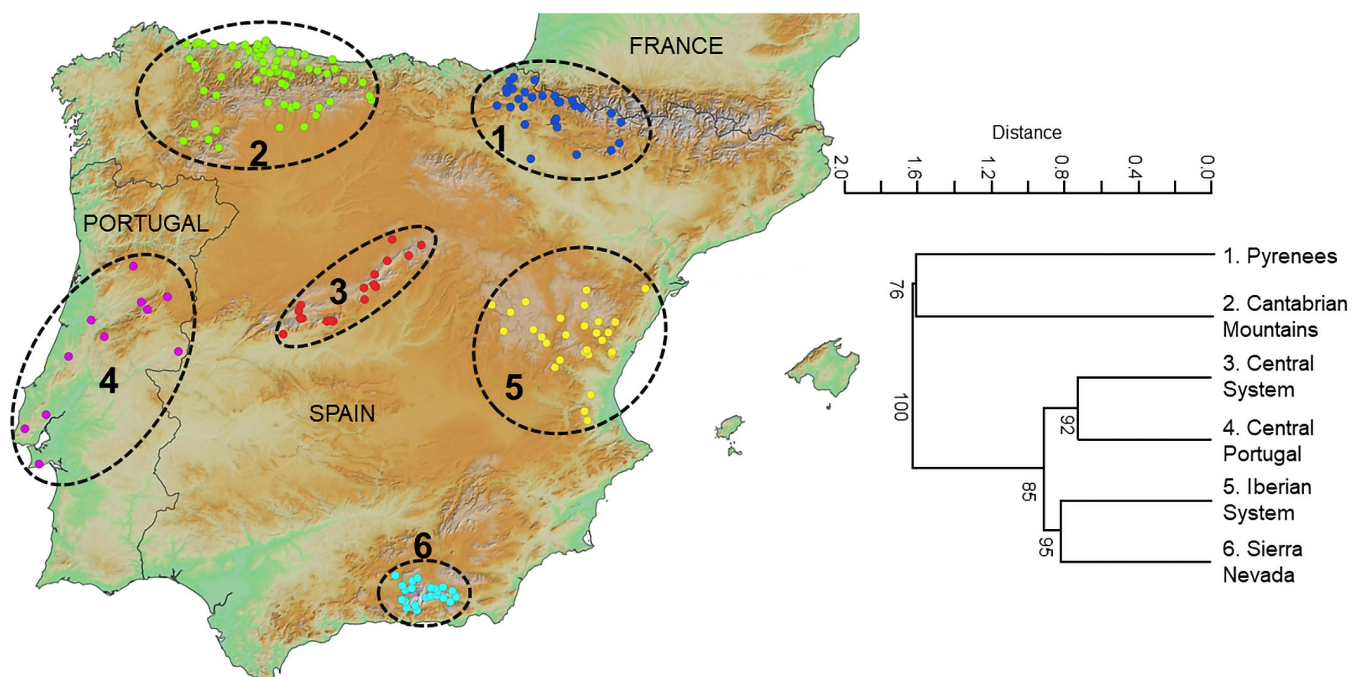

Figure 1. Sampling zones. (1) Pyrenees (dark blue dots); (2) Cantabrian Mountains (green dots); (3) Central System (red dots); (4) Central Portugal (pink dots); (5) Iberian System (yellow dots); (6) Sierra Nevada (pale blue dots). Dendrogram with the sampling zones in the Iberian Peninsula. Numbers at nodes indicate bootstrap support for that particular branch.

Table 1. Mean values (± SD) of analyzed variables in male and female *Cordulegaster boltonii*. n: sample size; Ax: antenodal cross-veins; Px: postnodal cross-veins; NI: nodal index as Ax/Px; AT: number of cells in the anal triangle; AL: number of cells in the anal loop. Range of values in brackets. F: ANOVA; KW: Kruskal-Wallis test; *p*: probability.

	n	Forewing			Hindwing				
		Ax	Px	NI	Ax	Px	NI	AT	AL
Males	588	18.25±1.30 (14–22)	13.45±1.67 (8–19)	1.37±0.16 (0.94–1.88)	12.85±1.11 (9–16)	13.08±1.60 (8–17)	0.99±0.13 (0.69–1.50)	4.37±0.86 (2–7)	5.06±0.93 (3–10)
Females	91	19.88±1.52 (17–23)	15.06±1.77 (11–19)	1.34±0.16 (0.95–1.92)	13.92±1.11 (12–17)	14.30±1.64 (11–19)	0.98±0.11 (0.63–1.33)		7.76±1.40 (5–12)
Test		KW = 75.522	$F_{1,633}$ = 65.58	KW = 3.34	$F_{1,677}$ = 73.84	$F_{1,677}$ = 45.20	KW = 0.076		KW = 188.907
p		0.0001	<0.0001	0.068	<0.0001	<0.0001	0.7827		0.0001

Table 2. Mean values (± SD) of analyzed variables in male *Cordulegaster boltonii* from Iberia vs. out of Iberia. n: sample size; Ax: antenodal cross-veins; Px: postnodal cross-veins; NI: nodal index as Ax/Px; AT: number of cells in the anal triangle; AL: number of cells in the anal loop. Range of values in brackets. F: ANOVA; KW: Kruskal-Wallis test; *p*: probability.

	n	Forewing			Hindwing				
		Ax	Px	NI	Ax	Px	NI	AT	AL
Iberia	483	18.06±1.27 (14–22)	13.19±1.59 (8–19)	1.38±0.16 (0.94–1.88)	12.71±1.07 (9–15)	12.85±1.53 (8–17)	1.00±0.13 (0.69–1.50)	4.28±0.83 (2–7)	4.90±0.78 (3–7)
Out of Iberia	105	19.14±1.03 (16–22)	14.69±1.49 (11–18)	1.30±0.19 (1.06–1.67)	13.48±1.09 (11–16)	14.12±1.47 (10–17)	0.96±0.11 (0.71–1.18)	4.85±0.86 (3–7)	5.80±1.16 (4–10)
Test		KW = 62.328	$F_{1,574}$ = 75.95	KW = 18.15	$F_{1,586}$ = 43.98	$F_{1,586}$ = 59.78	KW = 5.654	KW = 27.397	KW = 54.786
p		0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0174	0.0001	0.0001

Differences between males of the six Iberian zones compared

Inside the Iberian Peninsula, the number of postnodal (Px) cross-veins and the nodal index (NI) differed between zones both in the forewing and the hindwing (Table 4). The number of antenodal (Ax) cross-veins in the forewing and the number of cells in the anal loop (AL) varied between zones, too (Table 4). The highest average values of Ax, Px, and AL were recorded in the Cantabrian Mountains. On the other hand, the average number of cells in the anal triangle (AT) was similar throughout the six zones analyzed.

To detect similarities between zones of the Iberian Peninsula, a dendrogram based on the significant variables of forewing (Ax, Px, NI) and hindwing (Px, NI, AL) (Table 4) was created. The six zones are distributed in two groups, one formed by the Pyrenees and Cantabrian Mountains, and the other by remaining four zones (Fig. 1). Pairwise comparisons revealed that males from the Pyrenees and Cantabrian Mountains differ from each other in Px of both forewing (t = 4.49, *p* < 0.001) and hindwing (*t* = 5.46, *p* < 0.001), but not in the other variables (*p* > 0.05). In the second group, Central System and Central Portugal showed high similarity to each other, and the same was the case for the Iberian System and Sierra Nevada.
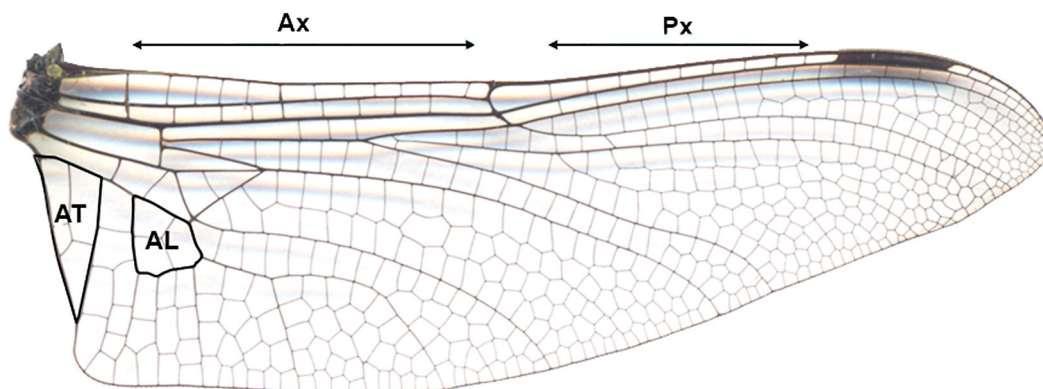

Figure 2. The hindwing of a *Cordulegaster boltonii* adult male. AT, anal triangle; AL, anal loop; Ax, antenodal cross-veins; Px, postnodal cross-veins.

Table 3. Number of *Cordulegaster boltonii* males (in brackets, percentage) as per number of cells in the anal loop. H': diversity (Shannon's index).

Zone	Number of cells in the anal loop						Total	H'
	3	4	5	6	7	> 7		
Out of Iberia	2 (2.1)	5 (5.3)	35 (37.2)	36 (38.3)	9 (9.6)	7 (7.4)	94	1.39
Pyrenees	4 (5.9)	8 (11.8)	44 (64.7)	12 (17.6)	0	0	68	1.01
Cantabrian Mountains	1 (0.8)	13 (10.2)	77 (60.6)	28 (22.0)	8 (6.3)	0	127	1.08
Central System	4 (3.6)	34 (30.6)	62 (55.9)	9 (8.1)	2 (6.3)	0	111	1.08
Central Portugal	0	2 (12.5)	13 (81.3)	1 (6.3)	0	0	16	0.60
Iberian System	4 (4.8)	21 (25.0)	46 (54.8)	11 (13.1)	2 (2.4)	0	84	1.18
Sierra Nevada	6 (8.3)	25 (34.7)	34 (47.2)	6 (8.3)	1 (1.4)	0	72	1.20

Table 4. Mean values (± SD) of analyzed variables in male *Cordulegaster boltonii* from six Iberian sampling zones: (1) Pyrenees; (2) Cantabrian Mountains; (3) Central System; (4) Central Portugal; (5) Iberian System; (6) Sierra Nevada. n: simple size; Ax: antenodal cross-veins; Px: postnodal cross-veins; NI: nodal index as Ax/Px; AT: number of cells in the anal triangle; AL: number of cells in the anal loop. Range of values in brackets. F: ANOVA; KW: Kruskal-Wallis test; *p*: probability.

Zone	n	Forewing			Hindwing				
		Ax	Px	NI	Ax	Px	NI	AT	AL
1	70	18.06±1.19 (16–21)	13.06±1.42 (10–16)	1.40±0.16 (1.06–1.73)	12.66±1.21 (10–15)	12.54±1.34 (8–16)	1.02±0.15 (0.77–1.50)	4.26±0.77 (3–6)	4.94±0.72 (3–6)
2	127	18.44±1.38 (14–22)	14.06±1.75 (9–19)	1.33±0.15 (0.94–1.80)	12.87±1.02 (10–15)	13.71±1.59 (10–17)	0.95±0.12 (0.69–1.27)	4.39±0.86 (2–7)	5.23±0.75 (3–7)
3	115	17.96±1.22 (16–21)	13.06±1.32 (10–16)	1.39±0.14 (1.07–1.80)	12.75±1.18 (9–15)	12.69±1.43 (9–15)	1.02±0.14 (0.73–1.50)	4.23±0.80 (3–6)	4.75±0.72 (3–7)
4	16	17.75±1.53 (15–20)	13.50±1.75 (11–17)	1.33±0.14 (1.12–1.54)	12.56±0.96 (11–14)	13.19±1.42 (11–16)	0.96±0.14 (0.75–1.18)	4.31±0.70 (3–5)	4.94±0.44 (4–6)
5	83	17.84±1.11 (15–20)	12.32±1.24 (8–15)	1.46±0.14 (1.20–1.88)	12.45±0.97 (10–15)	12.11±1.39 (9–16)	1.04±0.13 (0.69–1.30)	4.40±0.85 (3–7)	4.84±0.82 (3–7)
6	72	17.86±1.23 (15–21)	12.94±1.47 (10–17)	1.40±0.16 (1.06–1.80)	12.74±0.92 (11–15)	12.72±1.28 (10–16)	1.01±0.11 (0.75–1.27)	4.07±0.83 (3–6)	4.60±0.82 (3–7)
Test *p*		$F_{5,473}$ = 3.56 0.0036	KW = 59.418 0.0001	KW = 40.129 0.0001	$F_{5,477}$ = 1.75 0.1221	KW = 59.401 **0.0001**	KW = 28.373 **0.0001**	$F_{5,477}$ = 1.81 0.1088	KW = 31.53 0.0001

Discussion

According to Wootton (2020), *C. boltonii* is a hawking species, i.e., it needs high flight speed to capture its prey. This speed can be obtained with greater wings length, since in odonata, long wings favour high-speed manoeuverability. On the other hand, in anisopteran species, some wing areas influence wing shape and therefore flight mode (Blanke, 2018; Rajabi et al., 2016). The basal part of the hindwing helps to reduce the cost of flapping (Wootton, 2020), which means that species that make persistent and unbroken flights have a larger anal region than those that move less (Huang et al., 2020; Suárez-Tovar & Sarmiento, 2016). In the hindwing, the anal loop provides support to the anal region. Adult males of *C. boltonii* carry out numerous patrol flights daily over the streams they inhabit (Corbet, 2004), having a wide basal zone in their hindwings that lowers energy costs.

During the Quaternary glaciations, numerous species from central and northern Europe took refuge in areas of southern Europe (Hewitt, 2000; Schmitt, 2007), and the Iberian Peninsula was one of the most important refuges for both vertebrates and invertebrates (Gómez & Lunt, 2007). Although little geographical differentiation is presently observed, a moderate genetic diversity within *C. boltonii* suggests that large populations survived in diverse refugia during the last Ice Ages (Froufe et al., 2014).

Likewise, it has been suggested that in the past, the Iberian and North African populations of some species of Odonata and other insects were represented by common ancestors (Cabrero-Sañudo & Lobo, 2006; Ojeda et al., 2021; Simonsen et al., 2020; Todisco et al., 2010).

Males and females of *C. boltonii* differ in external adult morphology (Dijkstra & Lewington, 2006) and larval biometry (Casanueva et al., 2020; Ferreras-Romero & Corbet, 1999). Our data show that they also differ in wing venation. Furthermore, the wing venation of Iberian populations of *C. boltonii* differs from that found in populations outside the Iberian Peninsula.

The climate in the Iberian east and south is similar to that of North Africa. This fact may explain why their odonatofauna host a greater abundance of Ethiopian elements than that of northern Iberia on the one, and a lower proportion of Euro-Siberian elements on the other hand (Fontana-Bria et al., 2017). Our results demonstrate that the *C. boltonii* specimens from the Cantabrian Mountains and Pyrenees, the two northern zones (Euro-Siberians) studied in this work, appear grouped in our analysis and are separated from those from the four Mediterranean zones.

Recently, Santamaría et al. (2022) found that the numbers of cells in the anal triangle and the anal loop in Iberian *C. boltonii* were positively correlated with wing length, and the number of postnodal cross-veins was positively correlated with postnodal length. In the Iberian areas compared in this work, the highest number of postnodal cross-veins was recorded in the Cantabrian Mountains, and the highest number of cells in

the anal loop was recorded in the north (Pyrenees and Cantabrian Mountains) and west (Central Portugal). Conversely, the average number of cells in the anal triangle was similar throughout the six zones analyzed.

In the present study, the highest average values of Ax in the forewing, Px in both wings, and the number of cells in the AL of the hindwing were found in the Cantabrian Mountains (Zone 2). According to Boudot (2001), all populations existing in that zone belong to the nominate subspecies *C. b. boltonii*. On the other hand, the result of the clustered multivariante analysis does not allow to identify clear relationships between the remaining three Iberian subspecies proposed by Boudot (2001) and the other five population groups here studied.

Genetic analyses of several species of the genus *Cordulegaster* present in the western Palaearctic (Froufe
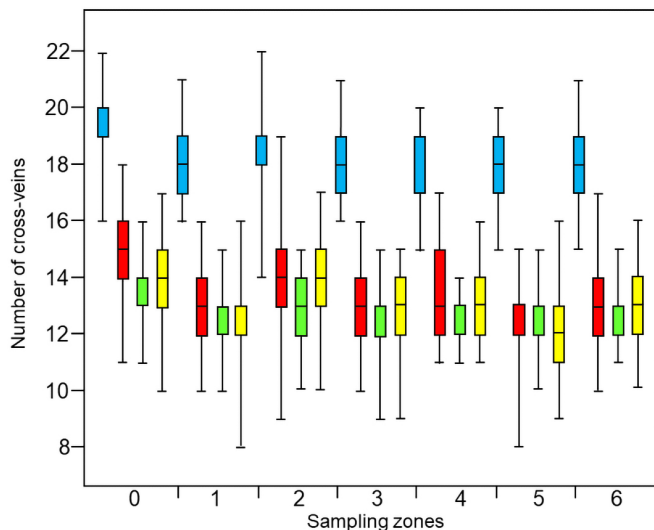
Figure 3. Boxplot of the number of cross-veins in the forewing and hindwing for the seven *Cordulegaster boltonii* zones analysed. Sampling zones: (0) Out of Iberian Peninsula; (1) Pyrenees; (2) Cantabrian Mountains; (3) Central System, 4) Central Portugal, 5) Iberian System, 6) Sierra Nevada. Blue: antenodal cross-veins in forewing; red: postnodal cross-veins in forewing; green: postnodal cross-veins in hindwing; yellow: postnodal cross-veins in hindwing. Bars: upper and lower values. Boxes: upper (75%) and lower (25%) percentiles. Line in box: median.
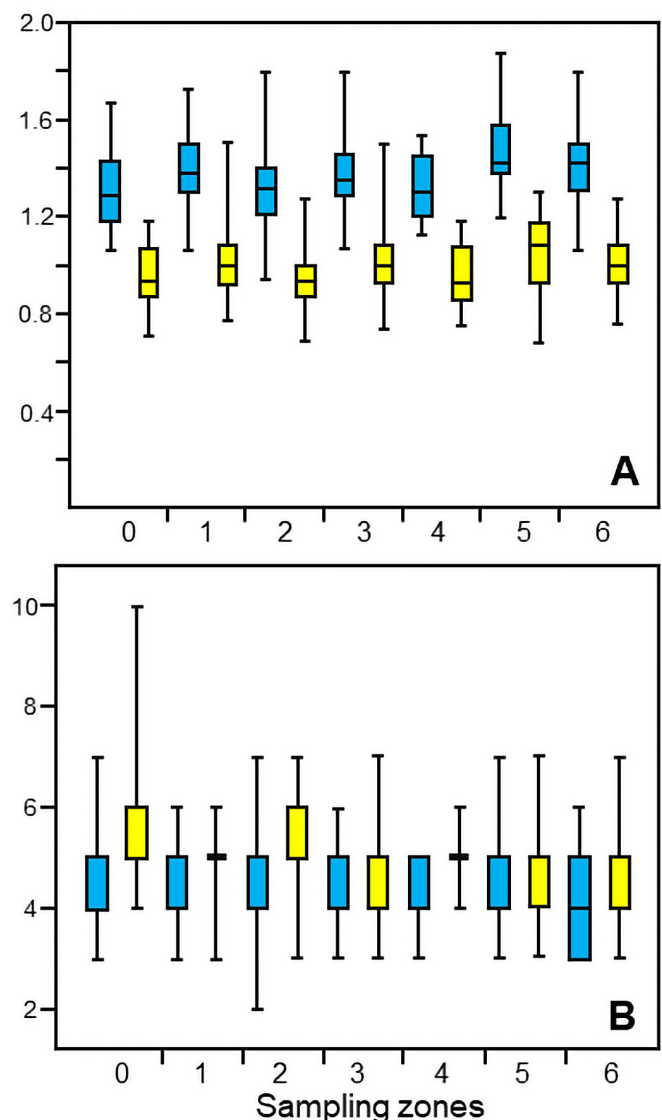
Figure 4. Boxplot of: (A) nodal index values in forewing (blue) and hindwing (yellow); (B) number of cells into the anal triangle (blue) and anal loop (yellow), for the seven sampling zones. (0) Out of Iberian Peninsula; (1) Pyrenees; (2) Cantabrian Mountains; (3) Central System; (4) Central Portugal; (5) Iberian System; (6) Sierra Nevada. Bars: upper and lower values. Boxes: upper (75%) and lower (25%) percentiles. Line in box: median.

et al., 2014) support the validity only of the subspecies *C. boltonii algirica* for populations from northern Africa. The results obtained in this work agree only partially with the above, since certain differentiations in wing venation seem to have occurred between the northern areas (Pyrenees and Cantabrian Mountains) and the rest of Iberia.

Finally, this work once again underscores the importance of museum collections, which continue to be useful for the study of intraspecific diversity (Short et al., 2018), either from a genetic (Mandrioli, 2008) or from other points of view (Suarez & Tsutsui, 2004).

References

Abbott, J. C. (ed.) (2006). *Dragonflies and damselflies (Odonata) of Texas*, Volume 1. Austin: Odonata Survey of Texas.

Abbott, J. C. & Hibbitts T. D. (2011). *Cordulegaster sarracenia*, n. sp. (Odonata: Cordulegastridae) from east Texas and western Louisiana, with a key to adult Cordulegastridae of the New World. *Zootaxa*, 2899, 60–68. doi:10.11646/zootaxa.2899.1.3

Askew, R. R. (2004). *The dragonflies of Europe* (revised edition). Colchester: Harley Books. doi:10.1163/9789004474383

Blanke, A. (2018). Analysis of modularity and integration suggests evolution of dragonfly wing venation mainly in response to functional demands. *Journal of the Royal Society Interface*, 15, 20180277. doi:10.1098/rsif.2018.0277

Boudot, J.-P. & Jacquemin, G. (1995). Revision of *Cordulegaster boltonii* (Donovan, 1807) in southwestern Europe and northern Africa with description of *C. b. iberica* ssp. nov. from Spain (Anisoptera: Cordulegastridae). *Odonatologica*, 24, 149–173.

Boudot, J.-P. (2001). Les *Cordulegaster* du Paléarctique occidental: identification et répartition (Odonata, Anisoptera, Cordulegastridae). *Martinia*, 17, 3–34.

Boudot, J.-P. & Holuša, O. (2015). *Cordulegaster boltonii* (Donovan, 1807). In J.-P. Boudot & V. J. Kalkman (Eds.), *Atlas of the European dragonflies and damselflies*. pp. 213–215. The Netherlands: KNNV publishing.

Bybee, S. M., Ogden, T. H., Branham, M. A. & Whiting, M. F. (2008). Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. *Cladistics*, 23, 1–38. doi:10.1111/j.1096-0031.2007.00191.x

Cabrero-Sañudo, F. J. & Lobo, J. M. (2006). Determinant variables of Iberian Peninsula Aphodiinae diversity (Coleoptera, Scarabaeoidea, Aphodiidae). *Journal of Biogeography*, 33, 1021–1043. doi: 10.1111/j.1365-2699.2006.01485.x

Carle, F. L. & Kjer, K. M. (2002). Phylogeny of *Libellula* Linnaeus (Odonata: Insecta). *Zootaxa*, 87, 1–18. doi: doi:10.11646/zootaxa.87.1.1

Casanueva, P., Santamaría, T., Hernández, M. A., Sánchez-Sastre, L. F., Teixeira, A., Bennas, N., El Haissoufi, M., Ferreras-Romero, M. & Campos, F. (2020). Biometric differences in Iberian and Maghrebi populations of *Cordulegaster boltonii* (Odonata: Cordulegastridae). *European Journal of Entomology*, 117, 260–264. doi: 10.14411/eje.2020.028

Corbet, P. S. (2004) (revised edition). *Dragonflies: behaviour and ecology of Odonata*. Colchester: Harley Books.

Corso, A. (2019). Morphological variability of *Cordulegaster trinacriae* in Italy (Odonata: Cordulegastridae). *Odonatologica*, 48, 175–201. doi: 10.5281/zenodo.3539730

Dijkstra, K-D. B. & Lewington, R. (2006). *Field Guide of the Dragonflies of Britain and Europe*. Gillingham: British Wildlife Publishing.

Dijkstra, K.-D. B. & Kalkman, V. J. (2012). Phylogeny, classification and taxonomy of European dragonflies and damselflies (Odonata): a review. *Organisms Diversity & Evolution*, 12, 209–227. doi: 10.1007/s13127-012-0080-8

Ferreras-Romero, M. & Corbet, P. S. (1999). The life cycle of *Cordulegaster boltonii* (Donovan, 1807) (Odonata: Cordulegastridae) in the Sierra Morena Mountains (southern Spain). *Hydrobiologia*, 405, 39–48. doi:10.1023/A:1003763819991

Fontana-Bria, L., Frago, E., Prieto-Lillo, E. & Selfa, J. (2017). Biogeographic evaluation of the dragonflies and damselflies in the Eastern Iberian Peninsula. *Arxius de Miscel·lània Zoològica*, 15, 8–29. doi:10.32800/amz.2017.15.0008

Fraser, F. C. (1934). *The fauna of British India. Odonata*. vol. II. London: Taylor and Francis.

Fraser, F. C. (1936). *The fauna of British India. Odonata*. Vol. III. London: Taylor and Francis.

Froufe, E., Ferreira, S., Boudot, J.-P., Alves, P. C. & Harris, D. J. (2014). Molecular phylogeny of the Western Palaearctic *Cordulegaster* taxa (Odonata: Anisoptera: Cordulegastridae). *Biological Journal of the Linnean Society*, 111, 49–57. doi:10.1111/bij.12190

Gómez, A. & Lunt, D. H. (2007). Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In: S. Weiss & N. Ferrand (Eds.), *Phylogeography of Southern European refugia*. pp. 155–188. Dordrecht, The Netherlands: Springer. doi:10.1007/1-4020-4904-8_5

Hammer, O., Harper, D. A. T. & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica*, 4, 9 pp.

Hernández, M. A., Casanueva, P., Nunes, L., Santamaría, T., Sánchez-Sastre, L. F., Ferreras-Romero, M. & Campos, F. (2022). Geographical variation of prementum size in Iberian *Cordulegaster boltonii* (Odonata: Cordulegastridae) populations. *International Journal of Odonatology*, 25, 56–61. doi:10.48156/1388.2022.1917169

Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature*, 405, 907–913. doi:10.1038/35016000

Hoffmann, J., Donoughe, S., Li, K., Salcedo, M. K. & Rycroft, C. H. (2018). A simple developmental model recapitulates complex insect wing venation patterns. *Proceedings of the National Academy of Sciences USA*, 115, 9905–9910. doi:10.1073/pnas.1721248115

Huang, S.-T., Wang, H.-R., Yang, W.-Q., Si, Y.-C., Wang, Y.-T., Sun, M.-L., Qi, X. & Bai, Y. (2020). Phylogeny of Libellulidae (Odonata: Anisoptera): comparison of molecular and morphology-based phylogenies based on wing morphology and migration. *PeerJ8*, e8567. doi:10.7717/peerj.8567

Kohli, M., Letsch, H., Greve, C., Béthoux, O., Deregnaucourt, I., Liu, S., Xin Zhou, X., Donath, A., Mayer, C., Podsiadlowski, L., Gunkel, S., Machida, R., Niehuis, O., Rust, J., Wappler, T., Yu, X., Misof, B. & Ware, J. (2021). Evolutionary history and divergence times of Odonata (dragonflies and damselflies) revealed through transcriptomics. *iScience*, 24 (11), 103324. doi:10.1016/j.isci.2021.103324

Leong, J. M. & Hafernik, J. E. Jr. (1992). Hybridization between two damselfly species (Odonata: Coenagrionidae): morphometric and genitalic differentiation. *Annals of the Entomological Society of America*, 85, 662–670. doi:10.1093/aesa/85.6.662

Mandrioli, M. (2008). Insect collections and DNA analyses: how to manage collections? *Museum Management and Curatorship* 23: 193–199. doi:10.1080/09647770802012375

Mayr, E. & Ashlock, P. D. (1991). *Principles of Systematic Zoology*. Second Edition New York: McGraw-Hill. INC.

Ninomiya, T. & Yoshizawa, K. (2009). A revised interpretation of the wing base structure in Odonata. *Systematic Entomology*, 34, 334–345. doi: 10.1111/j.1365-3113.2008.00455.x

Norberg, R. A. (1972). Pterostigma of insect wings: An inertial regulator of wing pitch. *Journal of Comparative Physiology A*, 81, 9–22. doi:10.1007/BF00693547

Ocharan, F. J. (1989). Variabilidad en poblaciones ibéricas de *Cordulegaster boltoni* (Donovan, 1807) (Odonata: Cordulegastridae). *Revista de Biología de la Universidad de Oviedo*, 7, 109–121.

Ocharan, F. J. & Torralba-Burrial, A. (2004). La relación entre los odonatos y la altitud: el caso de Asturias (norte de España) y la península ibérica (Odonata). *Boletín S.E.A.*, 35, 103–116.

Ojeda, D. I., John, M., Hammond, R. L., Savolainen, R., Vepsäläinen, K. & Kvamme, T. (2021). Phylogeny of the *Formicoxenus* genus-group (Hymenoptera: Formicidae) reveals isolated lineages of *Leptothorax acervorum* in the Iberian Peninsula predating the Last Glacial Maximum. *bioRxiv*. doi:10.1101/2021.11.05.467305

Pilgrim, E. M., Roush, S. A. & Krane, D. E. (2002). Combining DNA sequences and morphology in systematics: testing the validity of the dragonfly species *Cordulegaster bilineata*. *Heredity*, 89, 184–190. doi:10.1038/sj.hdy.6800112

Rajabi, H., Ghoroubi, N., Malaki, M., Darvizeh, A. & Gorb, S. N. (2016). Basal complex and basal venation of Odonata wings: structural diversity and potential role in the wing deformation. *PLoS ONE*, 11(8), e0160610. doi:10.1371/journal.pone.0160610

Rehn, A. C. (2003). Phylogenetic analysis of higher-level relationships of Odonata. *Systematic Entomology*, 28, 181–239. doi:10.1046/j.1365-3113.2003.00210.x

Riek, E. F. & Kukalová-Peck, J. (1984). A new interpretation of dragonfly wing venation based upon Early Upper Carboniferous fossils from Argentina (Insecta: Odonatoidea) and basic character states in pterygote wings. *Canadian Journal of Zoology*, 62, 1150–1166. doi:10.1139/z84-166

Rivas-Martínez, S. (1987). [*Map of vegetation series of Spain*]. Madrid: I.C.O.N.A.

Sánchez-Guillén, R. A., van Gossum, H. & Cordero Rivera, A. (2005). Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae). *Biological Journal of the Linnean Society*, 85, 471–481. doi:10.1111/j.1095-8312.2005.00506.x

Sánchez-Herrera, M., Beatty, C. D., Nunes, R., Salazar, C. & Ware, J. L. (2020). An exploration of the complex biogeographical history of the Neotropical banner-wing damselflies (Odonata: Polythoridae). *BMC Evolutionary Biology*, 20, 74. doi:10.1186/s12862-020-01638-z

Santamaría, T., Torres, A., Hernández, M. A., Casanueva, P., Sánchez-Sastre, L. F. & Campos, F. (2022). Some characteristics of the wings of *Cordulegaster boltonii* (Donovan, 1807) (Odonata: Cordulegastridae). *Journal of the British Dragonfly Society*, 38, 113–126.

Schmitt, T. (2007). Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Frontiers in Zoology*, 4, 11. doi:10.1186/1742-9994-4-11

Schorr, M. & Paulson, D. (2020). *World Odonata List*. Tacoma, USA: Slater Museum of Natural History, University of Puget Sound.

Short, A. E. Z., Dikow, T. & Moreau C. S. (2018). Entomological collections in the age of big data. *Annual Review of Entomology*, 63, 513–530. doi:10.1146/annurev-ento-031616-035536

Simonsen, T. J., Olsen, K. & Djernæs, M. (2020). The African-Iberian connection in Odonata: mtDNA and ncDNA based phylogeography of *Aeshna cyanea* (Müller, 1764) (Odonata: Aeshnidae) in Western Palaearctic. *Arthropod Systematics and Phylogeny*, 78, 309–320. doi:10.26049/ASP78-2-2020-06

Suarez, A. V. & Tsutsui, N. D. (2004). The value of museum collections for research and society. *BioScience*, 54, 66–74. doi:10.1641/0006-3568(2004)054[0066:TVOMCF]2.0.CO;2

Suárez-Tovar, C. M. & Sarmiento, C. E. (2016). Beyond the wing planform: morphological differentiation between migratory and non-migratory dragonfly species. *Journal of Evolutionary Biology*, 29, 690–703. doi: 10.1111/jeb.12830

Suhling, F., Sahlén, G., Gorb, S., Kalkman, V. J., Dijkstra, K-D. B. & van Tol, J. (2015). Order Odonata. In: J. Thorp & D. C. Rogers (Eds.), *Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates*. pp. 893–932. Amsterdan: Academic Press. doi:10.1016/B978-0-12-385026-3.00035-8

Suvorov, A., Scornavacca, C., Fujimoto, M. S., Bodily, P., Clement, M., Crandall, K. A., Whiting, M. F., Schrider, D. R. & Bybee, S. M. (2022). Deep Ancestral Introgression Shapes Evolutionary History of Dragonflies and Damselflies, *Systematic Biology*, 71 (3), 526–546. doi: 10.1093/sysbio/syab063

Tillyard, R. J. (1917). *The biology of dragonflies*. Cambridge: Cambridge University Press.

Todisco, V., Gartton, P., Cesaroni, D. & Sbordoni, V. (2010). Phylogeography of *Parnassius apollo*: hints on taxonomy and conservation of a vulnerable glacial butterfly invader. *Biological Journal of the Linnean Society*, 101, 169–183. doi:10.1111/j.1095-8312.2010.01476.x

Trueman, J. W. H. (1996). A preliminary cladistic analysis of odonate wing venation. *Odonatologica*, 25, 59–72.

Wootton, R. J. (1991). The functional morphology of the wings of Odonata. *Advances in Odonatology*, 5, 153–169.

Wootton, R. J. (1992). Functional morphology of insect wings. *Annual Review of Entomology*, 37, 113–140. doi:10.1146/annurev.en.37.010192.000553

Wootton, R. (2020). Dragonfly flight: morphology, performance and behaviour. *International Journal of Odonatology*, 23, 31–39. doi: 10.1080/13887890.2019.1687991

Supplementary Material

Supplementary document 1. Source of the entomological collections used, country, study zone and identities.

Supplementary document 2. Sampling sites of *Cordulegaster boltonii* adults in the Iberian Peninsula.