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NOTE

Biometric differences between several populations of *Cordulegaster boltonii* (Odonata: Cordulegastridae) in Ibero-Maghrebian area

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Key words. Odonata, Cordulegastridae, *Cordulegaster boltonii*, biometrics, dragonflies, mountain streams, Iberian Peninsula, Morocco

Abstract. Biometric data of the exuviae of female larvae of the dragonfly *Cordulegaster boltonii* collected in Portugal, Spain and Morocco were analysed to determine whether the size of three exuvial structures measured differed depending on the geographic localities of the populations. Based on the results recorded for the 16 populations studied, head width was negatively correlated with latitude and the greatest length of the gonapophysis was recorded for the Iberian populations at the centre of this peninsula. Multivariate cluster analysis revealed a clear separation of the Moroccan population. A second cluster separated the southernmost population (Sierra Nevada) from the remaining Iberian populations. Four population groups were distinguished: those located in watercourses in the north and central area of the Iberian Peninsula, those in Iberian watercourses in the East and Middle South, the Sierra Nevada and North Morocco. Some of these results coincide with the results of genetic studies of other authors.

INTRODUCTION

Morpho-biometric characters of Odonata have been used to confirm interspecific differences recorded by genetic analysis (Lee & Lin, 2012; Kohli et al., 2013). These characters enable species to be differentiated during the larval phase when other characters are not consistent or difficult to observe (Landwer & Sites, 2006), can be used to analyse the parallel evolution of species in the same genus (Stoks et al., 2005) and to relate genome size with body size (Ardila-García & Gregory, 2009). Similarly, they have been utilized to separate specimens or populations of the same species, either by analysing adults (e.g., Cabuga et al., 2017; Corso, 2019) or exuviae (Casanueva et al., 2017a).

During the larval stages odonates are preyed upon by other species, mainly fish (Morin, 1984; McPeek, 1990). Many species defend themselves by means of abdominal spines against predators (Arnqvist & Johansson, 1998; Johansson, 2002; Johansson & Wahlström, 2002; McCauley et al., 2008). This feature is phenotypically plastic and larger where there are predators, which does not incur costs such as a longer larval development time or increase in the body size of F-0 larvae (Johansson, 2002).

The larvae of *Cordulegaster boltonii* (Donovan, 1807) behave like burrowers (Corbet, 2004) and their physiognomy has been analysed by Verschuren (1989) and Boudot & Jacquemin (1995). Along with other anatomical structures, these authors measured the size of the lateral spines on abdominal segments 8 and 9, setae of these segments and the gonapophysis.

The aim of this work was to determine whether certain morpho-biometric characters of the exuviae (head width, gonapophysis length and relative size of the lateral spines) are useful for grouping *C. boltonii* populations over a wide geographical area. If so, do they support the results for the same area of a genetic analysis and/or morphometric analysis of other body structures.

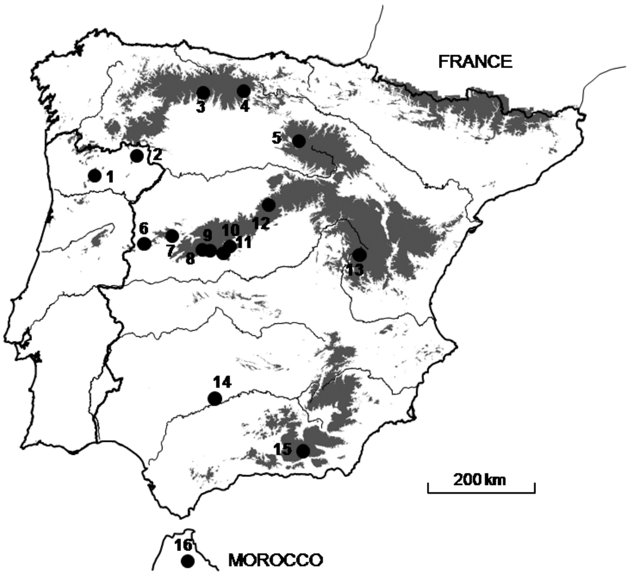

Fig. 1. Map showing the distribution of the 16 rivers sampled (black dots) on the Iberian Peninsula and in Morocco. 1 – Cova, 2 – Sabor, 3 – Curueño, 4 – Lazán, 5 – Arlanzón, 6 – Frío, 7 – Francia, 8 – Tormes, 9 – Barbellido, 10 – Gama, 11 – Alberche, 12 – Cega, 13 – Júcar, 14 – Bejarano, 15 – Monachil, 16 – Amsemlil (Morocco). Grey areas are mountain ranges: altitude > 1000 m a.s.l.

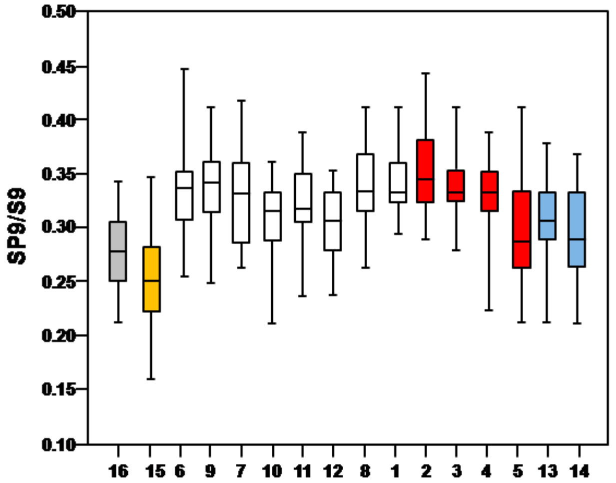

Fig. 2. Boxplot of the SP9/S9 values for the 16 *Cordulegaster boltonii* populations analysed. The numbers correspond to those in Fig. 1 and are arranged according to the dendrogram in Fig. 5. Grey – cluster A, orange – cluster B, white – cluster D1, red – cluster D2, blue – cluster C. Bars: upper and lower values. Boxes: upper (75%) and lower (25%) percentiles. Line in box – median.

MATERIAL AND METHODS

During 2016 and 2017 exuviae of final instar larvae of *C. boltonii* were collected from fourteen mountain rivers on the Iberian Peninsula and one in Morocco (Fig. 1, Table 1). In addition, exuviae that were collected from 1989–1992 from the population in the Bejarano River were also used. All samples were collected in May, June and July, which for this species is the period when most adults emerge (Ferreras-Romero & Corbet, 1999; Casanueva et al., 2017b). In the laboratory, the exuviae were measured, always by the same person (FC), using a Nikon SMZ800 binocular microscope with an eyepiece micrometre. Since males do not have a gonapophysis, only female exuviae were used.

Based on the work of Verschuren (1989), Boudot & Jacquemin (1995) proposes three criteria of exuviae for differentiating populations of *C. boltonii*: (a) form of the setae on the posterior edge of the 9th sternite, (b) ratio of the length of the lateral spine on the 9th sternite (SP9) and length of the 9th sternite (S9), which is SP9/S9, and (c) length of gonapophysis (LG). The first is a morphological character and therefore not quantifiable, while the other two are easily quantifiable. The last two were used in this study. An additional quantifiable character was also utilized; head width (He; i.e. the maximum distance between the lateral margins of the compound eyes), which does not vary during the emergence period (Casanueva et al., 2017c). These three variables are very poorly correlated, so collinearity is avoided (LG-SP9/S9: $r = 0.30$; LG-He: $r = -0.03$; He-SP9/S9: $r = -0.12$). The LG and He variables were adjusted to normality using the Shapiro-Wilk test, which then met the homogeneity of variance based on the Bartlett's test. Therefore, their average values were compared using an analysis of variance ANOVA. The SP9/S9 ratio values do not adjust to normality, so it was necessary to use the Kruskal-Wallis non-parametric analysis. In all cases the minimum significance value was $P < 0.05$.

A multivariate cluster analysis was used to group the variables by achieving the maximum homogeneity in each group and greatest difference between groups. For this, the nearest neighbour, similarity index or Euclidean distance was used as an algorithm. Clusters were joined based on the smallest distance between two groups. With a bootstrapping of N = 10000, the percentage support was calculated for each of the nodes.

Table 1. Geographic coordinates and sample sizes (N) of exuviae of female *Cordulegaster boltonii* from 16 populations. Altitude: m a.s.l.

River	Country	North	West	Altitude	N
1. Cova	Portugal	41°35′	7°47′	329	11
2. Sabor	Portugal	41°54′	6°44′	665	36
3. Curueño	Spain	42°50′	5°24′	1001	33
4. Lazán	Spain	43°01′	4°28′	1210	38
5. Arlanzón	Spain	42°13′	3°18′	1170	25
6. Frío	Spain	40°19′	6°38′	830	50
7. Francia	Spain	40°30′	6°02′	595	28
8. Tormes	Spain	40°20′	5°10′	1380	59
9. Barbellido	Spain	40°19′	5°12′	1450	49
10. Gama	Spain	40°25′	5°12′	1642	26
11. Alberche	Spain	40°26′	5°03′	1425	33
12. Cega	Spain	41°02′	3°49′	1255	43
13. Júcar	Spain	40°19′	1°48′	1245	22
14. Bejarano	Spain	37°56′	4°52′	400	42
15. Monachil	Spain	37°07′	3°30′	1016	49
16. Amsemlil	Morocco	35°15′	5°25′	1053	46

RESULTS

Measurements of 590 exuviae collected from sixteen Iberian and Maghrebi populations were analysed (Table 1). There were significant differences between populations for the SP9/S9 ratio (Kruskal-Wallis test, $H = 212.18$, $P = 0.000$), LG ($F_{15, 571} = 65.66$, $P = 0.000$) and He ($F_{15, 571} = 54.06$, $P = 0.000$). The SP9/S9 ratio did not clearly separate between the populations sampled, but the highest values were recorded for the northern and central populations and the lowest for the southernmost Iberian Peninsula (Monachil, located at Sierra Nevada) and Moroccan populations (Fig. 2). The populations at the centre of the Iberian Peninsula (Tormes and Alberche) had a longer gonapophysis than the other populations and the shortest was recorded for the Monachil population (Fig. 3). The head of the exuviae from Morocco was the widest (Fig. 4) followed by those from the Middle South and East

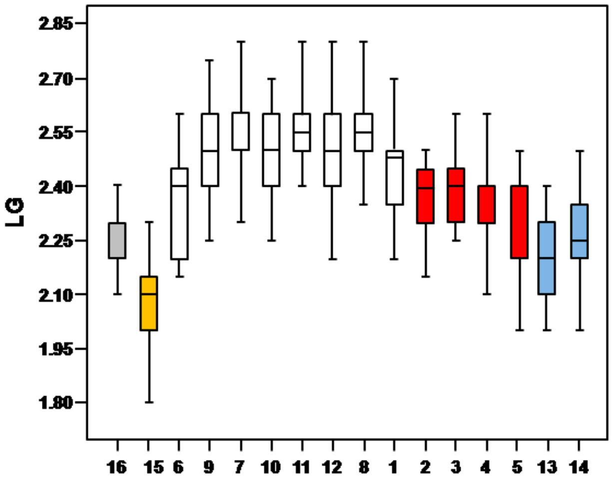

Fig. 3. Boxplot of the gonapophysis lengths (LG, in mm) for the 16 *Cordulegaster boltonii* populations analysed. The numbers correspond to those in Fig. 1 and are arranged according to the dendrogram in Fig. 5. Grey – cluster A, orange – cluster B, white – cluster D1, red – cluster D2, blue – cluster C. Bars: upper and lower values. Boxes: upper (75%) and lower (25%) percentiles. Line in box – median.

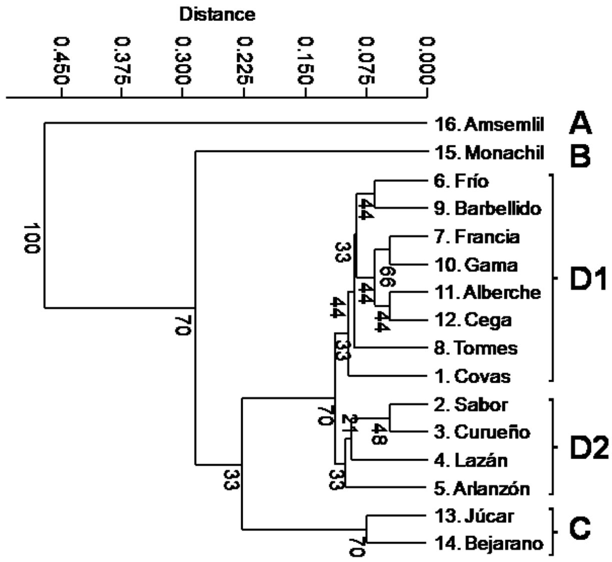

Fig. 5. Dendrogram of the 16 populations analysed based on the head widths, gonapophysis lengths and SP9/S9 ratios. The population numbers are the same as those in Fig. 1. Numbers at nodes refer to bootstrap support for that particular branch.

Iberian Peninsula populations (Bejarano and Júcar). The head width was negatively correlated with latitude.

Multivariate cluster analysis (Fig. 5) clearly separates the Moroccan population (Amsemlil) from the others. A second cluster separates, with a difference of 70%, the southernmost population (Monachil), which hereafter is called cluster B, from the other clusters, which are referred to hereinafter as clusters C and D. Clusters C and D differ by 33%. Cluster D includes the central and northern Iberian populations within which there are two groups that differ by 70%: cluster D1 including the seven populations in the centre and one from the north of Portugal, and cluster D2 including the four northernmost populations studied.

Since this analysis indicates that the Moroccan and Monachil populations differ from all others, they were not included in the non-parametric ANOVA used to determine whether clusters C, D1 and D2 differ from each other (Table 2). These analyses indicate that the variables He, GL and SP9/S9 differ significantly (P = 0.000), both when the three clusters are compared separately and when C is compared with D1 and D2 combined (Table 2). However, when clusters D1 and D2 were compared with each other, the SP9/S9 ratio did not differ significantly (P = 0.249).

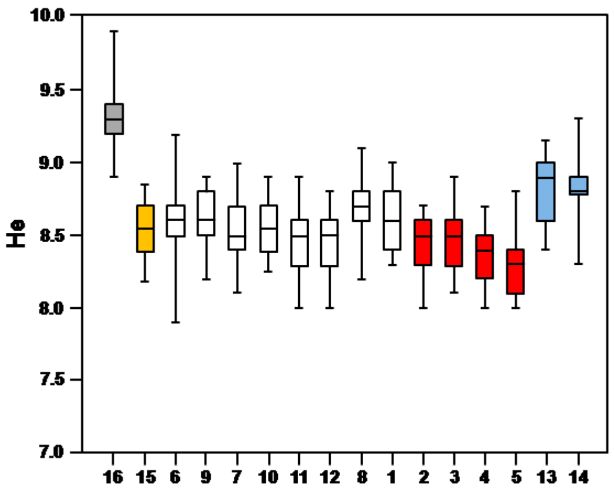

Fig. 4. Boxplot of the head widths (He, in mm) for the 16 *Cordulegaster boltonii* populations analysed. The numbers correspond to those in Fig. 1 and are arranged according to the dendrogram in Fig.5. Grey – cluster A, orange – cluster B, white – cluster D1, red – cluster D2, blue – cluster C. Bars: upper and lower values. Boxes: upper (75%) and lower (25%) percentiles. Line in box – median.

DISCUSSION

Based on the results the sixteen populations analysed form four groups: (A) North Africa, the population located in the Rif Mountains in northern Morocco, (B) southernmost Iberian Peninsula, Sierra Nevada population, (C) East and Middle South of the Iberian Peninsula, with two populations, and (D) Centre and North of the Iberian Peninsula, with twelve populations. In the D group there are two subgroups, one consisting of the four northernmost populations on the Iberian Peninsula and another consisting of seven populations in central Spain plus one in northern Portugal.

This grouping coincides only partially with the one proposed by Boudot & Jacquemin (1995) who analysed exuviae of *C. boltonii* from the Iberian Peninsula and Morocco. These authors suggest that the South Iberian populations have a mixture of the characteristics of the populations in North Africa and those at the centre of the Iberian Peninsula and those of the northern Iberian populations differ from those at the centre of the peninsular. On the other hand, Froufe et al. (2013) conclude that, of the populations they studied, only that in North Africa (*C. boltonii algirica*) is genetically different from the populations in Europe. This same difference also appears when the biometric characteristics of the exuviae were studied.

Table 2. Values of the ANOVA between clusters D1, D2 and C for head width (He), gonapophysis length (LG) and ratio of the lateral spine length (SP9) and length (S9) of the ninth abdominal segment of exuviae of female *Cordulegaster boltonii* from 16 populations. P – probability.

	D1–D2		D1–D2–C		(D1–D2)–C	
He	$F_{(1, 428)}$	66.84	$F_{(2, 492)}$	88.84	$F_{(1, 493)}$	96.11
	P	0.000	P	0.000	P	0.000
LG	$F_{(1, 428)}$	131.20	$F_{(2, 492)}$	166.38	$F_{(1, 493)}$	157.92
	P	0.000	P	0.000	P	0.000
SP9/S9	$F_{(1, 428)}$	1.330	$F_{(2, 492)}$	20.50	$F_{(1, 493)}$	39.61
	P	0.249	P	0.000	P	0.000

According to Froufe et al. (2013), it seems likely that North Africa was colonized twice, once by a common ancestor of *Cordulegaster princeps* (Jacquemin & Boudot, 1999) and *C. boltonii*, and more recently by *C. boltonii*. However, adults in Czech populations of *C. boltonii* only make short trips (< 10 km) (Hančíková, 2014). If the same occurs on the Iberian Peninsula and in Morocco, it is possible that the populations of *C. boltonii* on both sides of the Mediterranean Sea do not mix with each other, because they are separated by a sea. This isolation could have given rise to a separate subspecies in North Africa (*C. b. algirica*). Froufe et al. (2013) state that over a long term an allopatric speciation process could even be taking place. The results presented here show that the Iberian populations are clearly different from that located in North Africa (Morocco) (Fig. 5) and therefore support the hypothesis that they are separate population groups, without reproductive contact that allows gene flow. Furthermore, the population at Monachil (Sierra Nevada), the most southern and possibly also the most isolated of those studied on the Iberian Peninsula, is the most different from the other peninsular populations. In any case, in order to determine if this is true, it is necessary to study in more detail the genetical and anatomical characteristics of the populations of *C. boltonii* in North Africa and Sierra Nevada.

The Central System of populations on the Iberian Peninsula are clearly a distinct group. The eastern populations resemble those in the North. This indicates that they may have had a common origin, probably located in the North of the Iberian Peninsula. From there they would have spread south along two routes, one following the mountains in the North (populations 2, 3, 4, and 5) (Fig. 1) and the other the mountains in the centre, with both routes meeting in the mountains in northern Portugal (population 1).

In summary, genetic studies (Froufe et al., 2013) along with biometric analyses can be used for grouping or segregating different *C. boltonii* populations. This method could also be used for studying populations in other areas where this species is present, and even those of other species of *Cordulegaster* morphologically similar to *C. boltonii* (Corso, 2019).

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