Interspecific and intersexual variation in presacral vertebrae number in *Podarcis bocagei* and *P. carbonelli*

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Abstract. *Podarcis bocagei* and *P. carbonelli* are two closely related lizard species, endemic to the western Iberian Peninsula. Although genetic and morphological distinction between them is now well established, information on anatomical features is still very restricted. We studied presacral vertebrae number in both species, separately examining cervical and trunk vertebrae counts, in order to assess the interspecific and intersexual variation in these characters. There was no significant interspecific variation among lizards of the same sex for any of the vertebrae counts studied. However, important sexual variation existed, females presenting higher counts than males for both trunk and total presacral vertebrae, while males showed higher values of cervical vertebrae. Although our results lie within the known limits for other closely related species, they reveal that vertebrae number might present higher intraspecific variation than previously thought.

Keywords: anatomy, Lacertidae, Portugal, sexual dimorphism, skeleton.

Podarcis bocagei (Seoane, 1884) and *P. carbonelli* Pérez-Mellado, 1981 are members of the *P. hispanica* species complex (sensu Harris and Sá-Sousa, 2002) and were recently recognised as separate species (Sá-Sousa and Harris, 2002). Although several phylogenetic studies support their specific status (Harris and Sá-Sousa, 2002; Harris et al., 2002; Pinho, Harris and Ferrand, 2003; Pinho, Ferrand and Harris, 2004, 2006; Pinho, 2007) and their distinction based on external morphological features is now well documented (Sá-Sousa, 2001; Kaliontzopoulou, 2004; Kaliontzopoulou, Carretero and Llorente, 2005, 2007), information on many relevant anatomical and physiological traits is still lacking. The number of vertebrae is an especially interesting anatomic feature, which presents remarkable interspecific variability patterns across the Squamata and is related to radical evolutionary shifts within this group (Hoffstetter and Gasc, 1969). Within the lizard family Lacertidae, vertebrae count has been repeatedly used for phylogenetic inference (Arnold, 1973, 1983, 1989a, b, 1991, 1997; Salvador, 1982) and is thought to hold an important evolutionary potential (Van Damme and Vanhooydonck, 2002). Presacral vertebrae number is thought to remain more or less fixed among closely related species, but varies substantially when one compares lacertid genera. Sexual dimorphism in this character is one of the few derived morphological features distinguishing the Lacertidae from the rest of the Lacertoidea (Arnold, 2004; but see Greer 1987, 1990). Here, we report on the number of presacral vertebrae in *P. bocagei* and *P. carbonelli* and examine patterns of inter- and intraspecific variation.

We examined a total of 66 females and 96 males of *P. bocagei* collected in Mindelo-Vila Chã (UTM 29T NF27) and Espinho-Granja (UTM 29T NF24, NF34) and a total of 72 females and 96 males of *P. carbonelli* collected in Espinho-Granja, as well as in Torreira (UTM 29T NF21), all of them coastal localities in NW Portugal. The lizards were captured, sacrificed humanly and preserved in alcohol until their examination. The specimens were originally captured to analyse the reproductive biology and diet of these populations, tasks that could not be fulfilled without sacrificing the animals, but both species are very abundant in the study sites and examination of the populations in the years after capture did not reveal any demographic descent. Only adult specimens were examined because radiographies in small specimens were blurry and vertebrae could not be reliably

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counted. X-rays were taken using a Silhouette VR (General Electric) analogue machine, with an intensity of 41Kv and an exposure of 1mA/sec. In each specimen examined, we recorded the number of cervical vertebrae (VTBC) and trunk vertebrae (VTBTr), which sum up to the total number of presacral vertebrae (VTBTot). Normality was met after a square-root transformation (Kolmogorov-Smirnov test, $P > 0.05$ in all cases). Exploratory planned comparisons between lizards of the same species and sex from different populations did not reveal any significant effect of capture locality on any of the characters studied ($P > 0.1$ in all cases). We conducted two-way ANOVAs considering species and sex as factors, in order to quantify differences between groups in number of cervical, trunk and total presacral vertebrae. Additionally, to compare with existing data (Arnold, 1973), we considered modal values in each group, in order to examine the central tendencies observed. All statistical analyses were conducted using STATISTICA 7.1.

ANOVA comparisons showed that both species are sexually dimorphic considering cervical, trunk and total vertebral number, males presenting more cervical vertebrae and females presenting approximately two trunk vertebrae and at least one vertebra in total more than males (fig. 1, table 1). Post-hoc planned comparisons did not reveal significant differences in any vertebrae count between species considering individuals of the same sex either for males or for females. Results were slightly different when considering modes. Concerning cervical vertebrae, the most common value was 7 in both species and sexes (although at different frequencies, especially between sexes). On the other hand, the most frequent count for trunk vertebrae was the same for males of both species (18), but differed between females, being 20 for *P. bocagei* and 21 for *P. carbonelli*. Finally, modes coincided for the total number of presacral vertebrae, which was 27 for females and 25 for males of both species.

It is important to remark that our study revealed higher variability of vertebral counts than previously reported in these species. Both *Podarcis bocagei* (before elevation of *P. carbonelli* to the species level, i.e., Pérez-Mellado, 1998) and *P. hispanica* sensu lato have been reported to have 26-29 presacral vertebrae, as compared to ranges of 23-29 for both species examined here. This discrepancy is probably due to the small sample sizes previously examined (Arnold, 1973: $n = 19$ males, 16 females for *P. hispanica*).

The lack of interspecific variation observed could be due to either phylogenetic inertia, ecological divergence or both. The number of presacral vertebrae is highly preserved within *Podarcis*, mean vertebral counts being 26 to 27, while *P. hispanica* sensu lato, now considered an artificial taxon polyphyletic relative to *P. bocagei* and *P. carbonelli* (Harris and Sá-Sousa, 2002; Pinho, Ferrand and Harris, 2006), has been reported to present 26 or 27 (rarely 28) presacral vertebrae for males and 26 to 29 vertebrae (usually 28) for females (Arnold, 1973). It seems, consequently, that vertebral number is quite preserved between species of Iberian *Podarcis*, although modal values differ between *P. hispanica* (Arnold, 1973) and the species here examined. On the other hand, *P. bocagei* and *P. carbonelli* are both ground-dwelling species and might therefore share habitat-related constraints acting on presacral vertebrae number. A comparative study including all the members of the *P. hispanica* species complex could shed light to the causation of interspecific patterns of variability in this character.

Considering intraspecific patterns, our study revealed considerable sexual variation, taking into account that the characters examined were osteological. Absolute count of both total and trunk vertebrae was significantly higher in females than in males of both species. Obviously, the higher number of trunk vertebrae in females is related to their longer trunks (Kaliontzopoulou, 2004; Kaliontzopoulou, Carretero and Llorente, 2007), a pattern probably promoted by natural selection to enhance the availability of space for the allocation of eggs (Braña, 1996; Bauwens, Barbadillo and Gonzalez, 1997; Olsson et al., 2002). From a more mechanical point of view, a higher number of trunk vertebrae could correlate to the shortest limbs of females (Kaliontzopoulou, 2004; Kaliontzopoulou, Carretero and Llorente, 2007), since it is expected to increase body flex-

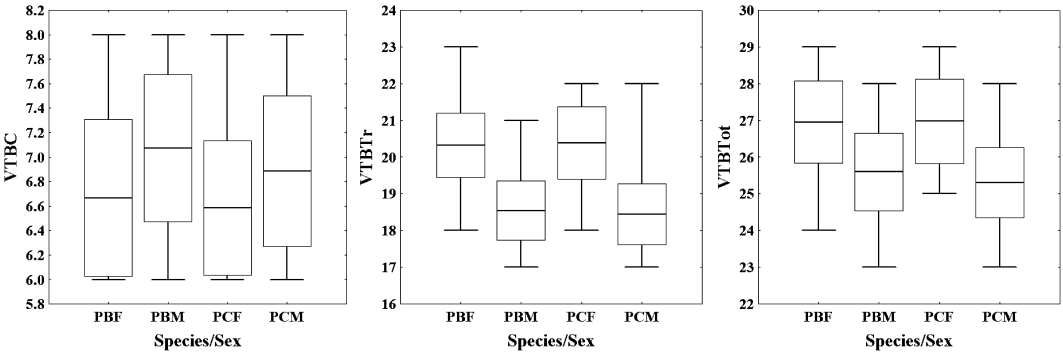

Figure 1. Box plots showing number of vertebrae in *Podarcis bocagei* (PB) and *Podarcis carbonelli* (PC) males (M) and females (F). VTBC: cervical vertebrae, VTBTr: trunk vertebrae, VTBTot: total presacral vertebrae. Lines represent mean values, boxes SD and whiskers Min-Max.

Table 1. Descriptive statistics (un-normalized variables) and results of ANOVA comparisons of vertebral counts for the factors species, sex and their interaction. Descriptive statistics are given as mean ± SD (top), range and mode (middle) and sample size (bottom). df = 1, 326 for all comparisons. VTBC: cervical vertebrae, VTBTr: trunk vertebrae, VTB: total presacral vertebrae. p-values are corrected for repeated tests using the False Discovery Rate procedure (Benjamini and Hochberg, 1995).

	Podarcis bocagei		Podarcis carbonelli		ANOVA (species, sex, species ∗ sex)	
	Females	Males	Females	Males	F	p-value
VTBC	6.67 ± 0.64	7.07 ± 0.60	6.58 ± 0.55	6.89 ± 0.61	4.00	0.108
	6-8 (7)	6-8 (7)	6-8 (7)	6-8 (7)	27.9	0.003
	66	96	72	96	0.6	0.488
VTBTr	20.32 ± 0. 88	18.54 ± 0.81	20.39 ± 0.99	18.44 ± 0.83	0.04	0.834
	18-23 (20)	17-21 (18)	18-22 (21)	17-22 (18)	367.01	0.003
	66	96	72	96	0.80	0.478
VTBTot	26.96 ± 1.12	25.59 ± 1.06	26.97 ± 1.15	25.30 ± 0.95	1.36	0.367
	24-29 (27)	23-28 (25)	25-29 (27)	23-28 (25)	162.13	0.003
	66	96	72	96	1.71	0.346

ibility (Hoffstetter and Gasc, 1969), and could also compensate for decreased mobility during pregnancy (Van Damme et al., 1989). Another interesting intraspecific pattern observed regards the number of cervical vertebrae, which is higher in males than in females of both species. Although it is difficult to formulate specific testable hypotheses on the matter, a higher number of cervical vertebrae might enhance neck flexibility and head mobility, which could in turn be favourable both for female immobilisation during mating and rival turning in male-male contests (Molina-Borja, Padrón-Fumero and Alfonso-Martín, 1999). Finally, a longer and more flexible neck would also be advantageous for males if behavioural display takes place (Molina-Borja, 1994, 2003), as is probably the case in *P. bocagei* (Ribeiro et al., 2006).

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