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Leukocyte differential counts and morphology from twelve European lizards

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Abstract
The present study reports the morphology of leukocytes of 12 European lacertid lizards (*Podarcis sicula*, *P. tiliguerta*, *P. melisellensis*, *P. bocagei*, *P. muralis*, *Algyroides nigropunctatus*, *Lacerta viridis*, *L. bilineata*, *L. trilineata*, *L. oxycephala*, *Timon lepidus*, and *Zootoca vivipara*) stained using May–Grünwald/Giemsa method. The morphology of white blood cells was very similar among species, suggesting a relative morphological uniformity within the lacertid lizards. For six species (i.e. *P. sicula*, *P. tiliguerta*, *P. melisellensis*, *P. bocagei*, *P. muralis*, and *A. nigropunctatus*), we determined the leukocyte differential counts, which may be considered representative of the normal values of the corresponding populations. These results may be useful either in clinical investigation to detect pathologies in wild individuals, as in management and conservation projects to assess the general health conditions of natural wild lizard populations.

Keywords: *Lacertidae*, *leukocyte morphology*, *leukocyte differential count*, *Haemogregarines*

Introduction

In clinical investigation, blood samples are of great diagnostic value, and can be easily obtained (e.g. Frye 1991). Haematological data provide clues to the existence of conditions that affect the cellular component of peripheral blood, and can be used to detect such condition as anaemia, parasitaemia, chronic stress and disorder of haemostasis (Mader 2000). Moreover, the haemogram is also of particular interest in ecological immunology (Sheldon & Verhulst 1996; Ots et al. 1998; Norris & Evans 2000), since it can support field researchers to analyse, in wild-living reptiles, the effects of health condition of individuals on different life history traits. For example, the leukograms of tortoises have been useful to explain why individuals differ from each other with respect to their reproductive decisions (Galeotti et al. 2005). Finally, the knowledge of the haematologic parameters of free-living individuals is important for assessing and managing their populations (Christopher et al. 1999; Dickinson et al. 2002). This is because haemograms supply a useful tool to easily detect pathologic processes within populations, which is a crucial point in managing endangered species (Martinez-Silvestre et al. 2005).

Many authors have described the blood cells of different reptile species, particularly tortoises (Alleman et al. 1992; O'Connor et al. 1994; Christopher et al. 1999; Kolle et al. 2001; Dickinson et al. 2002; Knotek et al. 2002, 2003; Knotkova et al. 2002; Azevedo & Lunardi 2003; Lopez-Olivera et al. 2003; Ugurtas et al. 2003; Keller et al. 2004), snakes (Bounous et al. 1996; Lamirande et al. 1999; Salakij et al. 2002a,b; Arikan et al. 2004, 2009a; Arikan & Çiçek 2010) and lizards (Pica et al. 1986; Divers & Redmayne 1996; Puerta et al. 1996; Eliman 1997; Tosunoglu et al. 2001; Sevinc & Ugurtas 2004; Pejrilova et al. 2004; Sacchi et al. 2007; Arikan et al. 2009b; Arikan & Çiçek 2010).

Although erythrocytes, thrombocytes, basophils, lymphocytes and monocytes are morphologically similar, remarkable differences occur in heterophils,

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eosinophils and monocytes among different reptile taxa, and frequently among species within a single taxon (Le Blanc et al. 2000; Mader 2000; Martinez-Silvestre et al. 2005). For example, heterophils vary in number and in nuclear lobules, whereas azurophils are often recognised as a distinct cell type from monocytes (Eliman 1997). Similarly, the eosinophils differ widely among reptiles for both shape and colouration of cytoplasmatic granules (Frye 1991; Mader 2000; Salakij et al. 2002a; Knotkova et al. 2002). Thus, some ambiguity still exists that can make the evaluation of the haemogram of reptile difficult to do (Cannon et al. 1996; Canfield 1998).

In addition, reptiles are the class among vertebrates in which immunology has been less investigated in respect, for example, to birds and mammals. Consequently, haematological studies are of great interest as anticipatory of immunological studies.

In this article we describe the morphology of circulating blood cells of 12 species of European lizards and we determine reference values for leukocyte differential counts for six of them.

Materials and methods

During summer 2007–2009, we collected 129 blood samples from both males and females of the following six lacertid species: *Podarcis sicula* (five males and five females, Latina, Italy), *P. tiliguerta* (four males and four females, Punta Sebera, Italy), *P. melisellensis* (seven males and five females, Kres, Croatia), *P. bocagei* (five males and six females, Oporto, Portugal), *P. muralis* (71 males and 10 females, Pavia, Italy), *Algyroides nigropunctatus* (five males and two females, Kres, Croatia). In addition, we collected blood samples from one to two individuals from *Lacerta viridis* (one male, Haj, Slovakia), *L. bilineata* (one male and one female, Pavia, Italy), *L. trilineata* (one male, Kres, Croatia), *L. oxycephala* (one female, Kres, Croatia), *Timon lepidus* (one juvenile, Oporto, Portugal), *Zootoca vivipara* (one female, Alpe Siusi, Italy). All individuals except *T. lepidus* were sexually mature, and appeared to be healthy and with no detectable abnormalities (such as injuries by predators).

Blood was collected immediately after capture from the orbital sinus using a heparinized glass capillary tube (see McLean et al. 1973). Air-dried smears were stained with May–Grünwald/Giemsa stain and scanned using a light microscope at 100× oil immersion following standard routines (Canfield 1998). In each microscope field, the red blood cells were counted and the leukocytes classified as lymphocytes, monocytes, eosinophils, heterophils, and basophils. In each smear, we counted 150–200 leukocytes and the corresponding red blood cells; when present, we also counted haemoparasites. The white blood cell count (WBC) and haemoparasite count were expressed as numbers per 10,000 erythrocytes.

The WBC, thrombocytes and haemoparasites counts were log-transformed to achieve normality, whereas the leukocyte percentages were arcsin-transformed. Linear models were used to check for differences in blood variables among species and between sexes, using sex, species and sex × species interaction as predictors. The initial models were subjected to a stepdown simplification procedure, where non-significant terms ($P > 0.05$) were removed sequentially, starting from the interaction terms, until the minimal adequate models, including only significant variables, were obtained (Crawley 1993). All statistical analyses were performed using the R 2.6.1 statistical software (R Development Core Team 2007), and unless otherwise stated, values are means ± SE.

Results

Blood cell morphology

Mature erythrocytes of lizards are nucleated ellipsoidal cells with rounded poles and uniform grey–blue cytoplasm, even though colour varied among smears probably as an effect of the heparin within capillary tubes or the time elapsed between collection and staining (Figures 1, 2). The nucleus is central and elongated with the long diameter parallel to that of the cell; it stains violet with a dense dark purple chromatin.

Heterophils (Figures 1, 2) are large, rounded cells with a light pink cytoplasm filled with small granules stained red to reddish-orange. In most cases, the granules are so numerous that they displace the nucleus against the side of the cell, while in others, the granules are less numerous and the nucleus is in the centre of the cell; the nucleus is light purple on staining and appears normally polylobed.

The eosinophils (Figures 1, 2) are as large as the heterophils and contain numerous small, weakly eosinophilic granules that often obscure the nucleus. The few intense eosinophilia in some cases could depend on the time elapsed between collection and staining. In some species, the granules are so thin and dense that their shape is not clearly defined, appearing as an undefined matrix filling the cytoplasm. The nucleus is variably positioned and appears dark purple on staining; sometimes it shows two or three lobes.

The basophils (Figures 1, 2) show the most conservative morphology among the 12 species analysed:

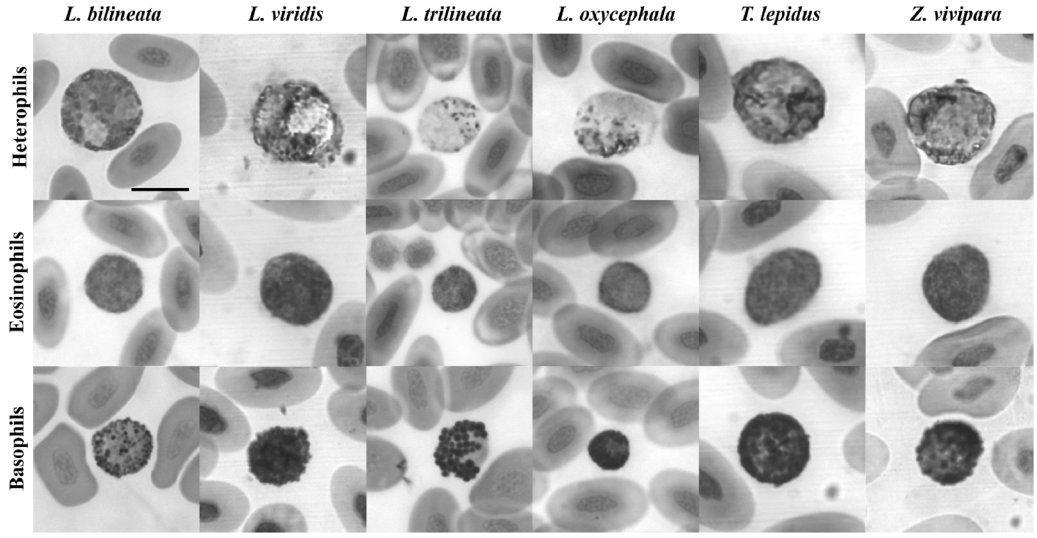

Figure 1. Heterophils, eosinophils and basophils of cells of the *Laceta bilineata*, *L. viridis*, *L. trilineata*, *L. oxycephala*, *Timon lepidus* and *Zootoca vivipara* (May–Grünwald/Giemsa stain). Scale bar: 10 μm.

they are as large as the other granulocytes and contain a round and a centrally positioned nucleus; the cytoplasm is densely filled by round granules that appear dark purple–black on staining.

In some individuals, we detected a fourth type of granulocyte completely different from the other types (Figure 3): it is a rounded cell with a non-segmented nucleus and an azurophilic cytoplasm densely filled by large, clear and rounded granules, which displace the nucleus off to the side of the cell.

The monocytes in all species are rounded cells with the diameter not larger than the long diameter of the erythrocytes. The cytoplasm is moderately granular and appears light pink on staining. The nucleus may appear 'C' shaped, centrally positioned, and with a violet pigmentation and a granular, dark purple chromatin.

The lymphocytes in European lizards, as for all other vertebrates, are mononuclear with an azurophilic scant cytoplasm covering a narrow area around the nucleus.

Finally, the thrombocytes have a round to oval nucleus staining dark purple and a uniform grey–blue cytoplasm, without granules, and frequently form small groups up to a dozen cells within the smear.

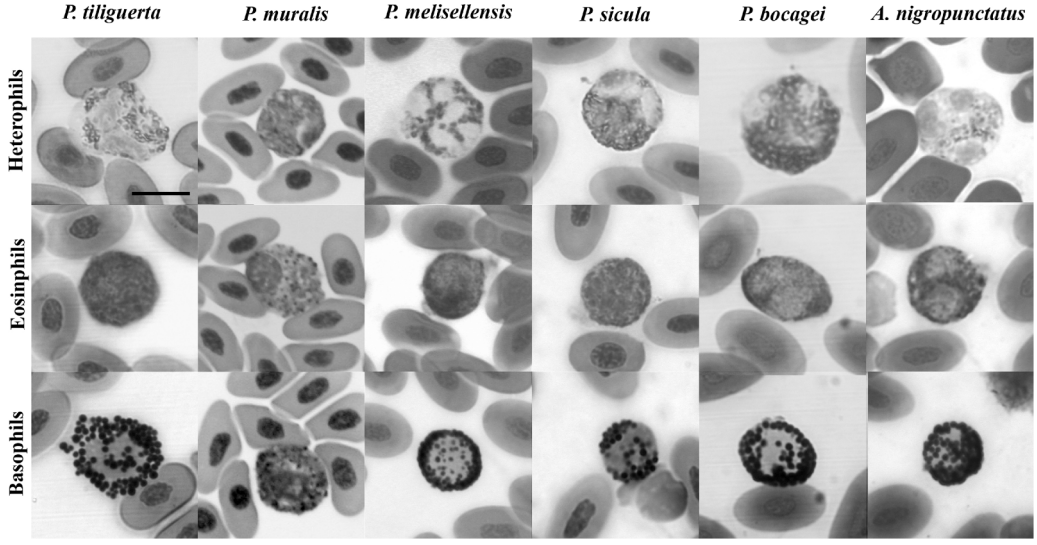

Figure 2. Heterophils, eosinophils and basophils of cells of the *Podarcis tiliguerta*, *P. muralis*, *P. melisellensis*, *P. sicula*, *P. bocagei*, and *Algyroides nigropunctatus* (May–Grünwald/Giemsa stain). Scale bar: 10 μm.

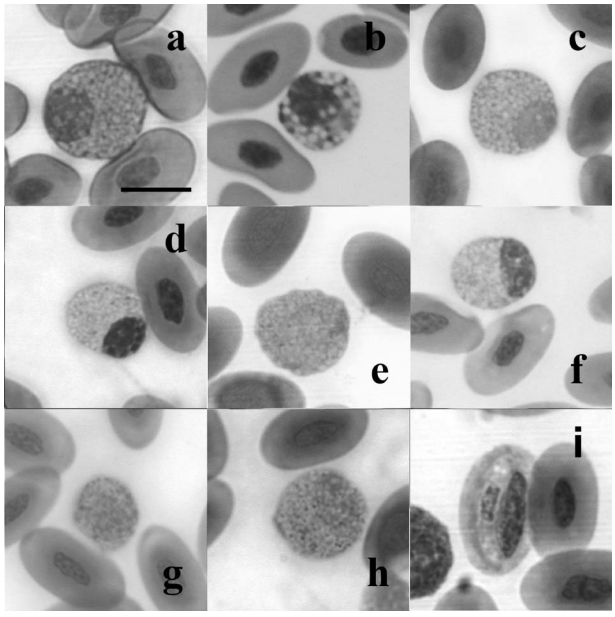

Figure 3. The fourth granulocyte type **(a–h)**, and an erythrocyte infected by haemogregarine spp. **(i)** (May–Grünwald/Giemsa stain); **(a)** *Podarcis tiliguerta*, **(b)** *P. muralis*, **(c)** *P. melisellensis*, **(d)** *P. sicula*, **(e)** *Algyroides nigropunctatus*, **(f)** *Lacerta bilineata*, **(g)** *L. trilineata*, and **(h)** *L. oxycephala*. Scale bar: 10 μm.

The thrombocytes are similar among all the species we investigated in this study.

Leukocyte differential count and haemoparasites

Total WBC (N/10,000 erythrocytes) ranged from 171 ± 22 in *P. melisellensis* to 280 ± 44 in *P. tiliguerta*, and we found a significant effect of the sex × species interaction ($F_{5,117} = 4.73$, $P < 0.001$), which reflected the lower concentration of leucocytes in Common wall lizard females in respect to males (Table I) and in respect to both males and females of all other species (Table I–Table VI). On the contrary, no significant differences were detected between the sexes in the other species, likely due to the lower sample of individuals analysed in respect to the *P. muralis* one. The number of heterophils differed significantly among species ($F_{5,123} = 3.54$, $P = 0.005$), and were more numerous in *A. nigropunctatus* (15.7%) in respect to *P. bocagei* (7.9%) and *P. tiliguerta* (7.1%, see Table I–Table VI). The eosinophils ranged from 7.7% (in *A. nigropunctatus*, Table II) to 16.8% (in *P. tiliguerta*, Table VI), but this range did not differ among species or between sexes. The percentage of basophils varied depending both on sex ($F_{1,122} = 7.07$, $P = 0.008$) and species ($F_{5,122} = 4.40$, $P = 0.001$). In all species apart from *A. nigropunctatus* (Table II), the basophils were detected more frequently in females than in males (11.1 and 9.2%, respectively). Irrespective of the sex, *P. melisellensis* had a significantly smaller percentage of basophils (5.1%) than all other species, whose basophils ranged from 7.4% in *P. muralis* to 13.6% in *P. tiliguerta* (Table I–Table VI). Finally, the lymphocytes were the most abundant type of leukocytes in all the six species analysed, ranging from 60.3% in *P. tiliguerta* to 73.6% in *P. melisellensis* (Table I–Table VI), but no significant differences were found among the species or between the sexes.

The number of thrombocytes differed significantly among the species ($F_{5,123} = 7.38$, $P < 0.001$), being lower in *P. melisellensis* in respect to the other species (Tables I–VI); no differences were detected between males and females (mean thrombocyte proportion among species, N/10,000 erythrocytes, males: 84 ± 33, females: 75 ± 29).

Haemoparasites (i.e. Haemogregarines, Figure 3i) were detected in all species, and parasite loads varied significantly among the species ($F_{5,123} = 5.12$, $P < 0.001$), but not between sexes. *P. sicula* and *P. melisellensis* had significant lower parasite concentrations than the other species (Figure 4).

Discussion

The blood cells of the 12 species of lacertids we analysed in this study can be basically classified as erythrocytes, thrombocytes and leukocytes that can be furthermore assigned to five main classes: heterophils, eosinophils, basophils, monocytes and lymphocytes. Lymphocytes and heterophils were the most common leukocytes in the blood, while monocytes and basophils were the rarest, as reported in other reptile groups (Saint-Girons 1970; Pica et al. 1986; Frye 1991; Campbell 1996; Mader 2000). The classification of the leukocytes in reptiles might be more difficult than in other vertebrates due to the high levels of morphological variation among species (reviewed in Canfield 1998), particularly with regard to the acidophilic granulocytes (Saint-Girons 1970; Divers & Redmayne 1996; Eliman 1997; Christopher et al. 1999; Kolle et al. 2001; Dickinson et al. 2002; Knotek et al. 2002, 2003; Knotkova et al. 2002; Arikan et al. 2004). However, the morphology of the blood cells was very similar in all 12 species of lacertids with regard to lymphocytes, basophils and monocytes but also to granulocytes, suggesting that blood cells have a relative morphological uniformity within Lacertids. Despite this uniformity, further investigations are needed, particularly in order to exclude the possibility for the morphology of the *Lacerta* species, *T. lepidus* and *Z. vivipara* blood to be unusual, as only a few individuals were analysed. In addition, reptiles are the only class

Table I. WBC, differential leukocyte count and thrombocyte count from the blood of *P. muralis*.

	Males ($N = 71$)		Females ($N = 10$)		Overall ($N = 81$)	
Blood cell type	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
WBC (N/10,000 erythrocytes)	222 ± 11	100 – 635	117 ± 10	58 – 168	209 ± 11	100 – 635
Heterophils (%)	15.5 ± 1.2	0.0 – 43.6	14.8 ± 2.7	1.3 – 28.5	15.5 ± 1.1	0.0 – 43.6
Eosinophils (%)	13.2 ± 1.0	0.0 – 41.6	16.0 ± 4.5	4.3 – 46.1	13.5 ± 1.0	0.0 – 46.1
Basophils (%)	6.7 ± 0.6	0.6 – 23.5	12.0 ± 1.8	4.8 – 21.4	7.4 ± 0.6	0.6 – 23.5
Type IV (%)	0.2 ± 0.1	0.0 – 1.3	0.0		0.2 ± 0.1	0.0 – 1.3
Monocytes (%)	0.4 ± 0.1	0.0 – 2.0	0.7 ± 0.5	0.0 – 4.9	0.4 ± 0.1	0.0 – 4.9
Lymphocytes (%)	63.9 ± 2.1	6.7 – 90.5*	56.6 ± 5.1	20.6 – 71.7	63.0 ± 2.0	6.7 – 90.5*
Thrombocytes (N/10,000 erythrocytes)	84 ± 15	0 – 770	87 ± 44	0 – 383	85 ± 14	0 – 770

*The low value of the inferior limit was due to a male showing high values of both heterophils and eosinophils, but without any sign of abnormality.

Table II. WBC, differential leukocyte count and thrombocyte count from the blood of *A. nigropunctatus*.

	Males ($N = 5$)		Females ($N = 2$)		Overall ($N = 7$)	
Blood cell type	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
WBC (N/10,000 erythrocytes)	227 ± 25	155 – 305	278 ± 71	207 – 349	241 ± 25	155 – 349
Heterophils (%)	17.4 ± 2.5	11.9 – 25.8	11.5 ± 3.6	7.9 – 15.0	15.7 ± 2.2	7.9 – 25.8
Eosinophils (%)	8.3 ± 1.6	3.8 – 13.8	6.0 ± 0.7	5.2 – 6.7	7.7 ± 1.2	3.8 – 13.8
Basophils (%)	9.5 ± 1.7	4.4 – 14.7	7.4 ± 3.7	3.7 – 11.1	8.9 ± 1.5	3.7 – 14.7
Type IV (%)	0.2 ± 0.2	0.0 – 1.1	0.3 ± 0.3	0.0 – 0.7	0.3 ± 0.2	0.0 – 1.1
Monocytes (%)	0.6 ± 0.2	0.0 – 1.3	0.0		0.4 ± 0.2	0.0 – 1.3
Lymphocytes (%)	63.9 ± 2.8	56.4 – 73.8	74.8 ± 6.9	68.0 – 81.7	67.0 ± 3.2	56.4 – 81.7
Thrombocytes (N/10,000 erythrocytes)	73 ± 17	25 – 112	65 ± 33	33 – 98	70 ± 14	25 – 112

Table III. WBC, differential leukocyte count and thrombocyte count from the blood of *P. bocagei*.

	Males ($N = 6$)		Females ($N = 5$)		Overall ($N = 11$)	
Blood cell type	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
WBC (N/10,000 erythrocytes)	304 ± 21	243–369	218 ± 31	138 – 328	265 ± 22	138 – 369
Heterophils (%)	6.9 ± 1.4	3.0 – 11.4	9.1 ± 2.9	0.0 – 16.9	7.9 ± 1.5	0.0 – 16.9
Eosinophils (%)	10.6 ± 1.6	7.1 – 15.2	15.2 ± 3.9	4.3 – 24.5	12.7 ± 1.9	4.3 – 24.5
Basophils (%)	13.5 ± 1.7	6.3 – 20.7	13.7 ± 3.3	7.3 – 25.3	13.6 ± 1.8	6.3 – 25.3
Type IV (%)	1.7 ± 0.2	0.4 – 3.6	0.9 ± 0.3	0.6 – 1.9	1.3 ± 0.4	0.4 – 3.6
Monocytes (%)	0.7 ± 0.2	0.5 – 1.2	0.6 ± 0.1	0.0 – 1.2	0.7 ± 0.1	0.0 – 1.2
Lymphocytes (%)	66.6 ± 2.8	58.8 – 73.8	60.5 ± 7.2	38.6 – 81.7	63.9 ± 3.8	38.6 – 81.7
Thrombocytes (N/10,000 erythrocytes)	115 ± 21	44 – 183	98 ± 17	38 – 133	108 ± 13	38 – 183

Table IV. WBC, differential leukocyte count and thrombocyte count from the blood of *P. melisellensis*.

	Males ($N = 7$)		Females ($N = 5$)		Overall ($N = 11$)	
Blood cell type	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
WBC (N/10,000 erythrocytes)	145 ± 16	104 – 210	207 ± 45	129 – 355	171 ± 22	104 – 355
Heterophils (%)	8.8 ± 1.4	2.6 – 13.5	15.1 ± 4.5	4.2 – 27.2	11.4 ± 2.2	2.6 – 27.2
Eosinophils (%)	9.3 ± 1.7	3.9 – 16.0	10.3 ± 2.8	3.8 – 19.7	9.7 ± 1.4	3.8 – 19.7
Basophils (%)	4.5 ± 1.2	1.3 – 9.1	5.9 ± 1.9	0.6 – 11.9	5.1 ± 1.0	0.6 – 11.9
Type IV (%)	0.0		0.3 ± 0.3	0.0 – 1.4	0.1 ± 0.1	0.0 – 1.4
Monocytes (%)	0.0		0.2 ± 0.1	0.0 – 0.7	0.1 ± 0.1	0.0 – 0.7
Lymphocytes (%)	77.4 ± 2.7	69.2 – 90.1	68.2 ± 7.2	47.7 – 85.6	73.6 ± 3.5	47.7 – 90.1
Thrombocytes (N/10,000 erythrocytes)	11 ± 8	0 – 59	13 ± 11	0 – 56	12 ± 6	0 – 59

Table V. WBC, differential leukocyte count and thrombocyte count from the blood of *P. sicula*.

	Males ($N = 5$)		Females ($N = 5$)		Overall ($N = 10$)	
Blood cell type	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
WBC (N/10,000 erythrocytes)	189 ± 36	113 – 302	220 ± 15	174 – 253	205 ± 19	113 – 302
Heterophils (%)	12.7 ± 5.6	6.2 – 35.0	5.4 ± 0.7	4.1 – 7.9	9.0 ± 2.9	4.1 – 35.0
Eosinophils (%)	9.9 ± 3.3	1.2 – 17.3	8.7 ± 1.1	4.4 – 10.5	9.3 ± 1.7	1.2 – 17.3
Basophils (%)	11.1 ± 4.5	3.7 – 28.3	13.5 ± 2.6	5.9 – 21.9	12.3 ± 2.5	3.7 – 28.3
Type IV (%)	0.2 ± 0.2	0.0 – 1.2	0.8 ± 0.5	0.0 – 2.0	0.5 ± 0.3	0.0 – 2.0
Monocytes (%)	1.5 ± 0.3	0.6 – 2.5	2.7 ± 1.1	0.0 – 5.7	2.1 ± 0.6	0.0 – 5.7
Lymphocytes (%)	64.5 ± 7.7	46.1 – 81.4	69.0 ± 4.4	59.9 – 80.5	66.8 ± 4.2	46.1 – 81.4
Thrombocytes (N/10,000 erythrocytes)	135 ± 74	0 – 334	101 ± 37	7 – 224	118 ± 40	0 – 334

Table VI. WBC, differential leukocyte count and thrombocyte count from the blood of *P. tiliguerta*.

	Males ($N = 4$)		Females ($N = 4$)		Overall ($N = 8$)	
Blood cell type	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
WBC (N/10,000 erythrocytes)	277 ± 84	55 – 436	283 ± 45	193 – 397	280 ± 44	55 – 436
Heterophils (%)	5.6 ± 1.6	1.4 – 8.3	8.7 ± 2.2	5.3 – 15.2	7.1 ± 1.4	1.4 – 15.2
Eosinophils (%)	16.8 ± 3.8	10.1 – 26.2	16.8 ± 5.4	6.9 – 28.5	16.8 ± 3.1	6.9 – 28.5
Basophils (%)	10.0 ± 2.6	6.5 – 17.7	13.7 ± 1.6	11.8 – 18.5	11.8 ± 1.6	6.5 – 18.5
Type IV (%)	3.9 ± 1.0	1.0 – 5.5	3.0 ± 1.5	0.9 – 7.3	3.4 ± 0.8	0.9 – 7.3
Monocytes (%)	0.3 ± 0.1	0.0 – 0.6	0.7 ± 0.4	0.0 – 2.0	0.5 ± 0.2	0.0 – 2.0
Lymphocytes (%)	63.5 ± 7.6	43.3 – 77.3	57.1 ± 10.4	28.5 – 73.9	60.3 ± 6.1	28.5 – 77.3
Thrombocytes (N/10,000 erythrocytes)	87 ± 9	62 – 102	85 ± 12	68 – 120	86 ± 7	62 – 120

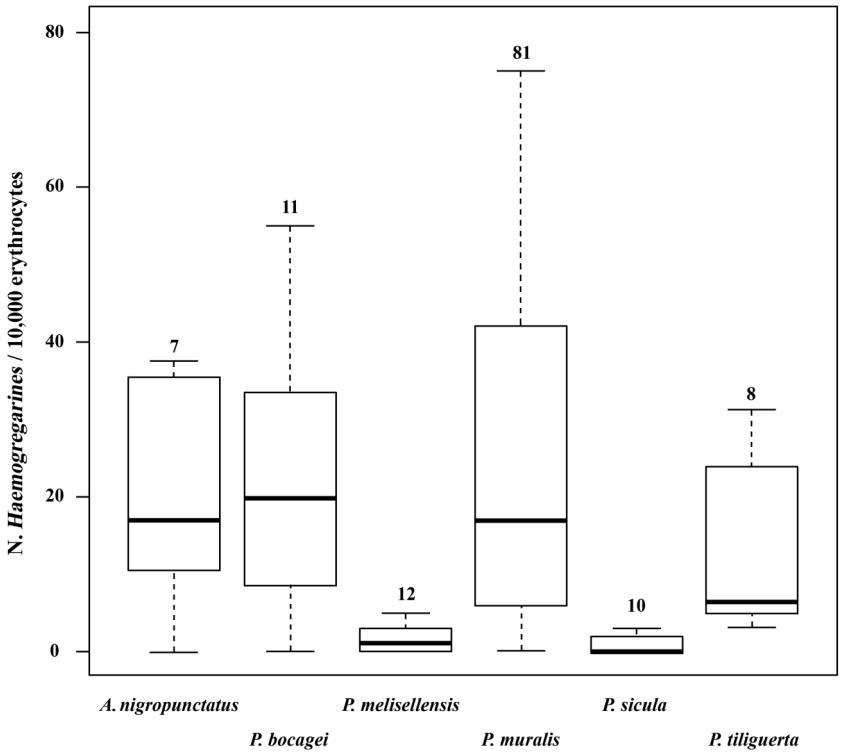

Figure 4. *Haemogregarine* loads (N/10,000 erythrocytes) in six European lacertid lizards. Numbers above boxes indicate sample sizes.

of vertebrates in which lymphocytes have not been characterised at the molecular level. Nevertheless, widespread evidences based on functional tests allow us to infer the existence of T and B lymphocytes (Cuchens & Clem 1979). In our 12 species, the morphology of lymphocytes is quite variable in size and nucleus/cytoplasm ratio, suggesting that different morphologies may actually correspond to different functional classes. Thus, our data need further improvements to clarify the morphology of live leucocytes by other approaches, such as flow cytometry.

As observed by Frye (1991), and recently in Mediterranean Geckos (Sacchi et al. 2007), we found a fourth type of granulocyte in all the 12 species, whose morphology differed highly from that of the heterophils, eosinophils and basophils. Frye (1991) names this cell type neutrophil, since granules do not stain selectively to May–Grünwald/Giemsa method. Alberio et al. (2005), reported in the lizard *Ameiva ameiva* four different granulocyte types, referring them as types I, II, III and IV. However, the essential differences between type I and III cells were the bilobed nucleus, the amount of heterochromatin and the more heterogeneous granules present in type III. Consequently, both cells were likely to constitute the same cell lineage, type III being a more mature state of type I granulocyte (Alberio et al. 2005). Despite this, the morphology of the fourth type we found in European lizards does not resemble any granulocyte types reported for *A. ameiva*. Further electron microscope analyses and investigations on the specific enzymatic activities of this cell type are needed to definitively assess if it is a distinct granulocyte rather than an activated or toxic state of the other granulocyte types and monocytes (Sacchi et al. 2007; Strik et al. 2007).

Recently, Arikan et al. (2009b) described the morphology of blood cells of 16 Lacertid lizards from Turkey, including *L. bilineata*, *L. trilineata*, *P. muralis* and *P. sicula*. Surprisingly, these authors did not find the heterophils in five species, including *P. sicula*, and reported a low occurrence of basophils in all species. Moreover, they provided no information about the sample sizes, or the sex of the lizards. Contrary to Arikan et al. (2009b), in our blood survey the heterophils were detected in all 10 individuals of *P. sicula* analysed, with percentages similar to those observed in the other four *Podarcis* species and also to those reported by Pica et al. (1986). In addition, in all species the basophils were not rare, as they were present with percentages between 5.1 and 12.3%. In two species (i.e. *P. sicula* and *P. tiliguerta*), basophils were as frequent as the heterophils and eosinophils. Finally, the percentages of granulocytes we find in our study were similar to those generally reported for the granulocytes of reptiles (Frye 1991; Mader 2000), and the leukocyte differential counts

we obtained for *P. muralis* perfectly matched that previously published by Duguy (1967). The heterophils and basophils are easily discernible from the other granulocytes because of their cytoplasmatic granules staining orange and dark purple. Thus the lack of heterophils as the rarity of the basophils reported by Arikan et al. (2009b) might reflect the low sample of individuals they probably considered or, alternatively, the occurrence within the sample of some anomalous individual affected by undetected pathologies.

A second relevant result of this study was the definition of the leukocyte differential counts for six of the 12 species analysed. The percentages of the leukocytes were quite similar among species, particularly within the genus *Podarcis*. Indeed, the more evident significant differences regarded the heterophils, which were more frequent in *A. nigropunctatus* than in all the other species, and the basophils, which were more abundant in males than in females in all the species but *A. nigropunctatus*. Since all sampled lizards appeared in good condition and no evident pathologies were detected, we assume that the differential counts here provided can be considered as representative of the normal values of the sampled populations. However, these data are based on small samples (with the exception of *P. muralis*), collected from single populations, so caution should be exercised when extrapolating the results to other natural populations of the six species.

Even though preliminary, our data represent a first important step in order to obtain an accurate set of baseline reference intervals for clinically healthy free-ranging lizards in different sites and under a variety of environmental conditions (Christopher et al. 1999). Once obtained, haemograms will be useful tools in the diagnosis and monitoring of lizard health and diseases, both for conservation and scientific purposes.

Finally, haemogregarines were detected in all six species analysed in detail. Parasite loads did not differ between sex but only among species. Overall, a four species group (i.e. *A. nigropunctatus*, *P. bocagei*, *P. tiliguerta* and *P. muralis*) showed higher parasite loads then the other two (*P. melisellensis* and *P. sicula*). This difference may be due to the time difference among blood sampling of species (and consequently a difference in the cycle of the parasite), rather than a true difference in the susceptibility to haemogregarines among species. Therefore, further analyses basing on a repeated blood sampling of individuals in different period of the breeding season would be necessary to fully understand the causes of the difference in parasite loads among species we found in our survey. Finally, we did not find any parasite infecting other blood cells than erytrocytes, contrary to the recent confirmation that haemogregarines are able to infect monocytes (Bonadiman et al. 2010).

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