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Incidence patterns of ectodermic lesions in wild populations of Common Wall Lizard (*Podarcis muralis*)

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Abstract. Skin lesions frequently present in adult lizards may be due to a variety of causes, both physical and infectious, including excessively high humidity and environmental temperature, malnutrition, concurrent disease etc. On the other hand, skin lesions in lizards could be simple evidence of various behavioural patterns and biotic interactions. However, studies on frequencies of dermal lesions and their anatomical and environmental correlates in lacertid lizards are rare. Here, we use *Podarcis muralis* to analyse the relations between occurrence of ectodermal lesions and three possible indicators of environmental stress (body condition index – BCI, infestation by ticks and tail condition) by evaluating differences among local populations at uni- and multivariate level. Our results showed that BCI, together with body size and sexual size dimorphism, varied between populations but had no direct influence on the presence of lesions. Males had higher frequencies of lesions and ticks but lower frequencies of broken tails than females. All three parameters varied between sites likely due to differences in predation/parasite exposures and agonistic interactions with conspecifics between sexes and populations. Results of multivariate analyses suggested that the occurrence of lesions is decoupled from the other morphological stress indicators. Detected associations indicated that relations between presence of lesions and other analysed variables are rather complex. Directions for further research on ectodermal lesions in lacertid lizards are provided.

Keywords: autotomy, body condition, cutaneous lesions, ectoparasites, lacertid lizard.

Introduction

There are many potential indicators of stress in wild animal populations. They include lower body condition index (Stevenson and Woods Jr., 2006), increased fluctuating asymmetry (Leung and Forbes, 1996), frequent infestations with endo- and ectoparasites (Oppliger et al., 1998) and poorer immunological status (Møller et al., 1998), when compared to populations from undisturbed environments.

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Reptiles potentially provide good models for studies focused on evaluation of stress in natural populations due to their abundance, richness, conspicuousness and ecological polyvalence (White et al., 1997; Woinarski and Ash, 2002). Among them, we chose a member of genus *Podarcis*, as its family (Lacertidae) represents the dominant group of lizards in Europe (Gasc et al., 1997) and the genus itself, widespread along the Mediterranean Basin, is especially interesting due to its ability to establish populations in human settlements and agroenvironments (Graziani et al., 2006; Ribeiro, 2011; Amaral et al., 2012, 2012b).

Cutaneous lesions can be manifested in a variety of ways (abscess, blisters/bullae, crusts, discoloration, oedema, erosions, necrosis, nodules, changes in skin colour or/and texture) and many factors can lead to these conditions (elaborated in Jacobson, 2007). However, skin lesions in lizards could be just simple evidence of different behavioural patterns and biotic interactions, such as courtship bites, male-to-male

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combats, unsuccessful predatory attacks etc. (Maderson, Baranowitz and Roth, 1978).

Dermatologic problems and their causes are well documented in species that are often kept as pets, with very little information on dermatologic problems in free ranging species (Cork and Stockdale, 1994; Martinez-Silvestre and Galán, 1999). Unfortunately, analyses at population level are rare (Martinez-Silvestre and Galán, 1999). Among ectotherm vertebrates, reptiles have been poorly investigated, with few similar studies being available for tortoises and virtually none for lizards (Gibbons et al., 2000).

While lizard lesions can be directly induced from environmental stress, this effect could be masked by other factors varying between localities such as climatic regimes, prey availability, parasitisation, predation pressure and intraspecific competition (Castilla and Labra, 1998; Van Damme et al., 1998; Martinez-Silvestre and Galán, 1999; Diego-Rasilla, 2003; Amo, Lopez and Martin, 2006; Pafilis et al., 2009; Amaral et al., 2012a). Our sampling was designed to minimise climatic variation among populations, while external parasitisation, inefficient predation and population density were assessed. Nevertheless, due to these multiple causes, this study is intended to be just a first approach to a complex topic. Specifically, our aim was to determine if there is a relation between the appearance of lizard skin lesions and four other variables, namely sex, body condition, infestation with ectoparasites and susceptibility to inefficient predators (evaluated by tail condition – intact or broken/regenerated) by analysing the frequency of lesions observed in two urban and two rural populations of Common Wall Lizard, *Podarcis muralis*. To our knowledge, there are no other analyses on the frequencies of dermal lesions in *P. muralis* populations and their anatomical and environmental correlates published so far.

Initial hypotheses were that: 1) individuals with dermal lesions would have a) lower BCI, b) higher infestation with ectoparasites and c) higher rates of broken/regenerated tails, since these three parameters could derive from a exposure to stress on both individual and populational level, and 2) males would be more prone to skin lesions when compared to females, due to more intense activity patterns resulting in more frequent encounters with conspecifics (agonistic) and predators.

Materials and methods

Species and study sites

For this study, adult specimens of *P. muralis* were sampled using standard noose techniques from mid-April-June 2011 from two urban and two non-urban environments, all met within a distance of 30 km. The first urban population (P1) was located in the Niš Fortress (N 43°19.477′ E 021°53.864′) in the centre of the City of Niš on the right bank of Nišava river in Southern Serbia. The habitat consisted of tall stone walls with open grass. The second population (P2) was collected at the Palilula ramp (N 43°18.788′ E 21°53.997′), on a railroad that passes through the City of Niš. Habitat consisted of small railroad rocks with small patches of grass along the railway up to one meter high. The third population (P3) was sampled in surroundings of Donji Dušnik village (N 43°10.263′ E 022°07.037′) at the base of Mountain Suva Planina, about 30 km from the City of Niš. Animals were collected on roofs of abandoned houses and watermills, on large and small piles of stones and fallen tree trunks. The sampling area was intersected with large number of small streams. The fourth population (P4) was sampled in Sićevo gorge (N 43°20.307′ E 022°05.000′), 15 km from the city in a habitat very similar to Donji Dušnik. The broader area of Niš (including non-urban study sites) has a temperate continental climate, with average annual temperature of 11.2°C and annual precipitation of 589.6 mm (www.hidmet.gov.rs). Due to the close distances, no relevant climatic differences existed among all four sites. Except for P2, all sampling sites were located near water.

Population abundance was estimated as number of newly seen lizards/hour/observer (Amaral, M.J., pers. comm.). A series of visual censuses were made by two observers walking through the study area. Three censuses were made at the same part of day and under similar ambient temperature conditions with no rain and low wind speed for all populations.

Considering lack of evidence that intra-specific aggression in *P. muralis* can lead to autotomy events (Diego-Rasilla, 2003) we used tail break frequencies as rough estimate of inefficient predation pressure.

Laboratory procedures

Immediately after the capture, lizards were transported to the laboratory of the Faculty of sciences and mathematics at University of Niš where they were sexed and measured for snout-vent length (SVL) and body mass. All individuals with developed secondary sexual traits and SVL larger than 49.78 mm (Aleksić and Ljubisavljević, 2001) were considered adults. Males were recognized by presence of hemipenises when palpated at the base of the tail and by well developed femoral pores at the basis of hind limbs. Body length was measured by dial calliper, with 0.01 mm precision, while body mass was weighted by Ohaus CS-200 compact digital scale, to the nearest 0.1 g. Presence of ticks as potential indicators of environmental stress (Amo, Lopez and Martin, 2006) was recorded after detailed inspection of lizard's skin, while handled. Tail was checked briefly, assigning individuals just as "intact" or "broken" (either regenerated or cut) regarding tail condition.

All individuals were checked for the presence of cutaneous lesions. Samples were collected from six individuals (using a sterile cotton swab and rubbing it over the lesions) to provide identification of microorganisms eventually responsible for the lesions. The isolates were cultured in Müller-Hinton agar (MH) for bacteria and on Sabouraud Dextrose agar (SD) for fungi and yeasts. Samples were incubated at 37°C. A small amount of material from colonies formed in MH agar was transferred to blood agar plate and to plate with Chapman agar with novobiocine for more detailed identification. These subcultures were incubated at the same temperature. Filamentous fungi were determined on the basis of morphometric characteristics of spores.

Upon completion of measurements, lizards were returned into their native habitats.

Statistical analyses

Distribution of values of analysed continuous variables (SVL and body mass) was checked for normality through Liliefors' tests. Regression residuals of body mass against log-transformed SVL were used to estimate (not to compare, see below) body condition index (Henle, 1990; Jakob, Marshall and Uetz, 1996). Calculations were carried out separately for males and females since sexual dimorphism was detected for some populations (see below). Tail condition of every individual was defined as categorical variable, with two possible states (intact or broken/regenerated). Both ectodermal lesions and ticks on lizard's body were also treated as categorical variables, with two states – presence/absence.

Comparisons of SVL were performed by means of two way ANOVAs while body condition index (body mass relative to SVL) was compared by means of two way ANCOVAs on body mass with SVL as covariate, both with population and sex (also tail condition, ticks infestation and lesions) as factors. Due to the categorical nature of the dependent variables, we used multiple correspondence analysis (Lebart, Morineau and Warwick, 1984) to represent both lizards groups and variable states simultaneously, as well as, log-linear analysis (Jobson, 1992) to infer the eventually synergic effects of population, sex, tail condition and tick infestation on the presence of skin lesions. All analyses were performed in Statistica 10.0 (StatSoft, 2011).

Results

A total of 317 individuals were collected from four populations (table 1). Lesions were found mainly on dorsal and ventral side of the head (fig. 1), but also in smaller degree on other parts of the body, mainly on ventral side (fig. 2). In the macroscopic examination, changes in scale colour and texture were seen. Scales modified by lesions were light to dark grey and scale surface was wrinkled. In undamaged lizards, scales were regularly spaced, collared, and scale surface was smooth. In several individuals we noticed scale necrosis.

Ectodermal lesions were recorded in all populations surveyed, though in various proportions depending sex and locality (table 1). Both bacteria and fungi were detected in ectodermal lesions: smooth, bright, lemon yellow-coloured bacterial colonies were developed on MH agar,

Table 1. Number of males and females per sample, values (mean ± SE) of snout-vent length (SVL) and body condition index (BCI, separately for each sex), number of individuals per sex and population (Pop.) with specified tail condition, tick parasitisation and ectodermal lesions on their skin.

Pop.	Locality	Sex	*n*	SVL	BCI	Intact tails	Broken tails	Ticks	Lesions
P1	Niš	M	48	59.47 ± 0.54	0.72 ± 0.14	26	22	0	32
	Fortress	F	40	56.89 ± 0.79	0.72 ± 0.10	14	26	0	12
P2	Palilula	M	42	57.72 ± 0.30	−0.58 ± 0.07	9	33	23	17
	Ramp	F	45	55.36 ± 0.41	−0.42 ± 0.06	10	35	10	9
P3	Donji	M	29	58.61 ± 0.97	−0.31 ± 0.09	13	16	3	11
	Dušnik	F	28	58.20 ± 0.69	−0.11 ± 0.12	11	17	0	0
P4	Sićevo	M	41	57.77 ± 0.71	−0.03 ± 0.07	20	21	5	18
	Gorge	F	44	58.54 ± 0.76	−0.16 ± 0.07	11	33	0	5

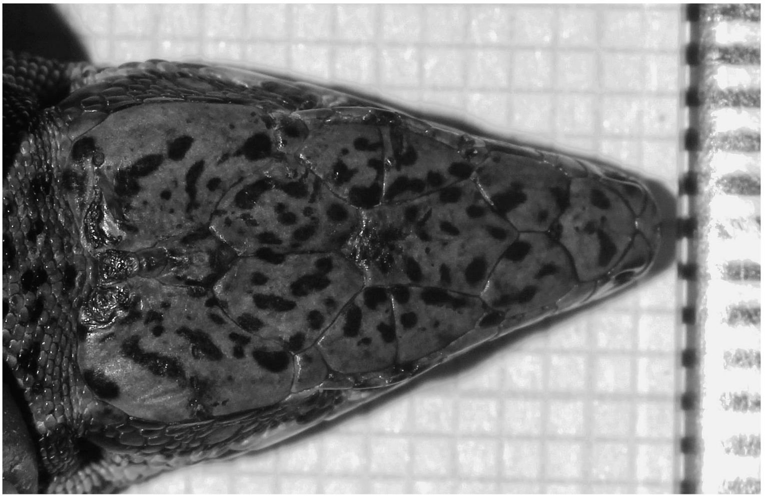

Figure 1. Dermatologic lesion on dorsal side of the head.

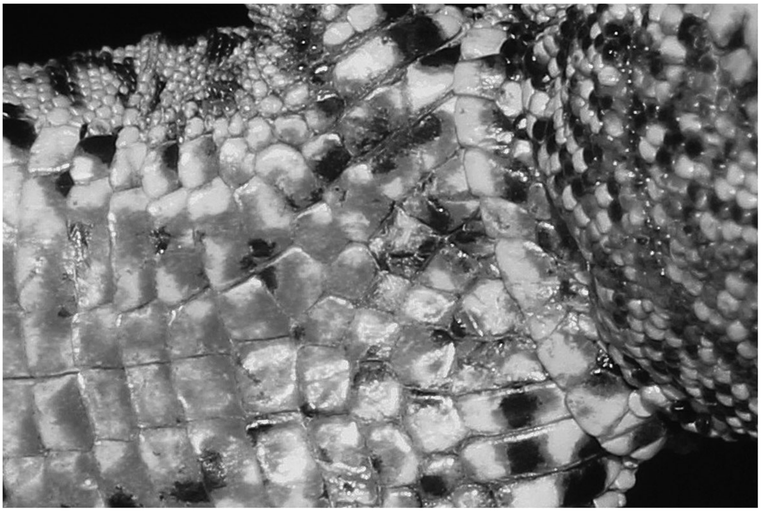

Figure 2. Dermatologic lesion on ventral side of the body.

typical of the genus *Staphylococcus*. Conventional diagnostic method confirmed presence of non-pathogenic bacteria *Staphylococcus epidermidis* in infected samples of lizard skin. Furthermore, filamentous fungi of genus *Alternaria* grew on SD agar, but their identification to species level was not possible.

Differences in body size (SVL) were detected between populations, sexes and their interaction (ANOVA population $F_{3,309} = 3.23$, $P < 0.021$; sex $F_{1,309} = 6.42$, $P < 0.011$; population × sex $F_{3,309} = 3.41$, $P < 0.021$). Namely, lizards from P2 tended to be smaller than those of the other three populations while P1 and P2 (but not P3 and P4) were sexually dimorphic with males attaining larger sizes than females (Scheffé post-hoc tests $P < 0.05$). Regarding body condition, there were differences between populations and sexes but these two factors (population and sex) did not interact (ANCOVA population $F_{3,308} = 70.96$, $P < 0.001$; sex $F_{1,308} = 46.72$, $P < 0.001$; population × sex $F_{3,308} = 1.74$, $P > 0.15$). Males were relatively heavier than females for the same SVL; individuals from P1 displayed the highest body condition and those from P2 the lowest (Scheffé post-hoc tests $P < 0.05$). When the tail condition and presence of ticks were evaluated together with sex (regardless the population), body condition was found to be higher in males and in individuals with intact tails and without ticks but did not differ between individuals with and without skin lesions (ANCOVA SVL as covariate, sex $F_{1,300} = 18.96$, $P < 0.001$; tail $F_{1,300} = 11.58$, $P < 0.001$; ticks $F_{1,300} = 23.41$, $P < 0.001$; all remaining factors interactions non-significant).

Relationships between the presence of the skin lesions and other factors were significant but complex as revealed by multiple correspondence analyses (fig. 3) and the log-linear models (table 2). Interpopulational and intersexual variation was recorded not only for the incidence of lesions but also for tail regeneration. However, only tail condition, modulated by sex, was associated *per se* to the lesions. This association was inverse; P3 and P4 (and also all females regardless the population) tended to have less skin lesions and ticks and higher frequencies of regenerated tails.

Population abundances were as follows: P1 – 4.0, P2 – 5.15, P3 – 3.55 and P4 – 4.01 lizards/hour/observer.

Discussion

Our first goal was to test if there was a relation between ectodermic lesions and some widely used stress indicators in analysed lizard populations (body condition, infestation by ectoparasites, tail condition). We first determined the general pattern of variation of body size and sexual size dimorphism in the populations under the study. Both parameters tend to be very plastic in lacertids (Cox, Butler and John-Alder, 2007), even between populations of the same species (Roitberg and Smirina, 2006; Roitberg, 2007). The same goes for *P. muralis* (Žagar et al., 2012) which seems to respond to local variations in the relative contributions of sexual, fecundity and natural selection (Kaliontzopoulou, Carretero and Llorente, 2010; Kaliontzopoulou, Carretero and Sillero, 2010). Not surprisingly, we recorded body size differences among populations, with lizards from the open area of the railroad reaching the smaller sizes. It is note-

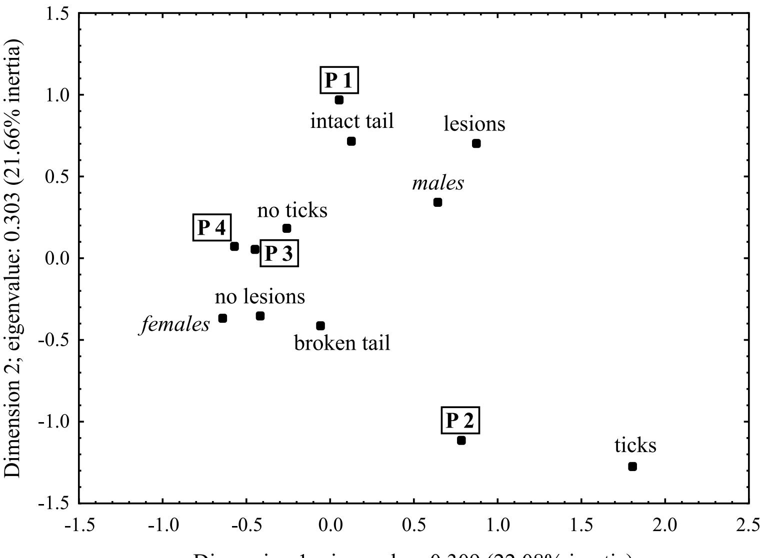

Figure 3. Plot of groups and variable states of *P. muralis* from southern Serbia by means of the multiple correspondence analysis using the first two axes. For P1-P4 see table 1.

Table 2. Results of the log-linear analyses of the influences of tail regeneration (tail), ectodermal lesions (lesions), tick parasitisation (ticks) according to the population and sex of *Podarcis muralis* from southern Serbia. Significant results in bold.

	df	Partial χ^2	P	Marginal χ^2	P
Population	**3**	**8.12**	**0.04**	**8.12**	**0.04**
Sex	**1**	0.03	0.87	0.03	0.87
Tail	**1**	**22.95**	**0.000002**	**22.95**	**0.000002**
Lesions	**1**	**34.62**	**<0.000001**	**34.62**	**<0.000001**
Ticks	**1**	**173.11**	**<0.000001**	**173.11**	**<0.000001**
population × sex	3	1.38	0.71	0.85	0.84
population × tail	**3**	**9.82**	**0.02**	**11.25**	**0.01**
population × lesions	**3**	**16.23**	**0.00**	**15.34**	**0.002**
population × ticks	**3**	**51.51**	**<0.000001**	**46.88**	**<0.000001**
sex × tail	**1**	**2.68**	**0.10**	**5.37**	**0.02**
sex × lesions	**1**	**27.62**	**<0.000001**	**33.84**	**<0.000001**
sex × ticks	**1**	**7.68**	**0.006**	**9.01**	**0.003**
tail × lesions	**1**	**1.68**	**0.20**	**4.66**	**0.03**
tail × ticks	1	0.20	0.65	0.14	0.71
lesions × ticks	1	2.95	0.09	3.69	0.05
population × sex × tail	3	2.28	0.52	2.89	0.41
population × sex × lesions	3	1.78	0.62	2.67	0.45
population × sex × ticks	3	1.14	0.77	1.86	0.60
population × tail × lesions	3	0.91	0.82	0.86	0.84
population × tail × ticks	3	1.49	0.68	2.13	0.55
population × lesions × ticks	3	1.39	0.71	1.96	0.58
sex × tail × lesions	1	0.08	0.77	0.004	0.95
sex × tail × ticks	1	0.36	0.55	1.99	0.16
sex × lesions × ticks	**1**	**2.46**	**0.12**	**5.54**	**0.02**
tail × lesions × ticks	1	0.10	0.76	0.02	0.89
population × sex × tail × lesions	3	1.26	0.74	0.88	0.83
population × sex × tail × ticks	3	0.47	0.93	0.66	0.88
population × sex × lesions × ticks	3	2.52	0.47	3.53	0.32
population × tail × lesions × ticks	3	1.44	0.70	0.97	0.81
sex × tail × lesions × ticks	1	0.28	0.59	0.12	0.72

Log-linear model (factors)	Test	Value	df	P
automatic (population-ticks/ population-tail/sex-tail/population-lesions/ sex-lesions/sex-ticks)	Max. likelihood χ^2	32.16	44	0.91
	Pearson χ^2	38.68	44	0.70
simplified (population-sex-tail/population-sex-lesions/ population-sex-ticks)	Max. likelihood χ^2	23.03	32	0.88
	Pearson χ^2	29.96	32	0.57

worthy that both urban populations displayed male biased sexual dimorphism whereas the two rural ones did not. Moreover, such variation was due to females (not to males) of the two urban populations, which were smaller than those from the rural areas. It is tempting to attribute such phenotypic differences to local variations in the relative contributions of sexual, fecundity and natural selection (Kaliontzopoulou, Carretero and Llorente, 2010). Namely, pregnant females could be more vulnerable to predators in disturbed habitats (see also tail regeneration) and simply not reaching bigger sizes. However, only a wider analysis with more populations could provide a robust support to this hypothesis.

Body condition index is an important measure of the fitness of an animal and could be used as an indicator of past foraging success, fighting ability, and ability to cope with environmental pressures (Jakob, Marshall and Uetz, 1996). Many aspects of the immune system are associated with body condition and nutrition, and individuals with high body condition should have better immune defence (Møller et al., 1998). Attenuation of body condition related with habitat deterioration has been reported in lacertids (Amo, López, and Martín, 2006, 2007). Certainly, the differences between sexes in BCI should be attributed to the higher robustness of males (Kaliontzopoulou, Carretero and Llorente, 2008) rather than to a poorer condition of the females. However, the differences between populations corroborated the pattern already found for SVL, since lizards at the railroad locality were not only smaller but in the worst condition. In all populations analysed here, lizards with intact tails and uninfected by ticks showed also better body condition; however, no association was found between presence of dermal lesions and body condition index. Similar findings are reported by Galán (1999) and Martínez-Silvestre and Galán (1999) in a comprehensive population study on *Podarcis bocagei* in north-west Spain: despite being frequent in some parts of the year, cutaneous lesions were not associated with survival probability, but were considered as affecting reproductive success.

However, some of the relations may have been masked by synergic interactions. Our multivariate analyses demonstrated that males and P1 tended to have higher frequencies of lesions and more intact tails, whereas females and P2 had more broken tails. Also, males and P2 carried more ticks whereas females, P3 and P4 had less ticks and fewer lesions. If differences in predator/parasite exposure between populations may be subjected to this pattern, it should be supported by specific assessment of both predators and parasites. Regarding the intersexual differences, males are also more vulnerable to predation since they spend time in patrolling and agonistic interactions related to reproduction (Cooper, Pérez-Mellado and Vitt, 2004; Tsasi et al., 2009).

Our analysis yields that individuals with higher BCI values more often had intact tails. Tail autotomy is the ability to shed the tail as a response to attempted predation, including that of conspecific (Maginnis, 2006). Although lizards greatly benefit from autotomy in terms of immediate survival, there are costs associated with this behaviour, such as reduction in social status, locomotor ability and energy reserves (Arnold, 1984, 1988; Bateman and Fleming, 2009). In our study, females more frequently had broken/regenerated tails. Determining what drives intersexual differences is problematic, as females could be either more successful in escaping inefficient predators or more susceptible to efficient predation (Bateman and Fleming, 2009). It is, however, doubtful that conspecific aggression would be responsible for the intersexual differences. Considering interpopulational differences, lizards from P2 had higher frequencies of broken/regenerated tails and those from P1 lowest which is most likely a matter of habitat exposure.

Statistical analysis also showed higher degree of lesions in males compared to females as well as differences between populations. Testosterone levels in males are higher during the mating season (Tokarz et al., 1998), when they become more aggressive and frequently interact with other males and females. Male combat often leads to scratches and injuries from bites and these wounds are often secondary colonized by bacteria, fungi and viruses which can lead to serious dermatological problems (Raynaud and Adrian, 1976). Moreover, males (and population P2 regardless sex) were also more prone to be invaded by ticks. Ticks infestations are common in reptiles, particularly in lizards and snakes (Burridge, 2005). Apart from spreading bacteria and viruses they can also contribute to the increase of dermatological problems, mainly ulcerations (Mader, 1996).

Extreme temperatures and humidity could be the cause of lesions (Martínez-Silvestre and Galán, 1999). Furthermore, four populations are settled within area of 30 km maximal distance from each other, on similar elevations and with minor differences in ambient temperature. As they belong to the same climate region (www.hidmet.gov.rs), we could exclude climate as factor that strongly induce skin lesions.

Finally, *Staphylococcus* infections may cause lesions in reptiles (Murphy and Armstrong, 1978; Wright, 1993). We did not find any record about the presence of *S. epidermidis* in reptiles, and it is possible that this finding is a result of sample contamination (Queck and Otto, 2008). Skin infections due to *Alternaria* spp. have been described in mammals (Cabanes et al., 1988; Roosje et al., 1994). Although *Alternaria* species themselves are able to promote these changes in the skin of lizards, it is equally likely that they colonised opportunistically lesions produced by other causes as conspecific aggression or predation failure.

In summary, it still seems premature to formulate here a definitive diagnosis regarding the causes of occurrence of these particular ectodermal lesions in analysed populations of Common Wall Lizard. Microorganisms identified there are not usual pathogens of lizard skin and cannot even be argued as causative factors for the development of lesions. Occurrence of lesions seems to be decoupled from other morphological traits widely recognized as indicators of population stress. However, additional analyses should be conducted including evaluation of seasonal and year-to-year variation in occurrence of lesions in populations under a wider variety of environmental circumstances, using more precise microbiological methods for sampling and detection of microorganisms at species level. If such information is successfully gathered, it would be of high value in the future not only in terms of conservation of the species (considered of community interest in EU legislation, and listed in the annex 4 of the Habitats directive of the European Council 92/43) but also for using this widespread species in Europe as an inexpensive bioindicator of environmental disturbance.

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