



Effects of biotic and abiotic stressors on asymmetries and head size in two sympatric lizard species

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Abstract

Organisms face numerous environmental stressors, which can affect developmental precision, including symmetry of various physical characteristics. Fluctuating asymmetry (FA) has therefore been suggested as a simple and efficient tool for assessing sub-lethal stress levels. We analyzed FA in two sympatric lizard species (*Iberolacerta horvathi* and *Podarcis muralis*) to determine potential effects of interspecific competition and urbanization, as proxies of stress, taking into account sexual dimorphism and environmental conditions. We sampled 16 syntopic and allotopic populations and used geometric morphometrics of head morphology. We detected significant but mixed effects on the head asymmetry from the environment and the syntopic occurrence that differed between species. *P. muralis* lizards had more asymmetric heads at higher altitudes, while *I. horvathi* lizards did at mid altitudes, which may be explained by *P. muralis* experiencing environmental stress of colder conditions at higher altitudes. The mid-altitude effect on asymmetries in *I. horvathi* might be explained by a lower availability of stony walls and higher abundance of *P. muralis*, thus higher competition. The asymmetry of supraciliary granules was affected by the presence of other species. However, lizards from allotopic populations attained larger asymmetries compared to lizards from syntopic populations, which was the opposite from what was expected. There was no effect of urbanization in *P. muralis*, which could be due to

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relatively low pollution and habitat degradation in study locations. Overall, we highlighted the possibility of using lizards and FA for bioindication of environmental stressors and especially improved the knowledge gap in the research of biotic stressors.

Keywords

Competition; directional asymmetry; fluctuating asymmetry; lizards; stress; urbanization

Introduction

Negative effects of either abiotic or biotic origin, have long been the focus of research to assist biodiversity conservation (Wood et al., 2000). With the escalation of anthropogenic pressure on natural populations there is an increasing need for efficient, easy to use, inexpensive and non-invasive indicators of population disturbance to aid conservation efforts (Burger, 2006). These indicators need to be sensitive, i.e., they can detect signs of population disturbance early before components of fitness have been strongly affected and before irreversible demographic damage has occurred (Bartell, 2006; Burger, 2006).

In this context, the degree of developmental stability (DS) has been suggested as a reliable indicator, because a wide range of environmental stressors has been shown to affect developmental precision (Beasley et al., 2013). DS is a characteristic of an organism to develop along a predetermined developmental pathway and resist random disturbances, which can occur during the development (Lens & Van Dongen, 2000). The majority of organisms have one or more axes of symmetry over which the body is a mirror image. However, they are rarely perfectly symmetrical and slight differences between the repeating structures occur. These random and subtle deviations from perfect symmetry are referred to as fluctuating asymmetry (FA; Palmer & Strobeck, 1986; Leamy & Klingenberg, 2005; Graham et al., 2010), which is one of the most commonly used measures of developmental (in)stability (Van Valen, 1962). The logic behind using FA as a measurement of DS is based on the assumption that both sides of bilaterally symmetrical individuals develop in the same environment and under the effect of identical genes, so that any differences between sides have to be a result of disturbances during the development (Clarke, 1993).

FA is a non-directional asymmetry, as opposed to the directional asymmetry where one side is larger than the other side (e.g., internal organs of vertebrates, handedness and position of owl ears; Møller & Swaddle, 1997). For a given sample or population, FA is computed as the variance of bilateral differences scored between individuals, either of size or of shape. It must be distinguished from anti-symmetry, another and less frequent kind of non-directional asymmetry, where signed bilateral differences do not have a Gaussian distribution (Klingenberg, 2015).

In the last two decades, there has been an increased interest in FA as a stress indicator due to its advantage over other indicators, such as direct measuring of net survival (fertility, survival), which is more expensive and impractical compared to observations of FA (Leung et al., 2000). Some studies have shown that asymmetry levels increase with the presence of stress factors, suggesting that FA can be used as an indicator of environmental and/or genetic stress and that it also might be useful for assessing the quality and health of individuals and populations (Leung & Forbes, 1996; Clarke, 1998). Common environmental stress factors caused by anthropogenic activity are exposure to pollutants, such as heavy metals and pesticide residues, temperature changes induced by climate change and habitat destruction (often as a consequence of urbanization; Lens et al., 1999). Biotic stress factors can include competition, inbreeding (and consequently higher homozygosity) and hybridization (Garrido & Pérez-Mellado, 2014). When exposed to stress, organisms need to invest energy in order to cope with it, thus there could be less energy available for stable development, which can lead to asymmetry of physical characteristics (Leung et al., 2000).

High levels of FA in lizards have so far been described as a consequence of various disturbance factors: inbreeding in populations from small islands (Soulé, 1967; Soulé & Yang, 1973), habitat fragmentation (Sarre, 1996), suboptimal incubation temperatures of eggs (Braña & Ji, 2000) and pollution (Tull & Brussard, 2007). Furthermore, the connection between FA and the female choice showed that females preferred more symmetric males (Martín & López, 2006), indicating its potential effect of FA on individuals' and population fitness. In some lizard species a correlation between FA and performance was observed, as higher asymmetry of hind limbs negatively affected their running speed (Martín & López, 2001; López & Martín, 2002). Recent studies (Lazić et al., 2013) and general overviews of this topic (Clarke, 1995; Møller & Swaddle, 1997) established FA as a valid indicator of DS and suggested it can be used as an early warning sign.

Studying head shape asymmetry was observed to be an especially good model system for examining the effects of environmental stress on the development, as the head is a developmentally complex body part involved in several important ecological and social activities in lizards, e.g., feeding, mating, acquiring and defending territories (Pianka & Vitt, 2003). Consequently, the shape of the head varies greatly between individuals within a population and among populations, indicating that it is potentially subject to selection pressure (Lazić et al., 2015).

Lizards, as ectothermic organisms, are good models to be used as indicator species for detecting the presence of environmental stress, since their development is more sensitive to environmental and genetic changes compared to endothermic organisms (Leary & Allendorf, 1989). Their advantage as bioindicator species is also the result of the many possible metric (limbs, head dimensions) and meristic (scales, femoral pores) traits, which are relatively easy to measure (Soulé, 1967). Moreover, lizards are usually abundant, which facilitates sample collection, and they inhabit places with varying levels of potential stressors (Crnobrnja-Isailović et al., 2005; Amaral et al., 2012; Lazić et al., 2013).

Interspecific interactions, direct or indirect, have been identified as one of the most important processes determining the structure of natural communities (Cody & Diamond, 1975; Bonsall & Hassell, 1997; Vrezec & Tome, 2004). Interspecific competition is a relationship between species, defined as a collection of reciprocal negative effects among ecologically similar species that results in a reduction of fecundity, growth and/or survivorship due to resource exploitation or interference (Smith & Smith, 1998). Prerequisites for competition between species are: they occupy the same space (occur in a zone of sympatry), are active in the same time and their fundamental niches overlap (at least partly) (Smith & Smith, 1998; Begon et al., 2006). In communities where two or more members of the same ecological guild coexist and resources they utilize are limited, they will either segregate in one or more dimensions of ecological niche to avoid interspecific interactions or they will interact (Begon et al., 2006). As competition effects are negative for all involved in the interaction, they represent biotic stress factors that may impact DS.

However, almost no information is available so far about the effects of interspecific interactions on asymmetry of organisms as a consequence of DS, although it could play an important role as a biotic stress factor (Thornhill, 1992; Witter & Swaddle, 1994; Graham et al., 2010). For example, Mallard and Barnard (2003) performed a study testing apparent competition on the conspecific and heterospecific level between two species of crickets and observed that limb FA was significantly correlated with the competitor's presence in one, but not in the other species. Such results suggest effects of competitor species on asymmetry (one species may be more dominant than the other in the interspecific competition) that hence can shape the relationships between both species in syntopic populations (Holt, 1977).

To fill this knowledge gap, we compared FA levels between allotopic and syntopic populations of two competing lizard species, which is the first such study in lizards known to us. We used a pair of ecologically and morphologically similar species of lacertid lizards that occur in sympatry in Slovenia and Croatia: the wall lizard, *Podarcis muralis* (Laurenti, 1768) and the Horvath's rock lizard, *Iberolacerta horvathi* (Méhelÿ, 1904). The two species exhibit a spatial pattern of occurrence in alternating allotopic and syntopic populations and opposite patterns in altitudinal distribution with *P. muralis* occurring in higher abundances at lower altitudes, while *I. horvathi* is most abundant at the highest elevations (De Luca, 1989; Žagar, 2016). Due to their similarities and restricted use of common spatial resources (Žagar et al., 2012, 2017; Osojnik et al., 2013), interspecific competition occurs in syntopic populations (Žagar et al., 2015a). Competitive interactions can be a source of biotic stress, therefore we expected that species will be more asymmetric in syntopic than in allotopic populations.

In addition to potential effects of interspecific interactions, we studied the influence of urbanization (only in *P. muralis*). Higher levels of FA were expected to be found in populations from urbanized (hence degraded and polluted) sites vs natural habitat sites. Since the species occur in a relatively high altitudinal gradient (approx. 1800 m) and exhibit an opposite trend in relative densities across altitudes



Figure 1. The locations of the studied populations (see Table 1 for the legend). For better visibility the area of Kočevska, where the density of sampling points was higher, is enlarged in (a).

(*I. horvathi* is more abundant at higher altitudes and *P. muralis* at lower altitudes), we included altitude as an environmental factor, and performed an additional test on a subset of populations to exclude the effect of altitude and potential differences due to phylogenetic distances. In general, we expected that populations from colder higher altitudes will have a higher level of asymmetries than lowland populations, but the effect should be stronger in the generalist species (*P. muralis*) that has a higher occurrence in lowlands and middle altitudes. Our study should improve our understanding of the effects of biotic and abiotic stressors on the occurrence of asymmetries in vertebrates and reveal the potential of using DS as bioindicator for environmental stress.

Materials and methods

Sampling and study design

Sampling sites were distributed across the area of sympatric occurrence of the two studied species, *Iberolacerta horvathi* and *Podarcis muralis*, across Slovenia and Croatia (fig. 1; De Luca, 1989; Sillero et al., 2014; Žagar, 2016). The study area is characterized by a large altitudinal gradient, heterogeneous topography, high forest cover and a mosaic of open areas with exposed rocky areas, which represent suitable habitat for both species (Žagar et al., 2013), while *P. muralis* also occurs in urbanized areas (Krofel et al., 2009). The climate is temperate continental with an

Table 1.

Locations of studied populations of *Podarcis muralis* and *Iberolacerta horvathi* in Slovenia and Croatia as labelled on the map (fig. 1). Populations labelled with * were used in the additional analysis on a subset of populations, since they occur at close geographical proximity and are genetically closely related. Abbreviations: F, female, M, male.

Map	Location name	Altitude	Presence of other	Urbanization	Sam	ple size
label			species		F	М
1	East from Ribnica*	741	Allotopic – P. muralis	Natural	40	58
2	Velike Bele stene*	1005	Allotopic – I. horvathi		63	69
3	Kuželjska stena*	840	Syntopic – I. horvathi		0	9
			Syntopic – P. muralis		1	1
4	Kameni zid*	1045	Syntopic – I. horvathi		1	11
			Syntopic – P. muralis		5	8
5	Sveti Štefan	308	Allotopic – P. muralis	Urban	20	15
	cemetery					
6	Fridrihštajn*	943	Allotopic – P. muralis		12	8
7	Ljubljana (Rožna	295	Allotopic – P. muralis	Urban	5	6
	Dolina)					
8	Dinara	1500	Syntopic – I. horvathi		1	2
			Syntopic – P. muralis		3	5
9	Bilpa	220	Syntopic – I. horvathi		5	4
			Syntopic – P. muralis		0	7
10	Mangart	2040	Allotopic – I. horvathi		5	1
11	Učka	1264	Syntopic – I. horvathi		3	13
			Syntopic – P. muralis		2	1
12	Vaganski vrh	1653	Allotopic – I. horvathi		10	9
13	Žurgarska stena*	890	Allotopic – I. horvathi		1	6
14	Planinska jama ¹	475	Syntopic – I. horvathi		1	6
			Syntopic – P. muralis		0	0
15	Orlovica	1270	Allotopic – I. horvathi		2	14
16	Donačka gora	835	Allotopic – P. muralis		2	1

alpine climatic influence from the north and influence of the Adriatic Sea from the southwest (Puncer, 1980; Perko & Orožen, 1998).

Sampled populations were distributed across an altitudinal span of approximately 1800 m (table 1). Each population was classified according to the presence of one or both species: (a) allotopic *I. horvathi*, (b) allotopic *P. muralis* or (c) syntopic (table 1, supplementary table S2). Synopic populations have similar abundances of both species at all sampled sites (Žagar A., pers. obs.). To test the effect of urbanization of the study site, we assigned selected sites of *P. muralis* to two categories: (a) urban and (b) natural (table 1, supplementary table S2). This factor could only be studied in *P. muralis*, because *I. horvathi* was not sampled in urbanized areas, but only in the natural environment in our study area.

Fieldwork photography and asymmetry methods

Lizards were collected using a noose in three years between 2013 and, 2015 (table 1), measured and photographed in the field and then released at the capture location. We collected four morphometric measurements of lizard heads: pileus length, head width, length and height. Measurements were always obtained by the same person (AZ) using digital calipers to the closest 0.01 mm. We photographed the dorsal side of the head of lizards in natural light with a Nikon Coolpix camera set to the macro function. In the process of photography, the camera was mounted with the lens directed downwards to the horizontal surface, where lizard was placed in the center of the optical field to take photographs at a fixed distance (20 cm) from the individual. Using the high-resolution photos and by inspecting photographs on the computer screen, we obtained a meristic trait – the number of supraciliar granules (SCGN). SCGN are located right above the eyes, below the supraocular scales; they have no evident function (Lazić et al., 2013). The same person (AA) counted the number of SCGN twice, each time in a different order with several days of rest between the first and second counting, to ensure independence of the trait counts.

Landmark-based methods of geometric morphometrics were used to analyze the variation in head size and shape (Bookstein, 1997). High-resolution photos of the dorsal side of the head were used to digitize 28 landmarks using tpsDig2 (Rohlf, 2005). Landmarks were set at the contact of big scales on lizards' heads (see Lazić et al., 2015 for landmark locations), which have a close relationship with the head bones underneath and develop according to them. Therefore, their contacts ensure a permanent position and are suitable as landmark locations. Landmarks were recorded twice by the same person (AA) in order to assess measurement error arising during digitization.

Data analysis

To test for the presence of directional asymmetry (DA) and/or FA in SCGN we used the two-way ANOVA model on log-transformed values with side as a fixed factor and individual as a random factor, as well as their interaction. In this ANOVA design, a significant effect of side alone would indicate the presence of DA and a significant interaction between side and individual would point to the existence of FA. As our results indicated the existence of FA (see *Results*), we calculated an individual asymmetry index (AI) for each trait as the unsigned right-left (R-L) difference between the log-transformed average of trait values across the two replicate counts of each individual, to account for measurement error. The values of AI did not deviate significantly from normality within each population for the studied trait (Kolmogorov-Smirnov test, P > 0.05 in all cases).

To test for the presence of FA and/or DA in lizard head shape, we used an approach similar to the one used for SCGN. Procrustes ANOVA was performed on replicated landmark configurations with individual, side and their interaction as factors. As our results showed significant individual-side interaction and thereby

indicated the presence of FA, we calculated an AI for each individual. To calculate the AI in head shape, we first calculated the difference in landmark coordinates between the left and the right side of the head by comparing original landmark configurations with the mirror-reflected copies. We used only positive original-to-reflected differences, and in cases in which these were negative, we used the reflected-to-original differences, obtaining an unsigned AI for each specimen. This matrix of unsigned asymmetry values was further used to calculate an AI index by taking the square root of sums of squared differences from all configurations of landmarks (Lazić et al., 2015).

To test for differences in the head shape asymmetry across species, sexes and while incorporating other environmental factors (altitude, presence of other species, and urbanization), we performed permutation ANOVA with 10,000 repetitions using the 'adonis' function from the *vegan* package (Oksanen et al., 2010). To test for differences in asymmetries of SCGN, head size and head shape across altitudes, allotopic/syntopic populations or urban/natural populations, we used a factorial ANOVA design including categorical factors: species, sex, and population or urbanization, and a continuous factor: altitude. For pairwise post-hoc comparisons we used the Bonferroni test. We performed an additional set of analysis only on a subsample of populations to reduce the effects of environment (altitude and geographical differences of distant locations, as well as phylogenetical differences). We used six locations from close geographical proximity (region Kočevsko, south Slovenia), at similar altitudes (700-1050 m a.s.l., specified in table 2), where we already know that individuals share common mitochondrial and nuclear haplotypes (Salvi et al., 2013; Cocca et al., 2021).

Results

We used photographs of adults of both species from 16 populations (fig. 1, table 1). In total, 436 individuals (200 of *P. muralis* and 236 of *I. horvathi*) were sampled.

Visual representation of the variation in head shape showed that most of the variation was concentrated at the base of the head in both species. Two-way ANOVA on the number of supraciliar granules (SCGN) revealed statistically significant individual variation (supplementary table S1). DA was not detected (insignificant side effect, supplementary table S1), while the presence of FA was confirmed, as indicated by the significant individual-side interaction (supplementary table S1). Similarly, Procrustes ANOVA on replicate landmark recordings revealed significant individual variation in head shape, DA and FA (supplementary table S1).

The results from the entire sample set showed statistically significant differences in head size across species and sexes (species-sex factor interaction, table 2). Males had bigger heads than females, but the difference in the head size between sexes was more pronounced in *P. muralis* (fig. 2A) than *I. horvathi* (fig. 2B); *P. muralis* males had significantly bigger heads than females. Head size also differed depending on the presence of other species (factor population, table 2), with individuals

Table 2.

Head size Head shape asymmetry SCGN df F F F df df р р р Altitude 1 7.99 0.004 1 0.14 0.696 1 1.51 0.205 1.58 0.217 Species 1 36.66 0.001 1 3.79 0.060 1 Sex 1 144.54 0.001 1.70 0.216 0.06 0.804 1 1 Population 46.00 0.001 2.85 4.63 0.030 1 1 0.110 1 1 5.71 0.019 8.91 0.007 0.01 0.906 Altitude: species 1 1 2.93 Altitude: sex 1 20.32 0.001 1 0.094 1 0.03 0.879 1 7.64 0.006 0.58 0.477 0.02 0.890 Species: sex 1 1 Altitude: population 1 1.79 0.189 1.14 0.271 0.04 0.815 1 1 Species: population 1 1.32 0.264 0.43 0.491 0.74 0.392 1 1 Sex: population 1 0.20 0.660 1 1.32 0.227 1 0.24 0.646 Altitude: species: sex 1 9.07 0.004 1 0.06 0.793 1 1.17 0.216 Altitude: species: population 1 54.34 0.001 1 12.22 0.004 1 0.45 0.454 Species: sex: population 1 0.21 0.658 1 0.29 0.578 1 0.01 0.925 319 Residuals 319 212 Total 334 334 225

Results from ANOVA comparing head size, head shape asymmetry and asymmetry of the number of supraciliar granules across species, sex, altitude and populations (allotopic/syntopic) from all sampled populations. Significant effects are marked in bold.

Abbreviations: df, degrees of freedom; F, F-test value; p, p-value; SCGN, the number of supraciliar granules.

from allotopic populations having a larger head size compared to syntopic populations in both species (fig. 2C). We also found significant effects on the head size with altitude but in combination with other factors (see altitude factor interactions, table 2), thus, head size also varied in a complex manner across altitude. Males but not females exhibited a linear negative trend of head size with altitude (fig. 3A, B), while on the species level we did not observe a linear relationship (fig. 3C, D). When we limited analysis to the six populations with similar altitudes and close geographical proximity we again observed statistically significant differences in head size between the sexes, species, sex-species interaction and presence of other species (table 3).

Head shape asymmetry differed between species; *I. horvathi* individuals had a more asymmetric head shape (table 1); however, it also depended on altitude of the locality (the interaction between species and altitude was statistically significant, table 2). Linear relationships revealed that the head shape asymmetry increased with altitude in *P. muralis* (fig. 4A), but not *I. horvathi*, where the head asymmetry was most pronounced at mid altitudes (fig. 4B). Presence of other species showed a significant effect on the head shape asymmetry only in combination with the factors altitude and species (table 2), while in the case of analysis limited to six populations, the head shape asymmetry only differed between species; *I. horvathi* had more asymmetrical heads compared to *P. muralis* (table 3).



Figure 2. Head centroid size between sexes of *Podarcis muralis* (A) and *Iberolacerta horvathi* (B), and between allotropic and syntopic populations of both species (C). F = female, M = male.

In the case of SCGN asymmetry we found a significant difference between allotopic and syntopic populations (table 2, fig. 5), which was also confirmed in the case of analysis limited to six populations (table 3). No differences



Figure 3. Head centroid size across altitude in females (A) and males (B), and in *Podarcis muralis* (C) and *Iberolacerta horvathi* (D).

Table 3.

		Head si	ze	He	Head shape asymmetry			SCGN		
	df	F	р	df	F	р	df	F	р	
Population	1	23.80	<0.001	1	< 0.01	0.960	1	3.84	0.046	
Species	1	24.33	< 0.001	1	52.04	0.020	1	3.51	0.070	
Sex	1	128.46	< 0.001	1	0.83	0.381	1	0.03	0.870	
Population: species	1	0.67	0.417	1	0.72	0.360	1	0.14	0.715	
Population: sex	1	0.06	0.792	1	< 0.01	0.979	1	1.09	0.290	
Species: sex	1	11.58	< 0.01	1	18.86	0.184	1	0.10	0.737	
Population: species: sex	1	< 0.01	0.739	1	0.75	0.356	1	0.01	0.745	
Residuals	269			269			218			
Total	276			276			225			

Results from ANOVA comparing head size, head shape asymmetry and the number of supraciliar granules asymmetry across species, sex, and populations (allotopic/syntopic) in genetically similar populations from south Slovenia. Significant effects are marked in bold.

Abbreviations: df, degrees of freedom; F, F-test value; p, p-value; SCGN, the number of supraciliar granules.

were found in SCGN asymmetry between species or sexes and across altitude (table 2).

Podarcis muralis males had larger heads than females in natural and urban populations, but we did not detect any effect of urbanization on head size, head shape asymmetry or SCGN asymmetry (table 4).



Figure 4. Levels of head shape asymmetry across altitude in *Podarcis muralis* (A) and *Iberolacerta horvathi* (B).



Figure 5. Asymmetry in SCGN across species and populations. IHOR = *Iberolacerta horvathi*, PMUR = *Podarcis muralis*.

Discussion

The interplay of various stressors can interfere with the process of an organism's development and thus lead to various phenotypic changes. These changes can be

Table 4.

Results from ANOVA comparing head size, head shape asymmetry and the number of supraciliar granules asymmetry between sexes and between natural and urban sites (urbanization). Df = degrees of freedom, F = F-test value, P = p-value. Significant effects are marked in bold.

		Head siz	æ		Head shap	e		SCGN			
	df	F	р	df	F	р	df	F	р		
Urbanization	1	1.71	0.166	1	0.29	0.654	1	0.41	0.560		
Sex	1	119.32	<0.001	1	0.24	0.702	1	0.17	0.676		
Urbanization: sex	1	0.96	0.35	1	199.58	0.168	1	0.05	0.796		
Residuals	129			129			98				
Total	132			132			101				

Abbreviations: df, degrees of freedom; F, F-test value; p, p-value; SCGN, the number of supraciliar granules.

detrimental to the organism, if they affect survival. Therefore, it is important to detect stress before changes in the environment lead to population extinction. One possible way of detecting early signs of stress is by using asymmetries of various morphological features. In this study we tested three stress effects, originating from biotic interactions (presence of competitor species) or human influence (urbanization), and the environment (colder conditions for ectotherms at higher altitudes). Two of these factors were found to affect levels of head shape asymmetry and one factor affected the scale asymmetry.

Firstly, our results showed considerable differences in head size between species and between sexes. *P. muralis* individuals had larger heads, which corresponds with previously known behavioral differences between species that showed that *P. muralis* is more dominant in the case of agonistic interactions (Žagar et al., 2015a; Žagar et al., 2017). Sexual dimorphism in head size is pronounced in many lizards, which has been attributed to sexual selection for stronger and more aggressive males (Kaliontzopoulou et al., 2007; Žagar et al., 2012). Biting is involved in territorial defense and in mating, when males grab females to hold them during copulation (Pianka & Vitt, 2003). Stronger bite force of larger-headed lizards also enables them to ingest harder prey, which can reduce intersex and interspecific competition for the same food sources (Verwaijen et al., 2002). Head size could also be related to the use of shelters (smaller, more flattened heads could allow the use of smaller shelters; Kaliontzopoulou et al., 2010).

Drop of temperature with rising altitude can represent more restricted environmental conditions for ectotherm organisms, and thus we expected that populations from colder higher altitudes will display a higher level of asymmetries than lowland populations in both species, but more pronounced in the generalist species P. muralis, that exhibits a predominantly lowland and middle altitude distribution and is known to be less adapted to cold conditions (i.e., has a physiology that is less adapted to shorter activity periods compared to I. horvathi, Žagar et al., 2015b). Indeed, altitude had a significant effect, but in a complex manner in combination with the biotic factor (presence of other species), and species. We confirmed our expectations:we found that P. muralis lizards had more asymmetric heads at higher altitudes, but I. horvathi lizards did not. A cold environment may be more stressful to the more generalist species *P. muralis*, which might experience environmental stress at higher altitudes due to its physiology being adapted to warmer conditions where activity periods are longer (Žagar et al., 2015b). On the other hand, the highaltitude specialist *I. horvathi* did not exhibit higher levels of head shape asymmetry at higher altitudes, thus confirming its adaptation to a colder environment that actually represents its optimal habitat in the study area (Žagar, 2016).

However, we detected a mid-altitude effect on asymmetries in *I. horvathi*. The highest levels of head asymmetry at mid altitude in *I. horvathi* might be related to the lower availability of favorable habitats for lizards. Namely, the lowest proportion of open areas with rock walls is found at mid altitudes compared to higher altitudes, as well as the lowest altitudes, where the species occurs only at the

entrances of karstic caves with vertical walls (Žagar, 2016). Mid altitudes also represent the area of highest overlap among the two species, as *P. muralis* is more abundant at lower and middle altitudes and *I. horvathi* is a mountain species with highest abundances at higher altitudes (Sillero et al., 2014; Žagar, 2016). The higher abundance of *P. muralis* together with limited stone wall open areas at mid altitude may also induce higher competition between the two species. And it is known that under these conditions (where sun-exposed areas as resources for basking are limited) *P. muralis* has a competitive advantage over *I. horvathi* (Žagar et al., 2015a). The higher interspecific competition for sun-exposed areas that may occur here could result in greater stress to *I. horvathi*, which possibly contributes to the higher levels of asymmetry found with our results. We assume it is also likely that other environmental factors (i.e., habitat structures used for hiding places, food availability, parasites, etc.) besides temperature are responsible for such a result, where we would need a more controlled set of populations to study the effects exempt of different potential effects that are present in natural populations.

Next, when we compared allotopic and syntopic populations on the subset of populations (to exclude the factor altitude and avoid potential effects of phylogenetically distant populations), we obtained variable results for head shape, head asymmetry and scale asymmetry. We first found that in both species the head size was affected by the presence of competitor species; lizards had larger heads when one species occurred alone (allotopy). This suggests that in areas without interspecific competitor, species may invest more energy in growth. The presence of an interspecific competitor can result in a higher resource competition (Begon et al., 2006) and can have a stressful effect on both species, which could result in smaller heads. We also observed a pronounced sex difference with males having bigger heads that females (following earlier obtained results on this species, Žagar et al., 2012). Bigger heads may be more pronounced in allotopy due to the fact that sexual selection might be stronger in allotopic populations. It is known that higher conspecific competition drives the larger size of heads, which correlates with stronger bite forces and dominance in male-male combats for females (Žagar et al., 2017).

Next, we found that in *I. horvathi* head shape was more asymmetric compared to *P. muralis*, which suggests that *I. horvathi* could be under greater stress or be more susceptible to stress effects than *P. muralis*. Such an interspecific difference suggests that the ability to buffer environmental disturbance may differ between species, and it may be connected with a more generalist character of *P. muralis*, which is very adaptable and tolerates more anthropogenic influence than most other European lizards (Arnold and Oveden, 2004). The results obtained are also interesting, because these could have an indirect effect on species' competitive interaction via the 'apparent competition' mechanism (Holt, 1977). The studied species are in fact known to potentially compete under syntopic conditions and limited microhabitat conditions connected with thermoregulation (Žagar et al., 2015a). However, in the case of SCGN asymmetries, we found – quite contrary to our expectations that competition could increase stabilizing selection – that animals tend to be more

symmetric in the presence of the other species. However, many other factors may also affect stabilizing selection, while our predictions were set to this expectation due to our study aim and sampling in syntopic and allotopic populations. Possible explanations for this finding in the light of the effects of competition might be that individuals that suffer more from interspecific competition may have a higher mortality and less reproductive success and thus lower adaptive value. Therefore, such individuals do not succeed in the population and are consequently more rare. This was also explained in some other studies (Thornhill, 1992; Møller et al., 1998; Tomkins & Kotiaho, 2001) and may also provide the explanation for why we did not detect them in our populations. Another explanation may also lie in the fact that conspecific competition exhibits higher stress pressure than interspecific competition, which is reflected in higher asymmetry in allotopic populations. We also acknowledge that this result could be due to imbalance of the sample, which is in favor of allotopic populations. Also, SCGN - as far as we are aware - have no evident functional significance, meaning that selection for DS in this character may be relaxed.

Finally, we tested if populations of *P. muralis* from urbanized (hence degraded and possibly polluted) sites show higher FA levels compared to natural habitat sites. Results provided no evidence that the urbanization influences DS of morphological traits in *P. muralis* in our study area. On one hand, this could mean that the two populations classified as urbanized in our study (railway tracks in the city of Ljubljana and the cemetery in the village of Fara) are not degraded or polluted to the level that it would disrupt developmental homeostasis and affect the developmental path of this species. To confirm this, comparison with populations from more degraded or polluted habitats would be required. To increase the detection of FA, it would also be useful to analyze a larger spectrum of physical characteristics, including already established features for measuring FA (e.g., length of hind legs, number of femoral pores, border points on lateral side of head, etc.; López & Martín, 2002; Crnobrnja-Isailović et al., 2005; Martín & López, 2006; Kaliontzopoulou et al., 2007; Lazić et al., 2013).

In conclusion, our study presents novel data on the effects of biotic stressors, such as interspecific competition, on the DS in lizards. The effects were found on the level of head shape asymmetry, but not in the scale asymmetries. Since the studied species differ in altitudinal preferences, we managed to understand the combination of effects of potential competition and environmental conditions that change across the altitude. These results emphasize the need to include variable relevant effects (biotic and abiotic) when trying to understand the occurrence of asymmetries as bioindicators of stress. We clearly highlighted the possibility of using lizards and FA for bioindication of environmental stressors.

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Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.15657840

References

- Amaral, M.J., Bicho, R.C., Carretero, M.A., Sanchez-Hernandez, J.C., Faustino, A.M.R., Soares, A.M.V.M. & Mann, R.M. (2012) The use of a lacertid lizard as a model for reptile ecotoxicology studies: part 2 – biomarkers of exposure and toxicity among pesticide exposed lizards. *Chemo-sphere*, 87, 765-774. DOI:doi.org/10.1016/j.chemosphere.2012.01.048.
- Arnold, E.N. & Ovenden, D. (2004) A Field Guide to the Reptiles and Amphibians of Britain and Europe. Collins, London, UK.
- Bartell, S.M. (2006) Biomarkers, bioindicators, and ecological risk assessment a brief review and evaluation. *Environ. Bioindic.*, 1, 60-73. DOI:doi.org/10.1080/15555270591004920.
- Beasley, D.A.E., Bonisoli-Alquati, A. & Mousseau, T.A. (2013) The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. *Ecol. Indic.*, 30, 218-226. DOI:doi.org/10.1016/j.ecolind.2013.02.024.
- Begon, M., Townsend, C.R. & Harper, J.L. (2006) *Ecology: from Individuals to Ecosystems*. 4th Edition. Blackwell Publishing, Malden, MA, USA.
- Bonsall, M.B. & Hassell, M.P. (1997) Apparent competition structures ecological assemblages. *Nature*, 388, 371-373. DOI:doi.org/10.1038/41084.
- Bookstein, F.L. (1997) *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, Cambridge, UK.
- Braña, F. & Ji, X. (2000) Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). J. Exp. Biol., 286, 422-433. DOI:doi. org/10.1002/(SICI)1097-010X(20000301)286:4<422::AID-JEZ10>3.0.CO;2-D.
- Burger, J. (2006) Bioindicators: types, development, and use in ecological assessment and research. *Environ. Bioindic.*, 1, 22-39. DOI:doi.org/10.1080/15555270590966483.
- Clarke, G.M. (1993) Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. *Environ. Pollut.*, 82, 207-211. DOI:doi.org/10.1016/0269-7491(93) 90119-9.
- Clarke, G.M. (1995) Relationships between developmental stability and fitness: application for conservation biology. *Conserv. Biol.*, 9, 18-24. DOI:doi.org/10.1046/j.1523-1739.1995.09010018.x.

- Clarke, G.M. (1998) The genetic basis of developmental stability. IV. Individual and population asymmetry parameters. *Heredity*, 80, 553-561. DOI:doi.org/10.1046/j.1365-2540.1998.00326.x.
- Cocca, W., Žagar, A., Sillero, N., Jowers, M.J., Krofel, M., Lužnik, M., Podnar, M., Tvrtković, N., Carretero, M.A. & Crottini, A. (2021) Genetic diversity of Horvath's rock lizard meets current environmental restrictions. *Conserv. Genet.*, 22, 483-498. DOI:doi.org/10.1007/s10592-021-01351-4.
- Cody, M.L. & Diamond, J.M. (1975) Ecology and Evolution of Communities. Harvard University Press, London, UK.
- Crnobrnja-Isailović, J., Aleksić, I. & Bejaković, D. (2005) Fluctuating asymmetry in *Podarcis muralis* populations from southern Montenegro: detection of environmental stress in insular populations. *Amphibia-Reptilia*, 26, 149-158. DOI:10.1163/1568538054253500.
- De Luca, N. (1989) Taxonomic and biogeographic characteristics of Horvath's rock lizard (*Lacerta horvathi* Mehely, 1904, Lacertidae, Reptilia) in Yugoslavia. *Scopolia*, 18, 1-48.
- Garrido, M. & Pérez-Mellado, V. (2014) Assessing factors involved in determining fluctuating asymmetry in four insular populations of the Balearic lizard *Podarcis lilfordi*. Salamandra, 50, 147-154.
- Graham, J.H., Raz, S., Hel-Or, H. & Nevo, E. (2010) Fluctuating asymmetry: methods, theory, and applications. *Symmetry*, 2, 466-540. DOI:doi.org/10.3390/sym2020466.
- Holt, R.D. (1977) Predation, apparent competition, and the structure of prey communities. *Theor. Popul. Biol.*, 12, 197-229. DOI:doi.org/10.1016/0040-5809(77)90042-9.
- Kaliontzopoulou, A., Carretero, M.A. & Llorente, G.A. (2007) Multivariate and geometric morphometrics in the analysis of sexual dimorphism variation in *Podarcis* lizards. J. Morphol., 268, 152-165. DOI:doi.org/10.1002/jmor.10494.
- Kaliontzopoulou, A., Carretero, M.A. & Llorente, G.A. (2010) Intraspecific ecomorphological variation: linear and geometric morphometrics reveal habitat-related patterns within *Podarcis bocagei* wall lizards. *J. Evol. Biol.*, 23, 1234-1244. DOI:doi.org/10.1111/j.1420-9101.2010.01984.x.
- Klingenberg, C.P. (2015) Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. *Symmetry*, 7, 843-934. DOI:doi.org/10.3390/sym7020843.
- Krofel, M., Cafuta, V., Planinc, G., Sopotnik, M., Šalamun, A., Tome, S., Vamberger, M. & Žagar, A. (2009) Razširjenost plazilcev v Sloveniji: pregled podatkov, zbranih do leta 2009. *Nat. Slov.*, 11, 61-99.
- Lazić, M.M., Kaliontzopoulou, A., Carretero, M.A. & Crnobrnja-Isailović, J. (2013) Lizards from urban areas are more asymmetric: using fluctuating asymmetry to evaluate environmental disturbance. *PLoS One*, 8, e84190. DOI:doi.org/10.1371/journal.pone.0084190.
- Lazić, M.M., Carretero, M.A., Crnobrnja-Isailović, J. & Kaliontzopoulou, A. (2015) Effects of environmental disturbance on phenotypic variation: an integrated assessment of canalization, developmental stability, modularity, and allometry in lizard head shape. *Am. Nat.*, 185, 44-58. DOI:10. 1086/679011.
- Leamy, L.J. & Klingenberg, C.P. (2005) The genetics and evolution of fluctuating asymmetry. Annu. Rev. Ecol. Evol. Syst., 36, 1-21. DOI:doi.org/10.1146/annurev.ecolsys.36.102003.152640.
- Leary, R.F. & Allendorf, F.W. (1989) Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends Ecol. Evol.*, 4, 214-217. DOI:doi.org/10.1016/0169-5347(89) 90077-3.
- Lens, L. & Van Dongen, S. (2000) Fluctuating and directional asymmetry in natural bird populations exposed to different levels of habitat disturbance, as revealed by mixture analysis. *Ecol. Lett.*, 3, 516-522. DOI:doi.org/10.1111/j.1461-0248.2000.00181.x.
- Lens, L., van Dongen, S., Wilder, C.M., Brooks, T.M. & Matthysen, E. (1999) Fluctuating asymmetry increases with habitat disturbance in seven bird species of a fragmented afrotropical forest. *Proc. Biol. Sci.*, 266, 1241-1246. DOI:doi.org/10.1098/rspb.1999.0769.

- Leung, B. & Forbes, M.R. (1996) Fluctuating asymmetry in relation to stress and fitness: effects of trait type as revealed by meta-analysis. *Ecoscience*, 3, 400-413. DOI:doi.org/10.1080/11956860. 1996.11682357.
- Leung, B., Forbes, M.R. & Houle, D. (2000) Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. Am. Nat., 155, 101-115. DOI:10.1086/303298.
- López, P. & Martín, J. (2002) Locomotor capacity and dominance in male lizards *Lacerta monticola*: a trade-off between survival and reproductive success? *Biol. J. Lin. Soc.*, 77, 201-209. DOI:doi. org/10.1046/j.1095-8312.2002.00103.x.
- Mallard, S.T. & Barnard, C. (2003) Competition, fluctuating asymmetry and sperm transfer in male gryllid crickets (*Gryllus bimaculatus* and *Gryllodes sigillatus*). *Behav. Ecol. Sociobiol.*, 53, 190-197. DOI:doi.org/10.1007/s00265-002-0537-4.
- Martín, J. & López, P. (2001) Hindlimb asymmetry reduces escape performance in the lizard Psammodromus algirus. Physiol. Biochem. Zool., 74, 619-624. DOI:10.1086/322925.
- Martín, J. & López, P. (2006) Links between male quality, male chemical signals, and female mate choice in Iberian rock lizards. *Funct. Ecol.*, 20, 1087-1096. DOI:doi.org/10.1111/j.1365-2435. 2006.01183.x.
- Møller, A.P. & Swaddle, J.P. (1997) Asymmetry, Developmental Stability and Evolution. Oxford University Press, Oxford, UK.
- Møller, A.P., Milinski, M. & Slater, P.J. (1998) Advances in the Study of Behavior: Stress and Behavior. Academic Press, London, UK.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2010) *vegan: Community Ecology Package*. R package version 1.17-4. Available at http://cran.r-project.org.
- Osojnik, N., Žagar, A., Carretero, M.A., García-Muñoz, E. & Vrezec, A. (2013) Ecophysiological dissimilarities of two sympatric lizards. *Herpetologica*, 69, 445-454. DOI:doi.org/10.1655/ HERPETOLOGICA-D-13-00014.
- Palmer, A.R. & Strobeck, C. (1986) Fluctuating asymmetry: measurement, analysis, patterns. Annu. Rev. Ecol. Syst., 17, 391-421. DOI:doi.org/10.1146/annurev.es.17.110186.002135.
- Perko, D. & Orožen, A.M. (1998) Slovenija: Pokrajine in Ljudje. Mladinska knjiga, Ljubljana, Slovenia.
- Pianka, E.R. & Vitt, L.J. (2003) Lizards: Windows to the Evolution of Diversity. University of California Press, Los Angeles, CA, USA.
- Puncer, I. (1980) Dinarski jelovo bukovi gozdovi na Kočevskem. Razprave 4. razr. SAZU, 22, 407-561.
- Rohlf, F.J. (2005) *tpsDig, Digitize Landmarks and Outlines, Version 2.05*. Department of Ecology and Evolution, State University of New York at Stony Brook, NY, USA.
- Salvi, D., Harris, D.J., Kaliontzopoulou, A., Carretero, M.A. & Pinho, C. (2013) Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard. *BMC Evol. Biol.*, 13, 147. DOI:doi.org/10.1186/1471-2148-13-147.
- Sarre, S. (1996) Habitat fragmentation promotes fluctuating asymmetry but not morphological divergence in two geckos. *Res. Popul. Ecol.*, 38, 57. DOI:doi.org/10.1007/BF02514971.
- Sillero, N., Campos, J., Bonardi, A., Corti, C., Creemers, R., Crochet, P.-A., Crnobrnja Isailović, J., Denoël, M., Ficetola, G.F., Gonçalves, J., Kuzmin, S., Lymberakis, P., de Pous, P., Rodríguez, A., Sindaco, R., Speybroeck, J., Toxopeus, B., Vieites, D.R. & Vences, M. (2014) Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia*, 35, 1-31. DOI:10. 1163/15685381-00002935.
- Smith, R.L. & Smith, T.M. (1998) *Elements of Ecology*. 4th Edition. Benjamin/Cummings Science Publishing, San Francisco, CA, USA.

- Soulé, M. (1967) Phenetics of natural populations. II. Asymmetry and evolution in a lizard. Am. Nat., 101, 141-160.
- Soulé, M. & Yang, S.Y. (1973) Genetic variation in side-blotched lizards on islands in the Gulf of California. *Evolution*, 27, 593-600. DOI:doi.org/10.2307/2407193.
- Thornhill, R. (1992) Fluctuating asymmetry, interspecific aggression and male mating tactics in two species of Japanese scorpionflies. *Behav. Ecol. Sociobiol.*, 30, 357-363. DOI:doi.org/10.1007/ BF00170603.
- Tomkins, J.L. & Kotiaho, J.S. (2001) Fluctuating Asymmetry. Encyclopedia of Life Sciences, pp. 1-5. Macmillan Publishers Ltd, Nature Publishing Group, London. DOI:doi.org/10.1038/npg.els. 0003741.
- Tull, J.C. & Brussard, P.F. (2007) Fluctuating asymmetry as an indicator of environmental stress from off-highway vehicles. J. Wildlife Manag., 71, 1944-1948. DOI:doi.org/10.2193/2006-397.
- Van Valen, L. (1962) A study of fluctuating asymmetry. *Evolution*, 16, 125-142. DOI:doi.org/10.2307/ 2406192.
- Verwaijen, D., Van Damme, R. & Herrel, A. (2002) Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Func. Ecol.*, 16, 842-850. DOI:doi. org/10.1046/j.1365-2435.2002.00696.x.
- Vrezec, A. & Tome, D. (2004) Altitudinal segregation between Ural Owl Strix uralensis and Tawny Owl S. aluco: evidence for competitive exclusion in raptorial birds. Bird Study, 51, 264-269. DOI:doi.org/10.1080/00063650409461362.
- Witter, M.S. & Swaddle, J.P. (1994) Fluctuating asymmetries, competition and dominance. Proc. R. Soc. Lond. B, 256, 299-303. DOI:doi.org/10.1098/rspb.1994.0084.
- Wood, A., Stedman-Edwards, P. & Mang, J. (2000) *The Root Causes of Biodiversity Loss*. Routledge, London, UK.
- Žagar, A. (2016) Altitudinal distribution and habitat use of the common wall lizard *Podarcis muralis* (Linnaeus, 1768) and the Horvath's rock lizard *Iberolacerta horvathi* (Méhely, 1904) in the Kočevsko region (S Slovenia). *Nat. Slov.*, 18, 47-62.
- Žagar, A., Osojnik, N., Carretero, M.A. & Vrezec, A. (2012) Quantifying the intersexual and interspecific morphometric variation in two resembling sympatric lacertids: *Iberolacerta horvathi* and *Podarcis muralis. Acta Herpetol.*, 7, 29-39.
- Žagar, A., Kos, I. & Vrezec, A. (2013) Habitat segregation patterns of reptiles in Northern Dinaric Mountains (Slovenia). *Amphibia-Reptilia*, 34, 263-268. DOI:10.1163/15685381-00002889.
- Žagar, A., Carretero, M.A., Osojnik, N., Sillero, N. & Vrezec, A. (2015a) A place in the sun: interspecific interference affects thermoregulation in coexisting lizards. *Behav. Ecol. Sociobiol.*, 69, 1127-1137. DOI:doi.org/10.1007/s00265-015-1927-8.
- Žagar, A., Simčič, T., Carretero, M.A. & Vrezec, A. (2015b) The role of metabolism in understanding the altitudinal segregation pattern of two potentially interacting lizards. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 179, 1-6. DOI:doi.org/10.1016/j.cbpa.2014.08.018.
- Žagar, A., Carretero, M.A., Vrezec, A., Drašler, K. & Kaliontzopoulou, A. (2017) Towards a functional understanding of species coexistence: ecomorphological variation in relation to wholeorganism performance in two sympatric lizards. *Funct. Ecol.*, 31, 1780-1791. DOI:doi.org/10. 1111/1365-2435.12878.

Animal Biology

Effects of biotic and abiotic stressors on asymmetries and head size in two sympatric lizard species

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Supplementary material

Table S1.

Results from ANOVA testing for the presence of directional (DA) and fluctuating asymmetries (FA) in the number of supraciliar granules and head shape of lizards from all sampled populations. Significant effects are marked in bold.

	SCC	ìN		Head	shape as	ymmetry
	df	F	р	df	F	р
Individual	327	5.50	<0.001	334	4.91	<0.02
Side	1	3.60	0.58	1	50.12	<0.02
Individual : side	293	8.12	<0.001	334	1.12	<0.02
Measurement error	616			670		

Abbreviations: df, degrees of freedom; *F*, *F*-test value;*p*, *p* value, SCGN, the number of supraciliar granules.

Table S2.

Individual label	Species	Sex	SVL	Population	Image	Individual	Туре
A_IHOR_M_01	IHOR	М	50.3	ALLOTOPIC	1	1	natur
A_IHOR_M_02	IHOR	М	57.4	ALLOTOPIC	1	2	natur
A_IHOR_M_03	IHOR	М	54.2	ALLOTOPIC	1	3	natur
A_IHOR_M_06	IHOR	М	56.6	ALLOTOPIC	1	4	natur
A_IHOR_M_08	IHOR	М	55.8	ALLOTOPIC	1	5	natur
A_IHOR_M_09	IHOR	М	55.2	ALLOTOPIC	1	6	natur
A_IHOR_M_10	IHOR	М	51.1	ALLOTOPIC	1	7	natur
A_IHOR_M_11	IHOR	М	61.1	ALLOTOPIC	1	8	natur
A_IHOR_M_12	IHOR	М	52.9	ALLOTOPIC	1	9	natur
A_IHOR_M_13	IHOR	М	51.2	ALLOTOPIC	1	10	natur
A_IHOR_M_14	IHOR	М	56.6	ALLOTOPIC	1	11	natur
A_IHOR_M_15	IHOR	М	53.2	ALLOTOPIC	1	12	natur
A_IHOR_M_17	IHOR	М	49.7	SYNTOPIC	1	13	natur
A_IHOR_M_18	IHOR	М	53.7	SYNTOPIC	1	14	natur
A_IHOR_M_19	IHOR	М	51.9	SYNTOPIC	1	15	natur
A_IHOR_M_20	IHOR	Μ	49	SYNTOPIC	1	16	natur
A_IHOR_M_21	IHOR	М	52.4	SYNTOPIC	1	17	natur
A_IHOR_M_22	IHOR	Μ	48.8	SYNTOPIC	1	18	natur
A_IHOR_M_23	IHOR	М	51	ALLOTOPIC	1	19	natur
A_IHOR_M_24	IHOR	М	56.6	ALLOTOPIC	1	20	natur
A_IHOR_M_26	IHOR	М	55	ALLOTOPIC	1	21	natur

Background dataset used in the analysis.

A_IHOR_M_27	IHOR	М	51.5	ALLOTOPIC	1	22	natur
A_IHOR_M_28	IHOR	М	48.5	ALLOTOPIC	1	23	natur
A_IHOR_M_29	IHOR	М	58.2	ALLOTOPIC	1	24	natur
A_IHOR_M_30	IHOR	Μ	59.8	ALLOTOPIC	1	25	natur
A_IHOR_M_31	IHOR	Μ	53.5	SYNTOPIC	1	26	natur
A_IHOR_M_32	IHOR	Μ	49.7	SYNTOPIC	1	27	natur
A_IHOR_M_33	IHOR	Μ	53.2	ALLOTOPIC	1	28	natur
A_IHOR_M_35	IHOR	Μ	60.4	ALLOTOPIC	1	29	natur
A_PMUR_M_02	PMUR	М	47.9	SYNTOPIC	1	30	natur
A_PMUR_M_03	PMUR	М	51.1	ALLOTOPIC	1	31	urban
A_PMUR_M_05	PMUR	М	49	ALLOTOPIC	1	32	urban
A_PMUR_M_06	PMUR	М	56.5	ALLOTOPIC	1	33	urban
A_PMUR_M_07	PMUR	М	54.9	ALLOTOPIC	1	34	urban
A_PMUR_M_13	PMUR	М	56.8	ALLOTOPIC	1	35	natur
A_PMUR_M_14	PMUR	М	62	ALLOTOPIC	1	36	natur
A_PMUR_M_16	PMUR	М	52.2	ALLOTOPIC	1	37	natur
A_PMUR_M_19	PMUR	М	59.4	ALLOTOPIC	1	38	natur
A_PMUR_M_22	PMUR	М	61.6	ALLOTOPIC	1	39	natur
A_PMUR_M_23	PMUR	М	62.4	ALLOTOPIC	1	40	natur
A_PMUR_M_24	PMUR	М	56	ALLOTOPIC	1	41	natur
A_PMUR_M_25	PMUR	М	50.8	SYNTOPIC	1	42	natur
A_PMUR_M_26	PMUR	М	55.1	SYNTOPIC	1	43	natur
A_PMUR_M_27	PMUR	Μ	47.5	SYNTOPIC	1	44	natur
A_PMUR_M_29	PMUR	Μ	55.6	ALLOTOPIC	1	45	natur

A_PMUR_M_30	PMUR	М	55.2	ALLOTOPIC	1	46	natur
A_PMUR_M_31	PMUR	М	50.7	ALLOTOPIC	1	47	natur
C_IHOR_M_01	IHOR	М	47.2	ALLOTOPIC	1	48	natur
C_IHOR_M_02	IHOR	Μ	58.3	ALLOTOPIC	1	49	natur
C_IHOR_M_03	IHOR	Μ	58.4	ALLOTOPIC	1	50	natur
C_IHOR_M_04	IHOR	Μ	49.1	ALLOTOPIC	1	51	natur
C_IHOR_M_05	IHOR	Μ	47.4	ALLOTOPIC	1	52	natur
C_IHOR_M_07	IHOR	Μ	52.6	ALLOTOPIC	1	53	natur
C_IHOR_M_09	IHOR	Μ	54.2	ALLOTOPIC	1	54	natur
C_IHOR_M_10	IHOR	Μ	48.8	SYNTOPIC	1	55	natur
C_IHOR_M_11	IHOR	Μ	51	ALLOTOPIC	1	56	natur
C_IHOR_M_13	IHOR	М	57.1	ALLOTOPIC	1	57	natur
C_IHOR_M_14	IHOR	Μ	53.9	ALLOTOPIC	1	58	natur
C_PMUR_M_02	PMUR	М	59.1	ALLOTOPIC	1	59	natur
C_PMUR_M_03	PMUR	М	55.4	ALLOTOPIC	1	60	natur
C_PMUR_M_05	PMUR	Μ	53.4	ALLOTOPIC	1	61	natur
C_PMUR_M_09	PMUR	М	57.4	ALLOTOPIC	1	62	natur
C_PMUR_M_10	PMUR	М	59.8	ALLOTOPIC	1	63	natur
C_PMUR_M_11	PMUR	Μ	51.1	ALLOTOPIC	1	64	natur
IHOR-01	IHOR	Μ	52.3	SYNTOPIC	1	65	natur
IHOR-03	IHOR	М	51	ALLOTOPIC	1	66	natur
IHOR-05	IHOR	М	49.8	SYNTOPIC	1	67	natur
IHOR-06	IHOR	М	46.9	SYNTOPIC	1	68	natur
IHOR-07	IHOR	М	53	SYNTOPIC	1	69	natur

M_IHOR_F_04	IHOR	F	62.2	ALLOTOPIC	1	74	natur
M_IHOR_F_05	IHOR	F	59	ALLOTOPIC	1	75	natur
M_IHOR_F_07	IHOR	F	61.6	ALLOTOPIC	1	76	natur
M_IHOR_F_08	IHOR	F	61.3	ALLOTOPIC	1	77	natur
M_IHOR_F_10	IHOR	F	61.1	ALLOTOPIC	1	78	natur
M_IHOR_F_12	IHOR	F	55.1	ALLOTOPIC	1	79	natur
M_IHOR_F_24	IHOR	F	60.7	ALLOTOPIC	1	81	natur
M_IHOR_F_25	IHOR	F	55.3	ALLOTOPIC	1	82	natur
M_IHOR_F_26	IHOR	F	48.9	ALLOTOPIC	1	83	natur
M_IHOR_F_27	IHOR	F	56.5	ALLOTOPIC	1	84	natur
M_IHOR_F_28	IHOR	F	51.4	ALLOTOPIC	1	85	natur
M_IHOR_F_29	IHOR	F	56.3	ALLOTOPIC	1	86	natur
M_IHOR_M_23	IHOR	М	48.8	ALLOTOPIC	1	108	natur
M_IHOR_M_26	IHOR	М	53.3	ALLOTOPIC	1	110	natur
M_IHOR_M_29	IHOR	Μ	54.6	ALLOTOPIC	1	111	natur
M_PMUR_F_02	PMUR	F	51.7	SYNTOPIC	1	113	natur
M_PMUR_F_08	PMUR	F	62	ALLOTOPIC	1	119	natur
M_PMUR_F_09	PMUR	F	61.9	ALLOTOPIC	1	120	natur
M_PMUR_F_10	PMUR	F	68	ALLOTOPIC	1	121	natur
M_PMUR_F_11	PMUR	F	58	ALLOTOPIC	1	122	natur
M_PMUR_F_12	PMUR	F	56.6	ALLOTOPIC	1	123	natur
M_PMUR_F_13	PMUR	F	68.5	ALLOTOPIC	1	124	natur
M_PMUR_F_14	PMUR	F	59.2	ALLOTOPIC	1	125	natur
M_PMUR_F_15	PMUR	F	60.5	ALLOTOPIC	1	126	natur

M_PMUR_F_16	PMUR	F	59.9	ALLOTOPIC	1	127	natur
M_PMUR_F_17	PMUR	F	61	ALLOTOPIC	1	128	natur
M_PMUR_F_18	PMUR	F	59.4	ALLOTOPIC	1	129	natur
M_PMUR_F_19	PMUR	F	58	ALLOTOPIC	1	130	natur
M_PMUR_F_20	PMUR	F	50.2	SYNTOPIC	1	131	natur
M_PMUR_F_21	PMUR	F	52.1	SYNTOPIC	1	132	natur
M_PMUR_F_25	PMUR	F	55.6	ALLOTOPIC	1	133	natur
M_PMUR_F_29	PMUR	F	54	ALLOTOPIC	1	134	natur
W_IHOR_F_01	IHOR	F	56.4	SYNTOPIC	1	135	natur
W_IHOR_F_03	IHOR	F	62	ALLOTOPIC	1	136	natur
W_IHOR_F_04	IHOR	F	58.3	ALLOTOPIC	1	137	natur
W_IHOR_F_05	IHOR	F	55.7	ALLOTOPIC	1	138	natur
W_IHOR_F_06	IHOR	F	55.6	ALLOTOPIC	1	139	natur
W_IHOR_F_07	IHOR	F	66.7	ALLOTOPIC	1	140	natur
W_IHOR_F_08	IHOR	F	54.9	ALLOTOPIC	1	141	natur
W_IHOR_F_09	IHOR	F	54.1	ALLOTOPIC	1	142	natur
W_IHOR_F_10	IHOR	F	59.3	ALLOTOPIC	1	143	natur
W_IHOR_F_11	IHOR	F	59.9	ALLOTOPIC	1	144	natur
W_IHOR_F_13	IHOR	F	61.4	ALLOTOPIC	1	145	natur
W_IHOR_F_14	IHOR	F	50.5	ALLOTOPIC	1	146	natur
W_IHOR_M_01	IHOR	Μ	50.6	SYNTOPIC	1	147	natur
W_IHOR_M_04	IHOR	Μ	55.3	ALLOTOPIC	1	149	natur
W_IHOR_M_07	IHOR	М	53.6	ALLOTOPIC	1	152	natur
W_IHOR_M_09	IHOR	Μ	46.9	ALLOTOPIC	1	153	natur

W_IHOR_M_11	IHOR	М	56.1	ALLOTOPIC	1	154	natur
W_IHOR_M_12	IHOR	М	47.5	ALLOTOPIC	1	155	natur
W_PMUR_F_02	PMUR	F	57.6	SYNTOPIC	1	156	natur
W_PMUR_F_03	PMUR	F	54.7	ALLOTOPIC	1	157	natur
W_PMUR_F_04	PMUR	F	58.6	ALLOTOPIC	1	158	natur
W_PMUR_F_05	PMUR	F	51.8	ALLOTOPIC	1	159	natur
W_PMUR_F_06	PMUR	F	52.8	ALLOTOPIC	1	160	natur
W_PMUR_F_07	PMUR	F	60.9	ALLOTOPIC	1	161	natur
W_PMUR_F_08	PMUR	F	56.9	ALLOTOPIC	1	162	natur
W_PMUR_F_10	PMUR	F	54.7	ALLOTOPIC	1	163	natur
W_PMUR_F_11	PMUR	F	51.1	ALLOTOPIC	1	164	natur
W_PMUR_F_12	PMUR	F	59.3	ALLOTOPIC	1	165	natur
W_PMUR_F_13	PMUR	F	48.8	ALLOTOPIC	1	166	natur
W_PMUR_F_14	PMUR	F	50.7	ALLOTOPIC	1	167	natur
W_PMUR_F_15	PMUR	F	50	ALLOTOPIC	1	168	natur
W_PMUR_F_16	PMUR	F	59.1	ALLOTOPIC	1	169	natur
W_PMUR_M_01	PMUR	Μ	59.2	SYNTOPIC	1	170	natur
W_PMUR_M_02	PMUR	Μ	49.5	SYNTOPIC	1	171	natur
W_PMUR_M_04	PMUR	Μ	56.5	ALLOTOPIC	1	172	natur
W_PMUR_M_06	PMUR	Μ	47.2	ALLOTOPIC	1	174	natur
1	PMUR	Μ	52.3	ALLOTOPIC	1	175	natur
10	IHOR	М	59	ALLOTOPIC	1	176	natur
11	IHOR	М	53.8	ALLOTOPIC	1	177	natur
12	PMUR	М	58.2	ALLOTOPIC	1	178	natur

13	PMUR	М	54	ALLOTOPIC	1	179	natur
14	PMUR	Μ	50	ALLOTOPIC	1	180	natur
15	PMUR	Μ	60.1	ALLOTOPIC	1	181	natur
16	PMUR	М	51.9	ALLOTOPIC	1	182	natur
17	PMUR	Μ	58.1	ALLOTOPIC	1	183	natur
18	PMUR	М	52.3	ALLOTOPIC	1	184	natur
19	PMUR	Μ	56.1	ALLOTOPIC	1	185	natur
2	IHOR	Μ	54.7	ALLOTOPIC	1	186	natur
20	IHOR	F	60.4	ALLOTOPIC	1	187	natur
21	IHOR	F	58.9	ALLOTOPIC	1	188	natur
24	IHOR	F	66.1	ALLOTOPIC	1	189	natur
25	PMUR	М	57.6	ALLOTOPIC	1	190	natur
26	IHOR	F	57.6	ALLOTOPIC	1	191	natur
27	PMUR	М	59.5	ALLOTOPIC	1	192	natur
28	IHOR	F	58.5	ALLOTOPIC	1	193	natur
3	IHOR	М	51.8	ALLOTOPIC	1	194	natur
30	IHOR	F	61.7	ALLOTOPIC	1	195	natur
31	IHOR	F	51.1	ALLOTOPIC	1	196	natur
32	IHOR	F	59.3	ALLOTOPIC	1	197	natur
33	IHOR	F	64	ALLOTOPIC	1	198	natur
35	IHOR	F	66.1	ALLOTOPIC	1	199	natur
38	PMUR	М	52.9	ALLOTOPIC	1	200	natur
39	IHOR	F	61.3	ALLOTOPIC	1	201	natur
4	IHOR	М	54.6	ALLOTOPIC	1	202	natur

40	PMUR	М	60.8	ALLOTOPIC	1	203	natur
41	PMUR	Μ	57.4	ALLOTOPIC	1	204	natur
42	IHOR	F	61.3	ALLOTOPIC	1	205	natur
43	PMUR	М	48.1	ALLOTOPIC	1	206	natur
44	IHOR	F	57.4	ALLOTOPIC	1	207	natur
47	PMUR	F	56.7	ALLOTOPIC	1	208	natur
48	PMUR	F	63.6	ALLOTOPIC	1	209	natur
49	PMUR	F	61.6	ALLOTOPIC	1	210	natur
5	IHOR	Μ	53.9	ALLOTOPIC	1	211	natur
50	PMUR	F	55.1	ALLOTOPIC	1	212	natur
51	PMUR	F	60.2	ALLOTOPIC	1	213	natur
52	IHOR	F	58	ALLOTOPIC	1	214	natur
53	IHOR	F	60.3	ALLOTOPIC	1	215	natur
55	PMUR	F	60.2	ALLOTOPIC	1	216	natur
56	PMUR	F	60.8	ALLOTOPIC	1	217	natur
57	IHOR	F	61.4	ALLOTOPIC	1	218	natur
58	IHOR	F	54.6	ALLOTOPIC	1	219	natur
59	PMUR	F	59.9	ALLOTOPIC	1	220	natur
6	IHOR	Μ	53.9	ALLOTOPIC	1	221	natur
60	PMUR	F	57.8	ALLOTOPIC	1	222	natur
61	PMUR	F	61.1	ALLOTOPIC	1	223	natur
62	PMUR	F	57.3	ALLOTOPIC	1	224	natur
63	PMUR	F	60.8	ALLOTOPIC	1	225	natur
64	PMUR	F	58.3	ALLOTOPIC	1	226	natur

65	IHOR	F	56	ALLOTOPIC	1	227	natur
66	PMUR	F	60	ALLOTOPIC	1	228	natur
69	PMUR	Μ	54.9	ALLOTOPIC	1	229	natur
7	IHOR	Μ	52.1	ALLOTOPIC	1	230	natur
70	PMUR	F	62.4	ALLOTOPIC	1	231	natur
71	PMUR	F	56.3	ALLOTOPIC	1	232	natur
73	PMUR	F	54.9	ALLOTOPIC	1	233	natur
74	PMUR	F	58.6	ALLOTOPIC	1	234	natur
75	PMUR	F	60.8	ALLOTOPIC	1	235	natur
78	PMUR	Μ	58.9	ALLOTOPIC	1	236	natur
79	PMUR	Μ	57.4	ALLOTOPIC	1	237	natur
81	IHOR	Μ	58.4	ALLOTOPIC	1	238	natur
82	IHOR	Μ	54.1	ALLOTOPIC	1	239	natur
83	PMUR	F	58.8	ALLOTOPIC	1	240	natur
84	PMUR	F	56.3	ALLOTOPIC	1	241	natur
85	PMUR	F	53.3	ALLOTOPIC	1	242	natur
88	PMUR	F	56.7	ALLOTOPIC	1	243	natur
90	PMUR	F	55	ALLOTOPIC	1	244	natur
91	PMUR	F	59.1	ALLOTOPIC	1	245	natur
92	PMUR	F	49.5	ALLOTOPIC	1	246	natur
93	PMUR	F	53.8	ALLOTOPIC	1	247	natur
94	PMUR	F	53.9	ALLOTOPIC	1	248	natur
96	PMUR	F	55.9	ALLOTOPIC	1	249	natur
97	PMUR	F	59.9	ALLOTOPIC	1	250	natur

Α	IHOR	F	55.5	ALLOTOPIC	1	251	natur
A10	IHOR	F	61.2	ALLOTOPIC	1	252	natur
A2	IHOR	F	64.1	ALLOTOPIC	1	253	natur
A3	IHOR	F	60.3	ALLOTOPIC	1	254	natur
A4	IHOR	F	61.4	ALLOTOPIC	1	255	natur
A6	IHOR	F	52.5	ALLOTOPIC	1	256	natur
A8	IHOR	F	58.6	ALLOTOPIC	1	257	natur
A9	IHOR	F	53.8	ALLOTOPIC	1	258	natur
В	IHOR	F	62.2	ALLOTOPIC	1	259	natur
B1	IHOR	F	57.5	ALLOTOPIC	1	260	natur
B2	IHOR	F	58.1	ALLOTOPIC	1	261	natur
B3	IHOR	F	57.2	ALLOTOPIC	1	262	natur
B4	IHOR	F	63.6	ALLOTOPIC	1	263	natur
B5	IHOR	F	55.6	ALLOTOPIC	1	264	natur
B6	IHOR	F	58.4	ALLOTOPIC	1	265	natur
IHOR_1526	IHOR	М	52.18	ALLOTOPIC	1	290	natur
IHOR_1527	IHOR	Μ	47.73	ALLOTOPIC	1	291	natur
IHOR_1528	IHOR	Μ	57.14	ALLOTOPIC	1	292	natur
IHOR_1529	IHOR	М	61.79	ALLOTOPIC	1	293	natur
IHOR_1530	IHOR	Μ	48.05	ALLOTOPIC	1	294	natur
IHOR_1531	IHOR	Μ	47.49	ALLOTOPIC	1	295	natur
IHOR_1532	IHOR	Μ	58.17	ALLOTOPIC	1	296	natur
IHOR_1533	IHOR	Μ	53.74	ALLOTOPIC	1	297	natur
IHOR_1535	IHOR	Μ	56.26	ALLOTOPIC	1	298	natur

IHOR_1536	IHOR	М	48.55	ALLOTOPIC	1	299	natur
IHOR_1538	IHOR	Μ	59.96	ALLOTOPIC	1	301	natur
IHOR_F1	IHOR	F	48.88	SYNTOPIC	1	320	natur
IHOR_F11	IHOR	F	60.37	ALLOTOPIC	1	321	natur
IHOR_F13	IHOR	F	58.78	ALLOTOPIC	1	322	natur
IHOR_F14	IHOR	F	61.34	ALLOTOPIC	1	323	natur
IHOR_F19	IHOR	F	53.27	ALLOTOPIC	1	324	natur
IHOR_F2	IHOR	F	53.66	SYNTOPIC	1	325	natur
IHOR_F4	IHOR	F	54.7	SYNTOPIC	1	326	natur
IHOR_F5	IHOR	F	57.52	SYNTOPIC	1	327	natur
IHOR_F9	IHOR	F	56.95	ALLOTOPIC	1	328	natur
KZID_IHOR1501	IHOR	Μ	54.22	SYNTOPIC	1	333	natur
KZID_IHOR1502	IHOR	Μ	47.53	SYNTOPIC	1	334	natur
KZID_IHOR1503	IHOR	Μ	49.6	SYNTOPIC	1	335	natur
KZID_IHOR1505	IHOR	Μ	53.34	SYNTOPIC	1	337	natur
KZID_IHOR1506	IHOR	Μ	53.94	SYNTOPIC	1	338	natur
KZID_IHOR1507	IHOR	Μ	50.24	SYNTOPIC	1	339	natur
KZID_PMUR1501	PMUR	F	58.63	SYNTOPIC	1	340	natur
KZID_PMUR1502	PMUR	F	49.94	SYNTOPIC	1	341	natur
KZID_PMUR1503	PMUR	Μ	50.25	SYNTOPIC	1	342	natur
KZID_PMUR1504	PMUR	Μ	58.76	SYNTOPIC	1	343	natur
KZID_PMUR1505	PMUR	Μ	56.49	SYNTOPIC	1	344	natur
PMUR_1544	PMUR	Μ	59.76	ALLOTOPIC	1	366	natur
PMUR_1545	PMUR	Μ	58.17	ALLOTOPIC	1	367	natur

PMUR_1546	PMUR	М	54.93	ALLOTOPIC	1	368	natur
PMUR_1547	PMUR	Μ	55.44	ALLOTOPIC	1	369	natur
PMUR_1548	PMUR	М	51.04	ALLOTOPIC	1	370	natur
PMUR_1549	PMUR	Μ	61.57	ALLOTOPIC	1	371	natur
PMUR_1550	PMUR	Μ	58.39	ALLOTOPIC	1	372	natur
PMUR_1551	PMUR	Μ	55.96	ALLOTOPIC	1	373	natur
PMUR_1552	PMUR	Μ	59.72	ALLOTOPIC	1	374	natur
PMUR_F16	PMUR	F	59.49	ALLOTOPIC	1	388	natur
PMUR_F17	PMUR	F	61.16	ALLOTOPIC	1	389	natur
PMUR_F18	PMUR	F	55.26	ALLOTOPIC	1	390	natur
PMUR_F19	PMUR	F	60.95	ALLOTOPIC	1	391	natur
PMUR_F20	PMUR	F	59.56	ALLOTOPIC	1	392	natur
PMUR_F21	PMUR	F	59.96	ALLOTOPIC	1	393	natur
PMUR_F22	PMUR	F	50.55	ALLOTOPIC	1	394	natur
PMUR_F23	PMUR	F	57.69	ALLOTOPIC	1	395	natur
PMUR_F24	PMUR	F	62.24	ALLOTOPIC	1	396	natur
A_IHOR_M_01	IHOR	Μ	50.3	ALLOTOPIC	2	1	natur
A_IHOR_M_02	IHOR	Μ	57.4	ALLOTOPIC	2	2	natur
A_IHOR_M_03	IHOR	М	54.2	ALLOTOPIC	2	3	natur
A_IHOR_M_06	IHOR	М	56.6	ALLOTOPIC	2	4	natur
A_IHOR_M_08	IHOR	М	55.8	ALLOTOPIC	2	5	natur
A_IHOR_M_09	IHOR	М	55.2	ALLOTOPIC	2	6	natur
A_IHOR_M_10	IHOR	М	51.1	ALLOTOPIC	2	7	natur
A_IHOR_M_11	IHOR	Μ	61.1	ALLOTOPIC	2	8	natur

A_IHOR_M_12	IHOR	М	52.9	ALLOTOPIC	2	9	natur
A_IHOR_M_13	IHOR	М	51.2	ALLOTOPIC	2	10	natur
A_IHOR_M_14	IHOR	М	56.6	ALLOTOPIC	2	11	natur
A_IHOR_M_15	IHOR	Μ	53.2	ALLOTOPIC	2	12	natur
A_IHOR_M_17	IHOR	Μ	49.7	SYNTOPIC	2	13	natur
A_IHOR_M_18	IHOR	Μ	53.7	SYNTOPIC	2	14	natur
A_IHOR_M_19	IHOR	Μ	51.9	SYNTOPIC	2	15	natur
A_IHOR_M_20	IHOR	Μ	49	SYNTOPIC	2	16	natur
A_IHOR_M_21	IHOR	Μ	52.4	SYNTOPIC	2	17	natur
A_IHOR_M_22	IHOR	Μ	48.8	SYNTOPIC	2	18	natur
A_IHOR_M_23	IHOR	Μ	51	ALLOTOPIC	2	19	natur
A_IHOR_M_24	IHOR	Μ	56.6	ALLOTOPIC	2	20	natur
A_IHOR_M_26	IHOR	Μ	55	ALLOTOPIC	2	21	natur
A_IHOR_M_27	IHOR	Μ	51.5	ALLOTOPIC	2	22	natur
A_IHOR_M_28	IHOR	Μ	48.5	ALLOTOPIC	2	23	natur
A_IHOR_M_29	IHOR	Μ	58.2	ALLOTOPIC	2	24	natur
A_IHOR_M_30	IHOR	Μ	59.8	ALLOTOPIC	2	25	natur
A_IHOR_M_31	IHOR	Μ	53.5	SYNTOPIC	2	26	natur
A_IHOR_M_32	IHOR	Μ	49.7	SYNTOPIC	2	27	natur
A_IHOR_M_33	IHOR	Μ	53.2	ALLOTOPIC	2	28	natur
A_IHOR_M_35	IHOR	Μ	60.4	ALLOTOPIC	2	29	natur
A_PMUR_M_02	PMUR	М	47.9	SYNTOPIC	2	30	natur
A_PMUR_M_03	PMUR	М	51.1	ALLOTOPIC	2	31	urban
A_PMUR_M_05	PMUR	М	49	ALLOTOPIC	2	32	urban

A_PMUR_M_06	PMUR	М	56.5	ALLOTOPIC	2	33	urban
A_PMUR_M_07	PMUR	М	54.9	ALLOTOPIC	2	34	urban
A_PMUR_M_13	PMUR	М	56.8	ALLOTOPIC	2	35	natur
A_PMUR_M_14	PMUR	М	62	ALLOTOPIC	2	36	natur
A_PMUR_M_16	PMUR	М	52.2	ALLOTOPIC	2	37	natur
A_PMUR_M_19	PMUR	М	59.4	ALLOTOPIC	2	38	natur
A_PMUR_M_22	PMUR	Μ	61.6	ALLOTOPIC	2	39	natur
A_PMUR_M_23	PMUR	М	62.4	ALLOTOPIC	2	40	natur
A_PMUR_M_24	PMUR	М	56	ALLOTOPIC	2	41	natur
A_PMUR_M_25	PMUR	Μ	50.8	SYNTOPIC	2	42	natur
A_PMUR_M_26	PMUR	М	55.1	SYNTOPIC	2	43	natur
A_PMUR_M_27	PMUR	М	47.5	SYNTOPIC	2	44	natur
A_PMUR_M_29	PMUR	М	55.6	ALLOTOPIC	2	45	natur
A_PMUR_M_30	PMUR	М	55.2	ALLOTOPIC	2	46	natur
A_PMUR_M_31	PMUR	М	50.7	ALLOTOPIC	2	47	natur
C_IHOR_M_01	IHOR	М	47.2	ALLOTOPIC	2	48	natur
C_IHOR_M_02	IHOR	Μ	58.3	ALLOTOPIC	2	49	natur
C_IHOR_M_03	IHOR	Μ	58.4	ALLOTOPIC	2	50	natur
C_IHOR_M_04	IHOR	Μ	49.1	ALLOTOPIC	2	51	natur
C_IHOR_M_05	IHOR	Μ	47.4	ALLOTOPIC	2	52	natur
C_IHOR_M_07	IHOR	Μ	52.6	ALLOTOPIC	2	53	natur
C_IHOR_M_09	IHOR	М	54.2	ALLOTOPIC	2	54	natur
C_IHOR_M_10	IHOR	М	48.8	SYNTOPIC	2	55	natur
C_IHOR_M_11	IHOR	М	51	ALLOTOPIC	2	56	natur

C_IHOR_M_13	IHOR	М	57.1	ALLOTOPIC	2	57	natur
C_IHOR_M_14	IHOR	Μ	53.9	ALLOTOPIC	2	58	natur
C_PMUR_M_02	PMUR	Μ	59.1	ALLOTOPIC	2	59	natur
C_PMUR_M_03	PMUR	Μ	55.4	ALLOTOPIC	2	60	natur
C_PMUR_M_05	PMUR	М	53.4	ALLOTOPIC	2	61	natur
C_PMUR_M_09	PMUR	Μ	57.4	ALLOTOPIC	2	62	natur
C_PMUR_M_10	PMUR	М	59.8	ALLOTOPIC	2	63	natur
C_PMUR_M_11	PMUR	М	51.1	ALLOTOPIC	2	64	natur
IHOR-01	IHOR	Μ	52.3	SYNTOPIC	2	65	natur
IHOR-03	IHOR	Μ	51	ALLOTOPIC	2	66	natur
IHOR-05	IHOR	Μ	49.8	SYNTOPIC	2	67	natur
IHOR-06	IHOR	Μ	46.9	SYNTOPIC	2	68	natur
IHOR-07	IHOR	Μ	53	SYNTOPIC	2	69	natur
M_IHOR_F_04	IHOR	F	62.2	ALLOTOPIC	2	74	natur
M_IHOR_F_05	IHOR	F	59	ALLOTOPIC	2	75	natur
M_IHOR_F_07	IHOR	F	61.6	ALLOTOPIC	2	76	natur
M_IHOR_F_08	IHOR	F	61.3	ALLOTOPIC	2	77	natur
M_IHOR_F_10	IHOR	F	61.1	ALLOTOPIC	2	78	natur
M_IHOR_F_12	IHOR	F	55.1	ALLOTOPIC	2	79	natur
M_IHOR_F_24	IHOR	F	60.7	ALLOTOPIC	2	81	natur
M_IHOR_F_25	IHOR	F	55.3	ALLOTOPIC	2	82	natur
M_IHOR_F_26	IHOR	F	48.9	ALLOTOPIC	2	83	natur
M_IHOR_F_27	IHOR	F	56.5	ALLOTOPIC	2	84	natur
M_IHOR_F_28	IHOR	F	51.4	ALLOTOPIC	2	85	natur

M_IHOR_F_29	IHOR	F	56.3	ALLOTOPIC	2	86	natur
M_IHOR_M_23	IHOR	М	48.8	ALLOTOPIC	2	108	natur
M_IHOR_M_26	IHOR	М	53.3	ALLOTOPIC	2	110	natur
M_IHOR_M_29	IHOR	Μ	54.6	ALLOTOPIC	2	111	natur
M_PMUR_F_02	PMUR	F	51.7	SYNTOPIC	2	113	natur
M_PMUR_F_08	PMUR	F	62	ALLOTOPIC	2	119	natur
M_PMUR_F_09	PMUR	F	61.9	ALLOTOPIC	2	120	natur
M_PMUR_F_10	PMUR	F	68	ALLOTOPIC	2	121	natur
M_PMUR_F_11	PMUR	F	58	ALLOTOPIC	2	122	natur
M_PMUR_F_12	PMUR	F	56.6	ALLOTOPIC	2	123	natur
M_PMUR_F_13	PMUR	F	68.5	ALLOTOPIC	2	124	natur
M_PMUR_F_14	PMUR	F	59.2	ALLOTOPIC	2	125	natur
M_PMUR_F_15	PMUR	F	60.5	ALLOTOPIC	2	126	natur
M_PMUR_F_16	PMUR	F	59.9	ALLOTOPIC	2	127	natur
M_PMUR_F_17	PMUR	F	61	ALLOTOPIC	2	128	natur
M_PMUR_F_18	PMUR	F	59.4	ALLOTOPIC	2	129	natur
M_PMUR_F_19	PMUR	F	58	ALLOTOPIC	2	130	natur
M_PMUR_F_20	PMUR	F	50.2	SYNTOPIC	2	131	natur
M_PMUR_F_21	PMUR	F	52.1	SYNTOPIC	2	132	natur
M_PMUR_F_25	PMUR	F	55.6	ALLOTOPIC	2	133	natur
M_PMUR_F_29	PMUR	F	54	ALLOTOPIC	2	134	natur
W_IHOR_F_01	IHOR	F	56.4	SYNTOPIC	2	135	natur
W_IHOR_F_03	IHOR	F	62	ALLOTOPIC	2	136	natur
W_IHOR_F_04	IHOR	F	58.3	ALLOTOPIC	2	137	natur

W_IHOR_F_05	IHOR	F	55.7	ALLOTOPIC	2	138	natur
W_IHOR_F_06	IHOR	F	55.6	ALLOTOPIC	2	139	natur
W_IHOR_F_07	IHOR	F	66.7	ALLOTOPIC	2	140	natur
W_IHOR_F_08	IHOR	F	54.9	ALLOTOPIC	2	141	natur
W_IHOR_F_09	IHOR	F	54.1	ALLOTOPIC	2	142	natur
W_IHOR_F_10	IHOR	F	59.3	ALLOTOPIC	2	143	natur
W_IHOR_F_11	IHOR	F	59.9	ALLOTOPIC	2	144	natur
W_IHOR_F_13	IHOR	F	61.4	ALLOTOPIC	2	145	natur
W_IHOR_F_14	IHOR	F	50.5	ALLOTOPIC	2	146	natur
W_IHOR_M_01	IHOR	Μ	50.6	SYNTOPIC	2	147	natur
W_IHOR_M_04	IHOR	Μ	55.3	ALLOTOPIC	2	149	natur
W_IHOR_M_07	IHOR	Μ	53.6	ALLOTOPIC	2	152	natur
W_IHOR_M_09	IHOR	Μ	46.9	ALLOTOPIC	2	153	natur
W_IHOR_M_11	IHOR	Μ	56.1	ALLOTOPIC	2	154	natur
W_IHOR_M_12	IHOR	Μ	47.5	ALLOTOPIC	2	155	natur
W_PMUR_F_02	PMUR	F	57.6	SYNTOPIC	2	156	natur
W_PMUR_F_03	PMUR	F	54.7	ALLOTOPIC	2	157	natur
W_PMUR_F_04	PMUR	F	58.6	ALLOTOPIC	2	158	natur
W_PMUR_F_05	PMUR	F	51.8	ALLOTOPIC	2	159	natur
W_PMUR_F_06	PMUR	F	52.8	ALLOTOPIC	2	160	natur
W_PMUR_F_07	PMUR	F	60.9	ALLOTOPIC	2	161	natur
W_PMUR_F_08	PMUR	F	56.9	ALLOTOPIC	2	162	natur
W_PMUR_F_10	PMUR	F	54.7	ALLOTOPIC	2	163	natur
W_PMUR_F_11	PMUR	F	51.1	ALLOTOPIC	2	164	natur

W_PMUR_F_12	PMUR	F	59.3	ALLOTOPIC	2	165	natur
W_PMUR_F_13	PMUR	F	48.8	ALLOTOPIC	2	166	natur
W_PMUR_F_14	PMUR	F	50.7	ALLOTOPIC	2	167	natur
W_PMUR_F_15	PMUR	F	50	ALLOTOPIC	2	168	natur
W_PMUR_F_16	PMUR	F	59.1	ALLOTOPIC	2	169	natur
W_PMUR_M_01	PMUR	М	59.2	SYNTOPIC	2	170	natur
W_PMUR_M_02	PMUR	М	49.5	SYNTOPIC	2	171	natur
W_PMUR_M_04	PMUR	М	56.5	ALLOTOPIC	2	172	natur
W_PMUR_M_06	PMUR	М	47.2	ALLOTOPIC	2	174	natur
1	PMUR	М	52.3	ALLOTOPIC	2	175	natur
10	IHOR	М	59	ALLOTOPIC	2	176	natur
11	IHOR	М	53.8	ALLOTOPIC	2	177	natur
12	PMUR	М	58.2	ALLOTOPIC	2	178	natur
13	PMUR	М	54	ALLOTOPIC	2	179	natur
14	PMUR	М	50	ALLOTOPIC	2	180	natur
15	PMUR	М	60.1	ALLOTOPIC	2	181	natur
16	PMUR	М	51.9	ALLOTOPIC	2	182	natur
17	PMUR	М	58.1	ALLOTOPIC	2	183	natur
18	PMUR	М	52.3	ALLOTOPIC	2	184	natur
19	PMUR	М	56.1	ALLOTOPIC	2	185	natur
2	IHOR	М	54.7	ALLOTOPIC	2	186	natur
20	IHOR	F	60.4	ALLOTOPIC	2	187	natur
21	IHOR	F	58.9	ALLOTOPIC	2	188	natur
24	IHOR	F	66.1	ALLOTOPIC	2	189	natur

25	PMUR	М	57.6	ALLOTOPIC	2	190	natur
26	IHOR	F	57.6	ALLOTOPIC	2	191	natur
27	PMUR	М	59.5	ALLOTOPIC	2	192	natur
28	IHOR	F	58.5	ALLOTOPIC	2	193	natur
3	IHOR	М	51.8	ALLOTOPIC	2	194	natur
30	IHOR	F	61.7	ALLOTOPIC	2	195	natur
31	IHOR	F	51.1	ALLOTOPIC	2	196	natur
32	IHOR	F	59.3	ALLOTOPIC	2	197	natur
33	IHOR	F	64	ALLOTOPIC	2	198	natur
35	IHOR	F	66.1	ALLOTOPIC	2	199	natur
38	PMUR	М	52.9	ALLOTOPIC	2	200	natur
39	IHOR	F	61.3	ALLOTOPIC	2	201	natur
4	IHOR	М	54.6	ALLOTOPIC	2	202	natur
40	PMUR	М	60.8	ALLOTOPIC	2	203	natur
41	PMUR	М	57.4	ALLOTOPIC	2	204	natur
42	IHOR	F	61.3	ALLOTOPIC	2	205	natur
43	PMUR	М	48.1	ALLOTOPIC	2	206	natur
44	IHOR	F	57.4	ALLOTOPIC	2	207	natur
47	PMUR	F	56.7	ALLOTOPIC	2	208	natur
48	PMUR	F	63.6	ALLOTOPIC	2	209	natur
49	PMUR	F	61.6	ALLOTOPIC	2	210	natur
5	IHOR	М	53.9	ALLOTOPIC	2	211	natur
50	PMUR	F	55.1	ALLOTOPIC	2	212	natur
51	PMUR	F	60.2	ALLOTOPIC	2	213	natur

52	IHOR	F	58	ALLOTOPIC	2	214	natur
53	IHOR	F	60.3	ALLOTOPIC	2	215	natur
55	PMUR	F	60.2	ALLOTOPIC	2	216	natur
56	PMUR	F	60.8	ALLOTOPIC	2	217	natur
57	IHOR	F	61.4	ALLOTOPIC	2	218	natur
58	IHOR	F	54.6	ALLOTOPIC	2	219	natur
59	PMUR	F	59.9	ALLOTOPIC	2	220	natur
6	IHOR	М	53.9	ALLOTOPIC	2	221	natur
60	PMUR	F	57.8	ALLOTOPIC	2	222	natur
61	PMUR	F	61.1	ALLOTOPIC	2	223	natur
62	PMUR	F	57.3	ALLOTOPIC	2	224	natur
63	PMUR	F	60.8	ALLOTOPIC	2	225	natur
64	PMUR	F	58.3	ALLOTOPIC	2	226	natur
65	IHOR	F	56	ALLOTOPIC	2	227	natur
66	PMUR	F	60	ALLOTOPIC	2	228	natur
69	PMUR	М	54.9	ALLOTOPIC	2	229	natur
7	IHOR	Μ	52.1	ALLOTOPIC	2	230	natur
70	PMUR	F	62.4	ALLOTOPIC	2	231	natur
71	PMUR	F	56.3	ALLOTOPIC	2	232	natur
73	PMUR	F	54.9	ALLOTOPIC	2	233	natur
74	PMUR	F	58.6	ALLOTOPIC	2	234	natur
75	PMUR	F	60.8	ALLOTOPIC	2	235	natur
78	PMUR	Μ	58.9	ALLOTOPIC	2	236	natur
79	PMUR	Μ	57.4	ALLOTOPIC	2	237	natur

81	IHOR	М	58.4	ALLOTOPIC	2	238	natur
82	IHOR	Μ	54.1	ALLOTOPIC	2	239	natur
83	PMUR	F	58.8	ALLOTOPIC	2	240	natur
84	PMUR	F	56.3	ALLOTOPIC	2	241	natur
85	PMUR	F	53.3	ALLOTOPIC	2	242	natur
88	PMUR	F	56.7	ALLOTOPIC	2	243	natur
90	PMUR	F	55	ALLOTOPIC	2	244	natur
91	PMUR	F	59.1	ALLOTOPIC	2	245	natur
92	PMUR	F	49.5	ALLOTOPIC	2	246	natur
93	PMUR	F	53.8	ALLOTOPIC	2	247	natur
94	PMUR	F	53.9	ALLOTOPIC	2	248	natur
96	PMUR	F	55.9	ALLOTOPIC	2	249	natur
97	PMUR	F	59.9	ALLOTOPIC	2	250	natur
А	IHOR	F	55.5	ALLOTOPIC	2	251	natur
A10	IHOR	F	61.2	ALLOTOPIC	2	252	natur
A2	IHOR	F	64.1	ALLOTOPIC	2	253	natur
A3	IHOR	F	60.3	ALLOTOPIC	2	254	natur
A4	IHOR	F	61.4	ALLOTOPIC	2	255	natur
A6	IHOR	F	52.5	ALLOTOPIC	2	256	natur
A8	IHOR	F	58.6	ALLOTOPIC	2	257	natur
A9	IHOR	F	53.8	ALLOTOPIC	2	258	natur
В	IHOR	F	62.2	ALLOTOPIC	2	259	natur
B1	IHOR	F	57.5	ALLOTOPIC	2	260	natur
B2	IHOR	F	58.1	ALLOTOPIC	2	261	natur

B3	IHOR	F	57.2	ALLOTOPIC	2	262	natur
B4	IHOR	F	63.6	ALLOTOPIC	2	263	natur
B5	IHOR	F	55.6	ALLOTOPIC	2	264	natur
B6	IHOR	F	58.4	ALLOTOPIC	2	265	natur
IHOR_1526	IHOR	Μ	52.18	ALLOTOPIC	2	290	natur
IHOR_1527	IHOR	Μ	47.73	ALLOTOPIC	2	291	natur
IHOR_1528	IHOR	Μ	57.14	ALLOTOPIC	2	292	natur
IHOR_1529	IHOR	Μ	61.79	ALLOTOPIC	2	293	natur
IHOR_1530	IHOR	Μ	48.05	ALLOTOPIC	2	294	natur
IHOR_1531	IHOR	Μ	47.49	ALLOTOPIC	2	295	natur
IHOR_1532	IHOR	Μ	58.17	ALLOTOPIC	2	296	natur
IHOR_1533	IHOR	Μ	53.74	ALLOTOPIC	2	297	natur
IHOR_1535	IHOR	Μ	56.26	ALLOTOPIC	2	298	natur
IHOR_1536	IHOR	Μ	48.55	ALLOTOPIC	2	299	natur
IHOR_1538	IHOR	М	59.96	ALLOTOPIC	2	301	natur
IHOR_F1	IHOR	F	48.88	SYNTOPIC	2	320	natur
IHOR_F11	IHOR	F	60.37	ALLOTOPIC	2	321	natur
IHOR_F13	IHOR	F	58.78	ALLOTOPIC	2	322	natur
IHOR_F14	IHOR	F	61.34	ALLOTOPIC	2	323	natur
IHOR_F19	IHOR	F	53.27	ALLOTOPIC	2	324	natur
IHOR_F2	IHOR	F	53.66	SYNTOPIC	2	325	natur
IHOR_F4	IHOR	F	54.7	SYNTOPIC	2	326	natur
IHOR_F5	IHOR	F	57.52	SYNTOPIC	2	327	natur
IHOR_F9	IHOR	F	56.95	ALLOTOPIC	2	328	natur

KZID_IHOR1501	IHOR	М	54.22	SYNTOPIC	2	333	natur
KZID_IHOR1502	IHOR	М	47.53	SYNTOPIC	2	334	natur
KZID_IHOR1503	IHOR	М	49.6	SYNTOPIC	2	335	natur
KZID_IHOR1505	IHOR	М	53.34	SYNTOPIC	2	337	natur
KZID_IHOR1506	IHOR	М	53.94	SYNTOPIC	2	338	natur
KZID_IHOR1507	IHOR	М	50.24	SYNTOPIC	2	339	natur
KZID_PMUR1501	PMUR	F	58.63	SYNTOPIC	2	340	natur
KZID_PMUR1502	PMUR	F	49.94	SYNTOPIC	2	341	natur
KZID_PMUR1503	PMUR	М	50.25	SYNTOPIC	2	342	natur
KZID_PMUR1504	PMUR	М	58.76	SYNTOPIC	2	343	natur
KZID_PMUR1505	PMUR	М	56.49	SYNTOPIC	2	344	natur
PMUR_1544	PMUR	М	59.76	ALLOTOPIC	2	366	natur
PMUR_1545	PMUR	М	58.17	ALLOTOPIC	2	367	natur
PMUR_1546	PMUR	М	54.93	ALLOTOPIC	2	368	natur
PMUR_1547	PMUR	М	55.44	ALLOTOPIC	2	369	natur
PMUR_1548	PMUR	М	51.04	ALLOTOPIC	2	370	natur
PMUR_1549	PMUR	М	61.57	ALLOTOPIC	2	371	natur
PMUR_1550	PMUR	М	58.39	ALLOTOPIC	2	372	natur
PMUR_1551	PMUR	М	55.96	ALLOTOPIC	2	373	natur
PMUR_1552	PMUR	М	59.72	ALLOTOPIC	2	374	natur
PMUR_F16	PMUR	F	59.49	ALLOTOPIC	2	388	natur
PMUR_F17	PMUR	F	61.16	ALLOTOPIC	2	389	natur
PMUR_F18	PMUR	F	55.26	ALLOTOPIC	2	390	natur
PMUR_F19	PMUR	F	60.95	ALLOTOPIC	2	391	natur

PMUR_F20	PMUR	F	59.56	ALLOTOPIC	2	392	natur
PMUR_F21	PMUR	F	59.96	ALLOTOPIC	2	393	natur
PMUR_F22	PMUR	F	50.55	ALLOTOPIC	2	394	natur
PMUR_F23	PMUR	F	57.69	ALLOTOPIC	2	395	natur
PMUR_F24	PMUR	F	62.24	ALLOTOPIC	2	396	natur

Abbreviations: IHOR = *Iberolacerta horvathi*, PMUR = *Podarcis muralis*, M = male, F =

female, SVL = snout to vent length (cm), natur = natural habitat, urban = urb