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Dead snakes and their stories: morphological anomalies, asymmetries and scars of road killed *Dolichophis caspius* (Serpentes, Colubridae) from Romania

George-Adelin Ile[1], Alexandra-Roxana-Maria Maier[1], Achim-Mircea Cadar[1], Severus-Daniel Covaciu-Marcov[1], Sára Ferenți[1]

1 *University of Oradea, Faculty of Informatics and Sciences, Department of Biology; 1, Universității, Oradea 410087, Romania*

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Corresponding author: Severus-Daniel Covaciu-Marcov (severcovaciu1@gmail.com)

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Abstract

We analysed several morphological characters of 84 road-killed *D. caspius* individuals from different areas of southern Romania. Most presented asymmetries in the total number of temporal scales, the temporal row and the periocular and labial scales. Almost a quarter of snakes had scars, located especially on the head and tail; many individuals had multiple injuries. The lowest rate of individuals with scars was found in the area with the least anthropogenic impact (Danube Gorge). This finding suggests that, in other areas in Romania, the species is threatened and lives in less optimal conditions. The number of individuals with asymmetries and scars differed according to the populated region, sex or size class. Most of the individuals were killed in August, due to the large number of road-killed juveniles.

Key Words

asymmetry traits, conservation, environment, morphology, road mortality

Introduction

As in many other species, snakes are in decline (Winne et al. 2007; Reading et al. 2010; Graitson et al. 2019; Reading and Jofré 2020). One of the major threats for snakes is mortality from road traffic when making a road crossing (Ciesiołkiewicz et al. 2006; Meek 2009, 2015; Lutterschmidt et al. 2019). This factor affects not only the common species (Borczyk 2004; Ciesiołkiewicz et al. 2006), but also species of conservation interest (Rosen and Lowe 1994), endemic species (Santhoshkumar et al. 2016) or those situated at the limit of their range (Covaciu-Marcov et al. 2012a). Road traffic does not only impact snakes, but also other animals (Ashley and Robinson 1996; Glista et al. 2007; Baxter-Gilbert et al. 2015; Ciolan et al. 2017). The high number of mortalities from different animal groups has permitted road mortalities to be employed as study material for feeding ecology (Drygala and Zoller 2013; Kolenda et al. 2019), phylogeography (Grill et al. 2009; Horcajada et al. 2018), temporal trends in populations (Meek 2020) and faunistic studies (Gonzáles-Gallina et al. 2016; Teodor et al. 2019).

In Romania, the Caspian Whipsnake, *Dolichophis caspius* Gmelin, 1789 is situated at the northern limit of its distribution range (Sillero et al. 2014). The species is frequently found dead on roads, not only in Romania (Covaciu-Marcov and David 2010; Covaciu-Marcov et al. 2012b; Sahlean et al. 2019), but in other regions (Tok et al. 2011). It is a large species (Fuhn and Vancea 1961) and, hence, corpses are easily noticeable from moving vehicles (Covaciu-Marcov et al. 2012b). In the last two decades, we collected numerous *D. caspius* corpses on different roads in Romania and accumulated a large database. Thus, we used road-killed *D. caspius* to analyse various parameters of external morphology, such as asymmetries, morphological anomalies or scars. In addition, we examined specimens for information on distribution-dependent risk of mortality.

Studies on asymmetry in reptiles are relatively new, geographically biased and focus mainly on lizards and less on snakes (Laia et al. 2015), although more recent studies have appeared in literature (Brown et al. 2017; Löwenborg and Hagman 2017). In order to remedy this deficiency, studies on this topic are necessary, the more so because asymmetry seems to measure developmental stability in stressful conditions (Laia et al. 2015). The main objectives were (1) to obtain meristic and metric data on morphology and compare these with existing data, (2) to analyse asymmetries, anomalies and scars on the studied individuals and (3) to examine for inter-regional differences in sex and size.

Methods

We examined 84 *D. caspius* individuals, from almost all over the territory occupied by this species in Romania, except from the small area it populates in southern Moldova (Sahlean et al. 2019). All snakes were found dead, killed mostly by cars on roads or sometimes by local people (Covaciu-Marcov and David 2010; Covaciu-Marcov et al. 2012b). Most were identified from a moving car at low speed. Some of the specimens had been preserved 20 years previously, but most were collected between 2018 and 2019. For all snakes, we had information on the locality, region and date of collection, except for two, in which we know only the geographic origin. They are deposited in the scientific collection of the Department of Biology, Faculty of Informatics and Sciences, University of Oradea.

Using the abundance of road-killed individuals, we estimated road mortality frequency with respect to period of the year. We calculated the frequency of occurrence in each season according to size. We examined several morphological traits, size (measured from head to tail), sex, head scalation, number of ventral and subcaudal scales, as well as the presence/absence of anomalies or scars. Sex was determined by dissection, after the method described by Pesantes (1994). Body-length was measured with a ruler, an action made difficult by the reduced flexibility of snakes due to long-term preservation. The snakes were divided into four size classes: 25–60 cm, 60–100 cm, 100–130 cm and 130–160 cm. Snakes were divided into three groups, according to their geographical origin, which were Dobruja, the Danube meadow and the Danube Gorge. Dobruja is situated south of the Danube, the other two regions lie north of the Danube. The Danube meadow and the most part of Dobruja are lowland areas, with high anthropogenic disturbance and intensive agriculture. The Danube Gorge is a mostly natural low mountain area.

For head scalation, we examined all the bilateral features (Fuhn and Vancea 1961). Temporal scales were described slightly differently by Fuhn and Vancea (1961) and Kotenko et al. (1986) in *D. caspius*. In the temporal region, we considered two parameters: number of scales connected to the parietal scale and the total number of scales situated between the parietal plate and the upper labials (Fuhn and Vancea 1961). We considered temporal scales as those that had more than 50% of their surface inside the temporal zone (delimited by the imaginary line drawn between the posterior corner of the parietal scale and the posterior margin of the last upper labial scale (Fig. 1A)). The temporal row was treated as in Brown et al. (2017). All scales around the eye (subocular, preocular, supraocular and postocular) were treated as perioculars, as applied previously (Bellaagh et al. 2010). The scales around the mouth (upper labials and lower labials) were treated as labials. The asymmetry was calculated as absolute value (|R-L|; R-right side, L-left side), a frequently-used parameter in asymmetry indices (Palmer 1994). The number of individuals with asymmetry or multiple asymmetry, regardless of the trait of occurrence, was reported as a percent from data subsets (sex, origin, size), similar to some asymmetry indices suggested by Palmer (1994), but separately for each trait. We noted anomalies of abnormal scale division or union and presence of scars. These were divided into scars on the head, body and tail (Frank and Dudás 2019). Tail integrity was also examined. Measurements were performed only when carcasses were in good condition. Severely damaged individuals were analysed within the limits of possibilities.

Using linear regression, we estimated the correlation between: size class and frequency of individuals with scars, size class and number of scars from each size class, respectively, seasonal frequency and average size of individuals. The Kruskal-Wallis test was used to estimate the differences between the number of ventral and subcaudal scales and their ratio (V/Scd) between the two sexes; the number of ventral and subcaudal scales and their ratio (V/Scd) between males and females from different categories (origin, size); number of individuals with scars and number of scars on individuals according to the three subsets (sex, origin and size). The One-way ANOVA test was used to estimate the differences amongst asymmetries (|R-L|) of the four traits over the three data subsets (sex, origin and size). The Kolmogorov-Smirnov test was used to estimate data distribution before calculating asymmetry. In order to detect directional asymmetry, we used a one-sample t-test on signed (R-L) differences between the right and left side of each trait. Calculations were made using the free PAST software (Hammer et al. 2001).

Results

The number and average size of snakes by month is presented in Fig. 1. Strong negative correlation was observed between the number of individuals in each month and

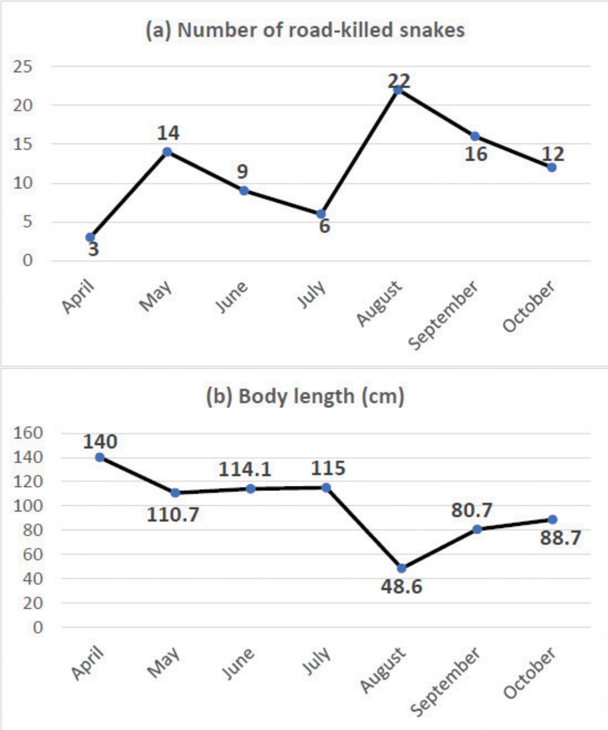

Figure 1. Number (**a**) and average body length (**b**) of individuals in each month.

their average size (r^2 = -0.93, p = 0.002, N = 82). Mortalities peaked in May and August (Fig. 1a). The average size of snakes was 90.3 cm (112.5 cm, if we exclude juveniles). The size of females varied between 27.5 and 150 cm, with an average size of 89.9 cm (101.1 cm, if we exclude juveniles). In males, the length varied between 27.5 and 160 cm, but the average size was 96.8 cm (121.7 cm, if we exclude juveniles).

Table 1. The average number and variation range of the three analysed traits: ventral (V) and subcaudal (Scd) scales and the V/Scd ratio for the total and according to sex (*significant differences between males and females (Kruskal-Wallis, p > 0.05).

		N	Ventrals (V)		Subcaudals (Scd)		V/Scd	
			Average	Range	Average	Range	Average	Range
Total		84	195.86	175–209	100.33	81–110	1.97	1.71–2.46
Sex	Males	36	196.84	183–209	102.26*	86–110	1.93*	1.79–2.33
	Females	34	198.66	175–209	95.90*	82–105	2.08*	1.75–2.43

Most of the corpses were in good condition, but in damaged corpses, only limited data were possible to obtain. The average number and range of ventral plates, subcaudal plates and the ratio between them (V/Scd) is presented in Table 1. The Kruskal-Wallis test indicated significant differences between males and females in number of subcaudal scales (p < 0.0001) and the V/Scd ratio (p = 0.0002), but not in ventral scales (p > 0.05, Table 1). The variation of three traits (V, Scd and V/Scd) in males and females did not differ significantly nor by the region or by size (p > 0.05). Most individuals (84.5%) had asymmetries in the next traits: the total number of temporal scales, temporal line, periocular and labial scales (Table 2). More than a half of the snakes presented combination asymmetries in two or more characters (Table 2), but none was asymmetric for all four traits. According to the Kolmogorov-Smirnov test, almost all values of the four traits (regardless the category) had normal distribution (p > 0.05) around a mean of zero (one-sample t-tests, p > 0.05). Directional asymmetry in the temporal line was indicated only in snakes from Dobruja (t-test: p = 0.028). Excluding this, the other asymmetries were fluctuating rather than directional or anti-symmetry. According to the ANOVA test, differences between the traits (temporal line, the total number of temporals, periocular and labials) were not significant regardless of the sex, origin or size (p varying between 0.06 and 0.98).

The temporal line pattern with three scales on both sides (Fig. 2a, b) is present in relatively few individuals, appearing in just 30 cases. However, the number of scales in the temporal row varies between two and five (on one side). In the case of snakes with three scales, these had an elongated, more or less identical shape, starting from the postocular (Fig. 2a, b). This pattern was the most common one and it is important for the description of the other cases. If the temporal row contains two scales, the second and the third scales (from the common pattern) are united, forming one single plate (Fig. 2c). In the case of four scales in the temporal row, one of the last two scales (of the common pattern) is divided, forming two new distinct scales (Fig. 2d, e). In the case of five scales, the last two (of the common pattern) are divided, forming four new distinct scales, but the first scale also can be divided (Fig. 2f). The examples with two, four or five

Table 2. The rate of asymmetric individuals and asymmetry traits according to sex, origin and size.

Category		N	Asymmetrical individuals (%)	Multiple asymmetries/ individual (%)	Asymmetry in traits (%)			
					Temporal line	Temporals total	Periocular	Labial
Total		84	84.52	54.76	48.81	78.57	10.71	9.52
Sex	Males	36	83.33	41.67	38.89	77.77	5.56	13.89
	Females	34	82.35	58.82	55.88	76.47	11.76	5.88
Site	Danube Gorge	28	96.43	50.00	39.28	85.71	17.86	10.71
	Danube Meadow	33	78.79	57.58	48.48	75.76	6.06	12.12
	Dobruja	23	78.26	56.52	60.87	73.91	8.7	4.35
Size	25–60	21	85.71	61.90	61.9	80.95	14.29	4.76
(cm)	60–100	17	94.12	52.94	47.06	82.35	17.65	11.76
	100–130	14	85.71	71.43	57.14	85.71	7.14	21.43
	130–160	19	89.47	47.36	36.84	78.95	10.53	10.53

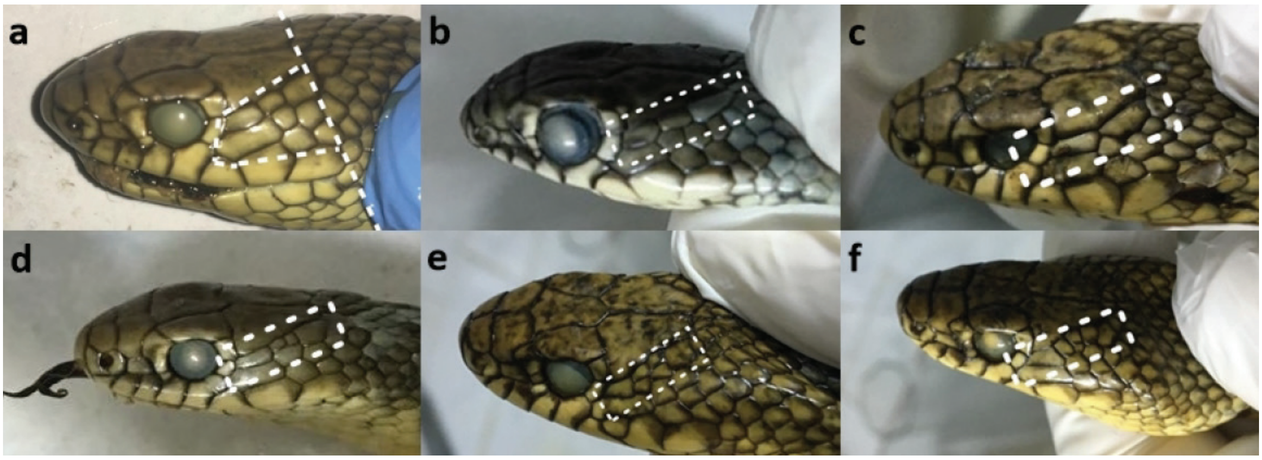

Figure 2. The temporal region delimited by the parietal and upper labial scales: **a, b** – the common pattern of three scales in the temporal row; **c, d, e, f** – variations of the temporal row with 2, 4 or 5 scales.

scales in the temporal row can be considered derivates of the three-scales pattern.

For the number of temporals, the variation was between 7 and 14 scales. The 11.90% of the individuals which had nine temporal scales, always presented two rows (double temporal row) with six scales. The first scale from the second row was always larger than the one above. The rest of the scales, up to nine, were positioned randomly in the space between the two rows and the upper labials (Fig. 2a).

Anomalies were present at the loreal, parietal, preocular, ventral and subcaudal scales. For the first two traits, we observed additional small divisions to the standard scales. On the only preocular, a transversal incomplete division was observed at the base of the scale near the subocular. This anomaly was present in 13 individuals, in eight it was observed on both sides. Anomalies of the ventral plates were represented by the incomplete longitudinal division and the subcaudal scales are united (Fig.3). Another anomaly type in the subcaudal plates was the tripling of the median joint spindle, with two rudimentary spindles which complete the main one.

Almost a quarter (24.69 %) of individuals had scars, especially on the head and tail (Table 3). Many individuals had multiple injuries, with one severely damaged individual having five scars on the body, four on the tail and broken tail tip. The scars on the head were present mostly on the parietal and temporal scales. In one individual, the rostral scale was injured. A strong positive, but non-significant correlation appeared between the size classes and ratio of scarred specimens ($r^2 = 0.80$, $p = 0.19$, $N = 71$) and the number of scars from each size class ($r^2 = 0.83$, $p = 0.17$, $N = 71$). There were no significant differences between the number of individuals with scars and the number of scars on individuals regardless of categories (sex, origin or size, Kruskal-Wallis: $p > 0.05$). Scars were observed even on juveniles of 25–30 cm length, but broken tails and multiple injuries are recorded only on individuals larger than 75 cm.

Discussion

Compared to previous studies (Bellaagh et al. 2010; Frank and Dudás 2018, 2019), we analysed a larger number of individuals of *D. caspius*. Biometric and morphological information has confirmed previously-described differences between sexes (Fuhn and Vancea 1961; Kotenko et al. 1986) with maximum length of males not exceeding the previously-described range, although seven females were longer than previously recorded (Fuhn and Vancea 1961). The variation range of ventral and subcaudal plates was equal or even extended most ranges mentioned until now (Fuhn and Vancea 1961; Dely 1978; Kotenko et al. 1986; Strugariu and Gherghel 2007). The values obtained by Kotenko et al. (1986) are comparable with the ones obtained here, but the average number of subcaudals (in females) was lower than in our study. The other traits had larger values. The V/Scd ratio was larger than in our study and differences between males and females were greater.

There are many reports on snakes` asymmetry (Herczeg et al. 2005; Shine et al. 2005; Danaisawadi et al. 2015; Brown et al. 2017), but to our best knowledge, only one study on *D. caspius* (Bellaagh et al. 2010). The absolute average values of the asymmetries in perioculars and lower labials in Bellaagh et al. (2010) are higher than our results. However, if we make an inter-trait comparison, temporal trait asymmetries greatly exceed the previous records (Bellaagh et al. 2010). Another study mentions an asymmetric temporal line in up to 50% of the individuals (Brown et al. 2017), nearly the same in our case. Many abnormal and asymmetric traits seem to be caused by incubation temperature (Arnold and Peterson 2002; Löwenborg et al. 2011) with postoculars and other traits affected by temperature (Arnold and Peterson 2002). Due to the proximity of the postocular and temporal scales, there is a possibility that temporal asymmetries, registered in this study, are the reflection of the non-optimal incubation temperature, especially since Romania is situated at the northern distribution limit of this species (Sillero et al.

Figure 3. Anomalies of the subcaudal (left) and ventral (middle and right) plates.

Table 3. The rate of individuals with scars on the whole body and body-parts (head, trunk, tail) and broken tail, according to sex, origin and size.

Category		N	Individuals with scars (%)	Multiple scars/ individual (%)	Scars (%)			
					Head	Body	Tail	Tail cut
Total		84	23.81	10.71	13.10	5.95	9.52	8.33
Sex	Males	36	25.00	11.11	13.88	5.56	11.11	8.33
	Females	34	32.35	14.71	17.65	8.82	11.76	11.76
Site	Danube Gorge	28	21.42	14.29	7.14	7.14	10.71	10.71
	Danube Meadow	33	24.24	15.15	12.12	9.09	12.12	12.12
	Dobruja	23	26.09	0.00	21.74	0.00	4.35	0.00
Size (cm)	25–60	21	19.05	0.00	14.29	0.00	4.76	0.00
	60–100	17	17.65	17.65	5.88	0.00	17.65	17.65
	100–130	14	35.71	7.14	14.29	14.29	7.14	7.14
	130–160	19	36.84	21.05	26.32	10.53	10.53	10.53

2014), that may influence the timing of offspring hatching (Sahlean and Strugariu 2018). It might then be expected that other features should also be affected, which is not the case. The more pronounced asymmetries in males are hard to explain. Males of the Caspian Whipsnake have larger heads than females (Fuhn and Vancea 1961; Dely 1978; Kotenko et al. 1986), consistent with other snake species (Zuffi and Carlino 2004). In addition, an increased growth rate in males with temporal asymmetry was reported in another species (Brown et al. 2017).

The directional asymmetry of the temporal line for the individuals from Dobruja is a result of their uneven distribution, just like in other cases (Palmer 1994). This is probably a consequence of a relatively small sample size (N = 22), where 11 individuals exhibit the extra scale on the left and two on the right side. The same character, studied in another species (Brown et al. 2017), showed directional asymmetry on the same left side. Brown et al. (2017) associated asymmetry with developmental disturbances. We expected a high level of asymmetry in individuals from the Danube meadow, with more anthropogenic habitats and less records of the species (Covaciu-Marcov et al. 2012b). However, despite our expectations, snakes from this region exhibited the lowest overall asymmetry and the highest number of symmetrical individuals. Surprisingly, the highest rate of individuals with asymmetries was recorded in the Danube Gorge, the area with large natural habitats, suggesting that the rate of asymmetries is not determined by the degree of human disturbance or the occurrence of natural habitats. Asymmetries could be caused by other factors, for example, due to random occurrence or influenced by the short time elapsed from the habitat disturbance, which was not enough to be expressed in high asymmetry rates. It is possible that the general high asymmetry rates of Caspian Whipsnakes in Romania and their reduced distribution range in the country (Sahlean et al. 2019) did not permit the correct discernment of regional differences and real causes of asymmetry. Moreover, in Romania, the species is at its northern distribution limit and most of its European distribution area was recently colonised after the last glacial period (Nagy et al. 2010).

The most frequent anomalies of the analysed snakes were the division or union of temporal scales. This is probably a consequence of the high variability of temporal scale patterns, described above. Other authors mention cases of scale anomalies in temporal areas in a different species (e.g. Idrisova 2018). A semi-divided ventral scale was observed on only one individual. This value is very low compared to other studies, as the appearance of this anomaly seems to be influenced by improper incubation temperature (Shine et al. 2005; Löwenborg et al. 2011; Löwenborg and Hagman 2017). Moreover, the semi-division of a ventral scale may appear concomitantly with an unpaired rib, affecting negatively the locomotory performance in terrestrial environments (Löwenborg et al. 2011; Löwenborg and Hagman 2017).

The presence of scars on 23.81% of the studied snakes offers indirect information about the interspecific relationships of snakes (Bonnet et al. 1999; Pleguezuelos et al. 2018; Frank and Dudás 2019). Other authors report similar (Placyk and Burghardt 2005; Frank and Dudás 2019) or even higher (Gregory and Isaac 2005) frequencies of body scarring. Tail autotomy was previously mentioned for *D. caspius* (Crnobrnja-Isailović et al. 2016), but individuals identified in this study without tails also presented different healed injuries. The scars of snakes seem to be caused by predators rather than by prey (Placyk and Burghardt 2005; Pleguezuelos et al. 2018), even if rodents are included in the trophic spectrum of the species (Fuhn and Vancea 1961) and some authors describe injuries due to rodents on snakes (Fearn 2011). More scars were present in females (Table 3), though in other cases, males had more scarring (Gregory and Isaac 2005). In addition, we expected more scars on males, due to their large size and potential longer life span and high levels of activity during the breeding period (Fuhn and Vancea 1961). The number of injuries is directly proportional to the snakes` size (Gregory and Isaac 2005). Although, in other cases, most of the scars were situated on the body (Frank and Dudás 2019), in our case, the head and the tail had 67% of the total scars. *Dolichophis caspius* is an aggressive species which bites when trapped (Fuhn and Vancea 1961), thus probably the head is exposed and the snakes, which escape confrontations, could remain with such scars. Additionally, snake species that adopt aggressive defence are more exposed to domestic animals, such as cats and dogs in anthropogenic regions (Meek 2012). Even if most studies confirm that scars are the result of interaction with other animals (Gregory and Isaac 2005; Fearn 2011; Frank and Dudás 2019), they could have been caused by direct or indirect anthropogenic factors. In Romania, *D. caspius* individuals are sometimes directly killed by humans (Covaciu-Marcov and David 2010), as found in other snake species in other regions (Meek 2012). At the same time, injuries could also be caused by different objects like barbed wire fences, debris etc. The lowest rate of individuals with scars was found in the Danube Gorge, the most natural area with the most reduced anthropogenic pressure.

The two mortality peaks in May and August (Fig. 3a) coincide with the breeding period (Fuhn and Vancea 1961) and the juveniles` hatching (Sahlean and Strugariu 2018). This is also reflected in the average size of snakes from each month, the smallest values being registered in August (Fig. 3b). Similar mortality-intensity peaks and the juveniles' presence at the end of summer were observed in other studies, too (Enge and Wood 2002; Shepard et al. 2008; DeGregorio et al. 2010). The average size of snakes (112.50 cm, if we exclude juveniles) could suggest the length at which snakes are the most vulnerable to road mortality. Below this value, snakes probably occupy less space on the road and are less likely observed by the drivers, who often run over snakes intentionally (Langley et al. 1989; Mesquita et al. 2015). Small-sized snakes were also rarely killed by cars in other regions for the same reasons (Lutterschmidt et al. 2019), though in other cases, most of the killed snakes were juveniles (Ciesiołkiewicz et al. 2006; DeGregorio et al. 2010).

In many snake species, the age of maturity can be reached at 68% (or even less in the case of large species) of their maximum length (Shine and Charnov 1991). This means that, for a maximum size of 184 cm in males and 127 cm in females (Fuhn and Vancea 1961), sexual maturity can be reached at approximately 125 and 86 cm, respectively (or even less, *D. caspius* being a large species). Some females, included in our study, measured 150 cm, a value that exceeds any found in scientific literature (Fuhn and Vancea 1961) and extends the size of sexual maturity in females to 102 cm. Sahlean and Strugariu (2018) collected females with a minimum SVL of 86 cm, indicating a total body length of about 119 cm. Thus, our results fit those previous records (Sahlean and Strugariu 2018).

The main hypothesis of our study, namely, that snake corpses provide useful information about life-traits, has been confirmed. We obtained information on morphology, asymmetry, differences according to sex, geographic region and, indirectly, on environmental conditions and on the period and length when they are the most threatened. The observation that the lowest rate of scars was registered in the region with the lowest anthropogenic impact suggests that *D. caspius* lives in less-optimal conditions in most of its distribution area in Romania. In addition, if high rates of mortalities continue, the data obtained can be employed to examine life history traits which, in turn, may have value for future conservation measures.

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