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Original scientific paper

MORPHOLOGICAL VARIABILITY AND SEXUAL DIMORPHISM OF THE FIRE SALAMANDER FROM THE GRADIŠKA AREA (REPUBLIC OF SRPSKA, BOSNIA AND HERZEGOVINA)

Dragojla Golub[1], Goran Šukalo[1], Branislav Babić[1]

[1]*University of Banja Luka, Faculty of Natural Sciences and Mathematics, Mladena Stojanovića 2, 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina*
***Corresponding author**: dragojla.golub@pmf.unibl.org

Abstract

The paper presents the results of the analysis of the morphological variability and sexual dimorphism in the fire salamander (*Salamandra salamandra*) population from the Gradiška area (Republic of Srpska, Bosnia and Herzegovina). Field research was conducted in the autumn and spring 2016 and 2017. A total of 70 adult individuals were analyzed, comprising of 40 males and 30 females. Twenty-two morphometric characters, body mass, one meristic trait (number of costal grooves on the lateral sides of the body), and qualitative traits related to body coloration were analyzed. By applying t-tests, statistically significant differences between the sexes were found for body mass, maximum body height, maximum body width, cloaca length, tail height, tail width, forelimb length, hind limb length, hand length, and foot length. The number of costal grooves on the left and right sides of the body ranged from eight to 12, with individuals having 10 and 11 costal grooves being the most common. Both individuals with the same or different numbers of costal grooves on the left and right sides of the body were observed. The majority of individuals (51%) had symmetric arrangement of yellow spots on the dorsal side of the body, while the presence of yellow spots was most prevalent (69%) on the ventral side of the body in terms of coloration.

Key words: *Salamandra salamandra,* morphometric and meristic characteristics, sexes, coloration

INTRODUCTION

Variability represents the changeability of certain traits within the same individual at different times, among different individuals within the same group, or between different generations of the same species. Morphological variability can be investigated at the level of morphometric characters (length measurements), meristic characters (countable traits), and qualitative traits (presence of specific colors, patterns on the body, etc.) (Tucić, 1987). Examining and quantifying intra- and inter-individual differences in morphological structures allows the discovery of patterns of phenotypic variation, as well as potential developmental and eco-evolutionary mechanisms leading to their divergence (Dryden and Mardia, 2016). Morphometric methods are applied to quantitatively analyze a specific morphological entity,

describing its shape and size or determining individual characteristics of that organism (Oxnard, 1978).

Sexual dimorphism, especially size-related sexual dimorphism, exists within many animal groups, with the greatest differences between sexes observed in ectothermic organisms. Apart from morphology (color, shape, and dimensions of the body), differences between sexes can also be reflected in behavior (territoriality, competition, courtship). The most common causes of size-related sexual dimorphism are three selective processes: sexual selection, fecundity selection, and ecological divergence between sexes (Darwin, 1871; Andersson, 1994; Shine, 1989; Milankov, 2007). Size differences between sexes have been documented in all three orders of amphibians, with females being larger than males in 61% of cases among anuran amphibians (Shine, 1979; Kupfer, 2007). Just as there is morphological variability within individuals of a single population, it can also be observed among geographically separated populations. Morphological traits that commonly exhibit geographic variability include size, shape, and proportions of various body parts (Mayr, 1965).

The fire salamander (*Salamandra salamandra*) is widespread across much of Western, Central, and Southern Europe. The species is found from sea level up to about 2500 meters altitude, but most commonly below 800 meters (Arnold, 2004). In the Balkans, it is widely distributed (Steward, 1969; Labus *et al*., 2012), typically inhabiting hilly and mountainous areas of moist deciduous forests with a thick layer of humus. Optimal habitats include beech forests, alder forests, moist birch and oak forests, and oak-hornbeam forests (Džukić, 1993; Meikl, 2010).

Globally, it is considered a taxon with declining populations (IUCN SSC Amphibian Specialist Group, 2023) and is protected under the Bern Convention (Appendix III). Habitat destruction, pollution, and collection for commercial purposes (primarily the pet trade) are considered major threats leading to reduced population densities in some areas (AmphibiaWeb, 2023). In Bosnia and Herzegovina (B&H), the fire salamander is not recognized as a strictly protected or protected species (Official Gazette of the Republika Srpska, 65/20; Official Gazette of the Federation of BiH, 21/20).

Numerous authors have studied the morphological variability of the fire salamander in the Balkan region (Radovanović, 1951; Đurović *et al*., 1979; Džukić, 1993; Kalezić *et al*., 2000; Bielen, 2003; Labus *et al*., 2012, 2013a, 2013b; Abazović, 2013; Šukalo *et al*., 2013, 2015; Đurić *et al*., 2016, etc.).

The aim of this study is to analyze the morphological, quantitative, and qualitative characteristics of the fire salamander in the wider Gradiška area (northwest B&H), in order to assess morphological variability and analyze sexual dimorphism within the same set of traits.

MATERIALS AND METHODS

Research area. Gradiška, is located in the northwest part of B&H and territorially belongs to the entity of the Republic of Srpska. The broader area of Gradiška includes Lijevče Polje and Potkozarje, bordered to the north by the Sava River, which also forms the border with Croatia. The climate is moderately continental, with an average monthly temperature of +11 °C. Besides the Sava River, the region is rich in hilly rivers, with the largest being Vrbaška,

Jablanica, Jurkovica, and Lubina. Forests are abundant in the area, with oak forests being the most prevalent. Along the northern coast of the Sava River and its major tributaries, there is a belt of pedunculate oak, interspersed with forests of sessile oak and common hornbeam (http://bih-x.info/regije-i-gradovi/gradovi-bih/gradiska/; www.fhmzbih.gov.ba).

Sampling. Collection of fire salamander specimens was conducted in the villages of Miloševo Brdo (157 m above sea level) and Bistrica (120 m above sea level), located approximately 12 km from Gradiška, situated on the southern slopes of Mount Prosara, not far from the eastern slopes of Mount Kozara (Figure 1). Specimens were captured in mid-October 2016 at the Bistrica land in early April 2017 at the Miloševo Brdo. All salamanders were captured during the daytime, between 08:00 and 17:00. A total of 81 adult fire salamander individuals were captured and analyzed. Sex was determined by macroscopic examination of the cloaca. Since reliable sex determination is only possible for individuals whose total body length exceeds 14 cm (Bogaerts *et al*., 2021), individuals with a total body length of less than 14 cm were excluded from the analysis. Therefore, the analysis included only 70 fire salamander individuals. After measurements, all specimens were returned to their habitats unharmed.

Figure 1. Geographical location of the researched sites
(https://maps-bosnia.com/bosnia-on-world-map;
https://en.wikipedia.org/wiki/File:Bosnia_and_Herzegovina_physical_map.svg)

Morphometric characteristics. The analysis of morphological variability in the fire salamander included the measurement of 22 morphometric characters (Figure 2): TD - total body length, STD – snout to vent length (distance from the tip of the snout to the anterior edge of the cloaca), VT - maximum body height, ŠT - maximum body width, DK - cloaca length (from the beginning to the end of the cloaca), DG - head length, ŠG - head width, VG - head height, DR - tail length (from the posterior edge of the cloaca to the tip of the tail), VR - tail height (immediately behind the cloaca), ŠR - tail width (immediately behind the cloaca), RIZMN - distance between legs (between the forelimbs and hind limbs, i.e., from the armpit to

the groin), DPN - forelimb length (from the armpit to the base of the hand), DZN - hind limb length (from the armpit to the base of the foot), DŠ - hand length (from the base of the hand to the end of the third toe), DS - foot length (from the base of the foot to the end of the fourth toe), DO - eye diameter, RIZMO - interorbital distance, RIZMN - distance between nostril, DZŽ - length of the parotoid gland, ŠZŽ - width of the parotoid gland, and DV - jaw length (from the snout to the corners of the mouth). A caliper with a precision of 0.1 mm was used for measuring the morphometric characters.

The body mass of each individual was also determined using a digital scale with a precision of 1 g.

The meristic character, the number of costal grooves on the lateral sides of the body, was determined macroscopically by counting them along the left and right sides of the body.

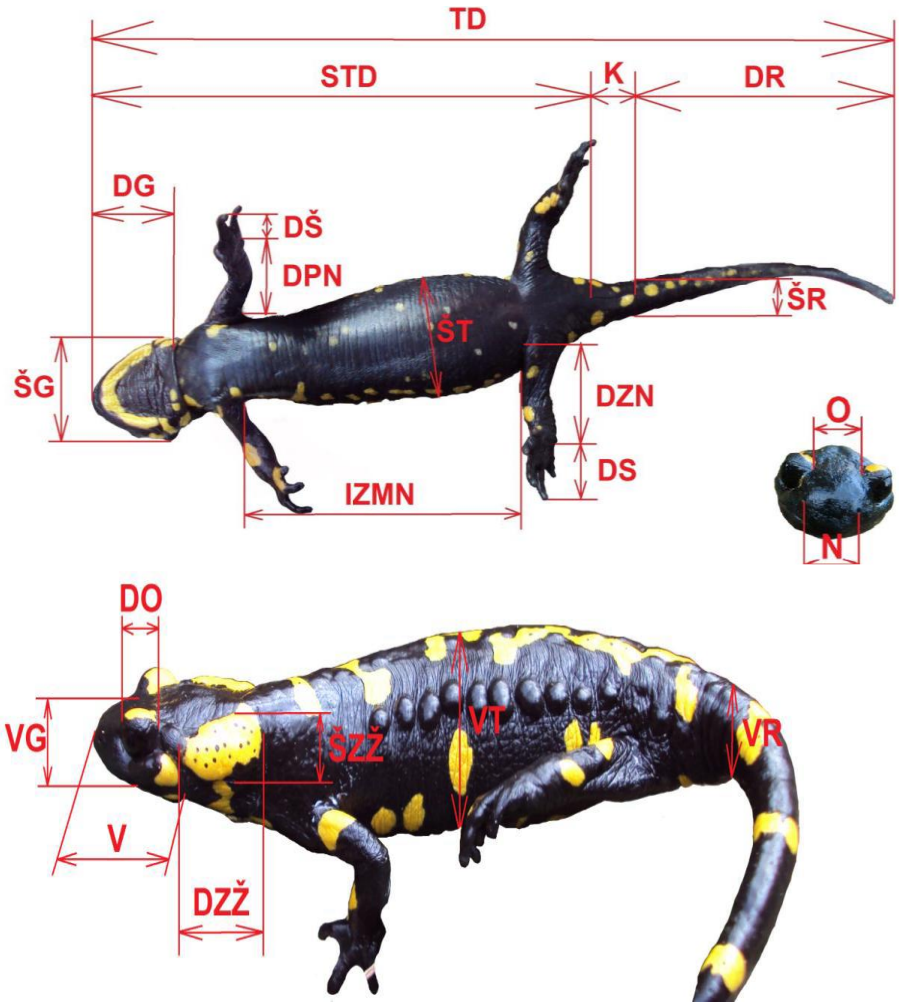

Figure 2. Scheme of the analyzed morphometric characters of the fire salamander (Abazović, 2013)

Qualitative Traits. The individuals were photographed from the dorsal and ventral sides of the body to define the type of coloration, which was determined according to Džukić (1993). Based on the type of yellow pattern on the dorsal side of the body, the fire salamanders were divided into three groups: animals with diffusely scattered yellow spots, animals with yellow spots showing a tendency toward mediodorsal merging, and animals with a more or less

symmetrical arrangement of yellow spots. Regarding the coloration of the ventral side, two main types were distinguished: animals with yellow spots on the ventral side and animals with a black ventral side, although several subtypes of ventral coloration were also noted (marbled yellow pattern and the manifestation of yellow pigment in the form of small transverse lines) (Džukić, 1993).

Statistical analysis. Descriptive statistical analysis was performed for all morphometric characters, calculating the range (minimum and maximum values), mean value, and standard deviation (SD). The statistical significance of differences in mean values of morphometric characters between males and females at a confidence level of $p<0.05$ was tested using the t-test (Skakić, 2001).

RESULTS AND DISCUSSION

Sex ratio

Of the total 70 individuals analyzed of the fire salamander, it was found that 30 (43%) were female and 40 (57%) were male (the sex ratio is approximately 1:1.3). Table 1 shows that males numerically dominate during autumn (October), while the number of females is slightly higher during the spring (April).

Table 1. The number of males and females of the fire salamander in relation to the sampling period

Date	Site	Number of males	Number of females
October, 2016	Bistrica	27	13
April, 2017	Miloševo brdo	13	17

Data supporting the higher number of males have been obtained by other authors as well: for northwest Croatia, 62% males and 38% females (Bielen, 2003), for the area around Teslić (B&H), 63% males and 27% females (Đurić *et al*., 2016), and for Mount Ozren (area around Doboj, B&H), 59% males and 41% females (Nešković, 2016). Abazović (2013) noted a slightly higher representation of females (F: 54%, M: 46%) in the area around Banja Luka (B&H), while Šukalo *et al*. (2015) found a higher representation of males (M: 63%, F: 37%) also in the Banja Luka area. Labus *et al.* (2013a, 2013b) provide results for the number of male and female individuals of the fire salamander for the central Balkans area (M: 38%, F: 62%) and Šar Mountain (M: 37%, F: 63%), where a higher representation of females in the sample is evident. Research at two sites in Serbia conducted by Kalezić *et al.* (2000) provides the following data: in the Despotovac area, males were more prevalent (M: 74%, F: 26%), while in the Vršac area, females dominated (M: 16%, F: 84%).

The dominant presence of males or females in a particular area is most often associated with the sampling period. In the spring (March-May), during the mating period, females are somewhat more frequently encountered. After reproduction, females rest for about a month, so from the end of May, males can mainly be found. Another significant activity occurs in the fall, associated with migration to winter shelters. During the fall, males and sexually immature

individuals are mostly active, while adult females carrying larvae have limited activity (Meikl, 2010).

Morphometric characters and body mass

The minimum, maximum, and mean values, as well as the standard deviation for 22 morphometric characters and body mass, for males and females, are provided in Table 2.

Table 2. Overview of the minimum, maximum, and mean values, as well as the standard deviation for body mass and morphometric characters of the fire salamander by sex (SD-standard deviation; p=level of significance: bold values indicate $p<0.05$; length values are expressed in millimeters, and body mass in grams. The meanings of the abbreviations for morphometric characters are found in the Materials and Methods section).

	MALES (N = 40)				FEMALES (N = 30)				
Trait	**Min. value**	**Max. value**	**Mean value**	**SD**	**Min. value**	**Max. value**	**Mean value**	**SD**	**p**
Body mass	18.91	44.45	29.62	6.18	24.20	50.40	37.56	6.91	**0.000**
TD	154.3	210.4	184.35	12.41	154.6	208.1	181.11	12.90	0.291
STD	85.5	121.3	103.82	7.16	91.4	119.6	105.06	6.03	0.444
VT	13.4	25.5	18.09	2.42	16.1	27.0	21.58	2.20	**0.000**
ŠT	17.0	25.5	19.98	2.08	19.8	29.1	23.25	2.34	**0.000**
DK	8.4	13.0	10.54	0.81	6.0	8.9	7.63	0.80	**0.000**
DG	20.0	32.5	27.51	2.50	23.6	30.0	27.45	1.40	0.915
ŠG	16.0	23.2	19.57	1.66	17.1	22.1	20.14	1.35	0.130
VG	6.0	10.0	7.88	0.93	6.0	10.3	8.10	1.18	0.367
DR	52.8	79.3	70.00	6.08	48.3	80.2	68.42	7.46	0.333
VR	7.0	10.3	8.29	0.91	7.4	10.9	9.11	0.94	**0.000**
ŠR	5.9	10.0	7.66	1.15	5.9	10.9	8.56	1.35	**0.003**
RIZMN	49.0	70.0	60.22	5.11	55.0	70.1	61.25	4.27	0.372
DPN	20.6	28.7	24.44	1.83	18.7	26.7	22.56	1.76	**0.000**
DZN	20.1	30.5	23.84	1.98	17.0	25.9	22.48	2.07	**0.007**
DŠ	10.4	16.2	14.06	1.35	10.0	15.2	13.14	1.16	**0.004**
DS	13.0	18.5	16.30	1.27	13.6	17.4	15.46	0.97	**0.003**
DO	4.7	15.4	5.95	1.64	4.1	6.4	5.31	0.55	0.051
RIZMO	8.1	11.0	9.86	0.68	8.6	11.1	9.81	0.62	0.766
RIZMNZ	5.9	8.8	7.33	0.60	6.5	8.5	7.32	0.53	0.990
DZŽ	1.1	16.2	13.51	1.02	11.6	17.3	13.65	1.40	0.633
ŠZŽ	4.6	7.9	6.25	0.64	5.0	8.0	6.11	0.69	0.413
DV	16.2	22.0	19,29	1.18	16.8	21.5	19.27	1.28	0.960

Using the t-test, statistically significant differences between sexes were found for nine morphometric characters (maximum body height, maximum body width, cloaca length, tail height, tail width, forelimb length, hind limb length, hand length, and foot length) and body mass (Table 2). Females have higher mean values for body mass, maximum body width, and maximum body height. The larger values of these three traits in females can be associated with the fact that females were mostly captured during spring, i.e., just before or during larval deposition. In amphibians, the fecundity of females generally favors their larger body size

compared to males (Kalezić *et al*., 2000). On the other hand, males have higher mean values for seven morphometric characters (cloaca length, tail height, tail width, forelimb length, hind limb length, hand length, and foot length), which can be linked to reproductive behavior and courtship. Sexual dimorphism in the fire salamander is most noticeable during mating, when males have an enlarged (swollen) cloaca. Also, the male's body is shorter and slimmer, and the limbs and tail are proportionally longer (Džukić, 1993; Labus *et al*., 2013a), which is consistent with our results.

Šukalo *et al.* (2015) noted statistically significant differences in body mass, cloaca length, forelimb length, hind limb length, and hand length for salamanders in the area around Banja Luka. Research results from the Teslić area (Đurić *et al.*, 2016) show that the mean values of nine morphometric characters differ significantly between sexes: snout to vent length, cloaca length, body mass, jaw length, distance between legs, parotoid gland length, tail length, total body length, and head width. Labus *et al.* (2013b) found statistically significant differences between the sexes of the fire salamander from the Šar Mountain in head width, forelimb length, hind limb length, and hand length. For *S. salamandra* from Mount Ozren, statistically significant differences were found in cloaca length, body mass, hand length, and foot length (Nešković, 2016).

Meristic trait

The number of costal grooves along the left and right sides of the body ranged from a minimum of eight to a maximum of 12 (Figure 3). Of the total 70 analysed individuals, 61% had the same number of costal grooves, while 39% had a different number of costal grooves. Analysing both the same and different numbers of costal grooves, it was found that on the left side of the body, the largest number of individuals had 11 and 10 costal grooves, and on the right side of the body, 10 and 11 costal grooves. Among individuals with the same number of costal grooves, those with 11 (46%) and 10 (33%) costal grooves were the most prevalent.

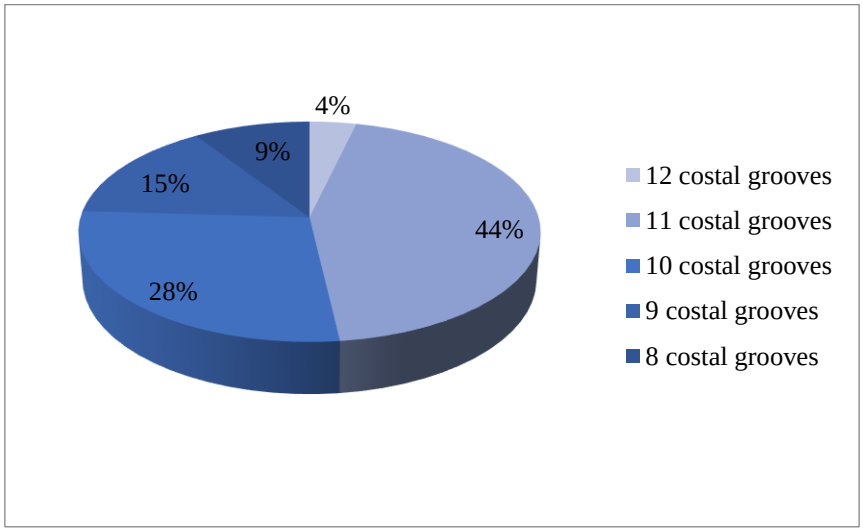

Figure 3. The percentage representation of fire salamander individuals with the same number of costal grooves along the left and right sides of the body.

For fire salamanders from the Teslić area, the number of costal grooves also varied from eight to 12 (Đurić *et al.*, 2016). In the analyzed sample from the Banja Luka area, the number of costal grooves varied from seven to 12 (Abazović, 2013), while for individuals from Mount Ozren, the number of costal grooves on the body sides varied from nine to 12 (Nešković, 2016). In all the mentioned studies, individuals with 10 and 11 costal grooves on the body sides were predominant.

Qualitative traits

In terms of the type of yellow pattern on the dorsal side of the body, individuals with a symmetric arrangement of yellow spots predominated in the analyzed sample (36 individuals, or 51%). Regarding the coloration of the ventral side of the body, the majority of individuals had yellow spots (48 individuals, or 69%) (Figure 4).

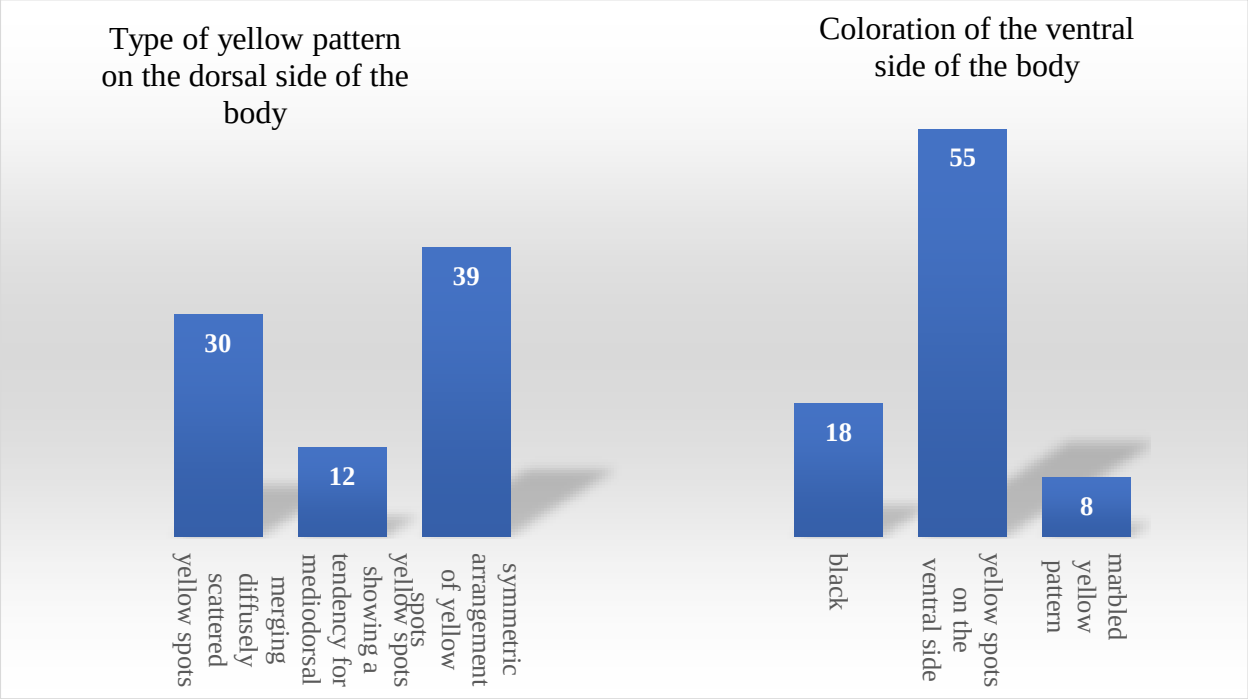

Figure 4. The representation of coloration types on the dorsal and ventral sides of the body of individuals of the fire salamander

The dominant type of yellow pattern on the dorsal side of the body in most other studies is characterized by a symmetric arrangement of yellow spots, except for the fire salamanders from the Teslić area and Mount Ozren, where the most prevalent type was diffusely scattered yellow spots (Đurić *et al*., 2016; Nešković, 2016). On the other hand, the highest number of individuals with yellow spots on the ventral side of the body was observed in most of the mentioned studies. An exception are Džukić's (1993) findings for populations of the fire salamander from Serbia, where a black ventral side was more common.

While it is generally believed that there is no difference in coloration between males and females (Džukić, 1993), some studies suggest the presence of sexual dichromatism, with males having a larger total area covered with yellow spots on their backs and tails compared to females (Balogova and Uhrin, 2015). On the other hand, some authors found a clear

relationship between the color pattern and two parameters representing ecosystem productivity and prey availability (Barzaghi *et al.*, 2022).

CONCLUSION

Statistically significant differences between sexes for the studied set of morphometric traits and body mass in the fire salamander were found in body mass, maximum body height, maximum body width, cloaca length, tail height, tail width, forelimb length, hind limb length, hand length, and foot length. The number of folds on the body sides varied from eight to 12, with individuals recorded having both the same and different numbers of folds. Regarding the dorsal color pattern, individuals with a symmetric arrangement of yellow spots predominated, while on the ventral side, individuals with yellow spots were dominant. The results presented in this study provide the first data on morphological characteristics of the fire salamander in the Gradiška area and serve as a basis for further biological and ecological research on this species, both in this area and beyond.

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